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The effects of resveratrol on cognition, cerebral blood flow, gastrointestinal microbiota and systemic inflammation in healthy weight, overweight and obese adults

Ellen Francesca Smith

PhD

2022

The effects of resveratrol on cognition, cerebral blood flow, gastrointestinal microbiota and systemic inflammation in healthy weight, overweight and obese adults

Ellen Francesca Smith

A thesis submitted in partial fulfilment of the requirements of the University of Northumbria at Newcastle for the degree of Doctor of Philosophy

Research undertaken in the Faculty of Health and Life Sciences, University of Northumbria

May 2022

Abstract

The stilbene polyphenol resveratrol has been shown to interact with several health-promoting mechanisms, which might impact cognitive performance. However previous research has indicated that resveratrol supplementation is not able to impact cognitive performance in young, healthy adults; despite consistent ability to modulate cerebral blood flow. Recent work suggests that resveratrol supplementation may be more beneficial in individuals who are compromised by age, disease, or overweight status. Specifically, obese individuals are characterised by a multitude of health issues including sustained inflammation, elevated cholesterol levels; excessive fat accumulation and neuroinflammation; here resveratrol supplementation offers a therapeutic option. Moreover, a recent shift in literature focus indicates the importance of gut microbial composition on host-health; and specifically, how this can be modified by dietary intervention. Obesity is associated with dysbiosis of the microbiota, disruption to the intestinal barrier and exacerbated pro-inflammatory response; where it is hypothesised that polyphenol intervention may have the capability to modulate microbial composition and exert health-promoting effects. With said health promoting effects of polyphenolic-gut-modulation potentially also extending to cognitive function, via the gut-brain-axis. Therefore, the two randomised, placebo-controlled, double-blind intervention trials included in this thesis aimed to investigate the effects of resveratrol supplementation on cognitive function, cerebral blood flow, inflammation and gastrointestinal microbiota in healthy adults of varying weight status.

The key findings here indicate that as in young, healthy adults, resveratrol supplementation results in clear modulation of CBF in healthy overweight-obese adults. Furthermore, it confirms that within this population, resveratrol is unable to exert cognitive enhancing effects, with limited evidence of a detrimental effect observed. Limited changes in microbial composition indicate that this is likely a promising avenue for future investigations of either resveratrol or other polyphenolic or dietary intervention.

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In loving memory of Julie Khan. Mama Jules to many and the heart of the BPN, we love and miss you so much – I couldn't have done it without you.

Author's Declaration

I declare that the work contained within this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others. The author, supervisors and external collaborators (Evolva AG) worked together to design the methodology of the experimental chapters. For these chapters, the data collection, statistical analysis and interpretation was the work of the author. The analysis of biological samples was completed by staff members within the Applied Sciences department of Northumbria University.

Any ethical clearance for the research presented in this thesis has been approved. Approval has been sought and granted for all studies presented throughout this thesis by the Faculty of Health and Life Sciences Ethics Committee at Northumbria University and were conducted according to the Declaration of Helsinki (1964).

I declare that the word count of this thesis is 97,935 words.

Name: Ellen Francesca Smith

Date: 30/05/2022

CHAPTER 1.
POLYPHENOLS INTRODUCTION

1.1. Definition, classification, synthesis and sources of polyphenols

1.1.1. Classification and synthesis of polyphenols

Polyphenols are a highly diverse, extensive group of phytochemicals, that are chemically characterised as compounds with phenolic structural features. Specifically, in order to be classified as a 'poly'phenol the structure must include two or more phenolic rings (Del Rio et al., 2013; Tsao, 2010). They are produced by plants as a 'secondary metabolite' and enhance the survival of the plant by contributing to pollination and pigmentation. They also provide a protective role, by defending the plant from environmental stressors, specifically pathogens, parasites, reactive oxygen species (ROS) and ultraviolet (UV) radiation (Kennedy, 2014b; Kennedy & Wightman, 2011). In terms of dietary sources, polyphenols are naturally occurring compounds found in fruits, vegetables and other plant sources (Septembre-Malaterre, Remize, & Poucheret, 2018), with more than 8,000 polyphenol compounds identified (Oak et al., 2018). As such, they are highly abundant within the human diet, primarily through the consumption of fruits, vegetables, cereals and beverages including tea, coffee and wine (Li et al., 2014; Tsao, 2010).

Phenolics are initially synthesised via the shikimic pathway from shikimic acid. Whilst some phenolic acids can be synthesised directly from shikimic acid, more complex phenolic structures utilise additional pathways including the acetate pathway (Kennedy, 2014a). In these cases, the chorismic acid derivative L-phenylalanine creates cinnamic acids via a series of hydroxylation and methylation reactions. Cinnamic acid then provides the base of all polyphenols, which combine a shikimate pathway-derived cinnamic acid starter unit (cinnamoyl-CoA) with two or three malonyl-CoA units, which are derived via the acetate pathway. This phenylpropanoid pathway results in the production of either chalcone synthase (the basis of all flavonoid polyphenols) or stilbene synthase (which forms stilbene polyphenols) (Jeandet et al., 2002; Kennedy, 2014a; Lijavetzky et al., 2008).

As Figure 1 demonstrates, polyphenols can be categorised into different classes based on the number of phenolic rings in their structure, the structural elements that bind these rings to each other and the substituents linked to the rings. The first categorisation of polyphenols is flavonoids or non-flavonoids, where all flavonoid share a common structure (C_6-C_3-C_6) of two 6-carbon rings, with a three-carbon bridge (Dewick, 2009). Flavonoids represent the largest and most diverse single group of secondary metabolites and can be subdivided into flavones,

flavonols, flavanones, isoflavones, chalcones, flavanols and anthocyanins, of which some of these subgroups can be further divided (Liu, He, Ma, & Chen, 2019). Whereas non-flavonoids includes structurally different compounds such as lignans (C_6-C_3-C_3-C_6) and stilbenes (C_6-C_2-C_6) (Farzaei, Rahimi, & Abdollahi, 2015).

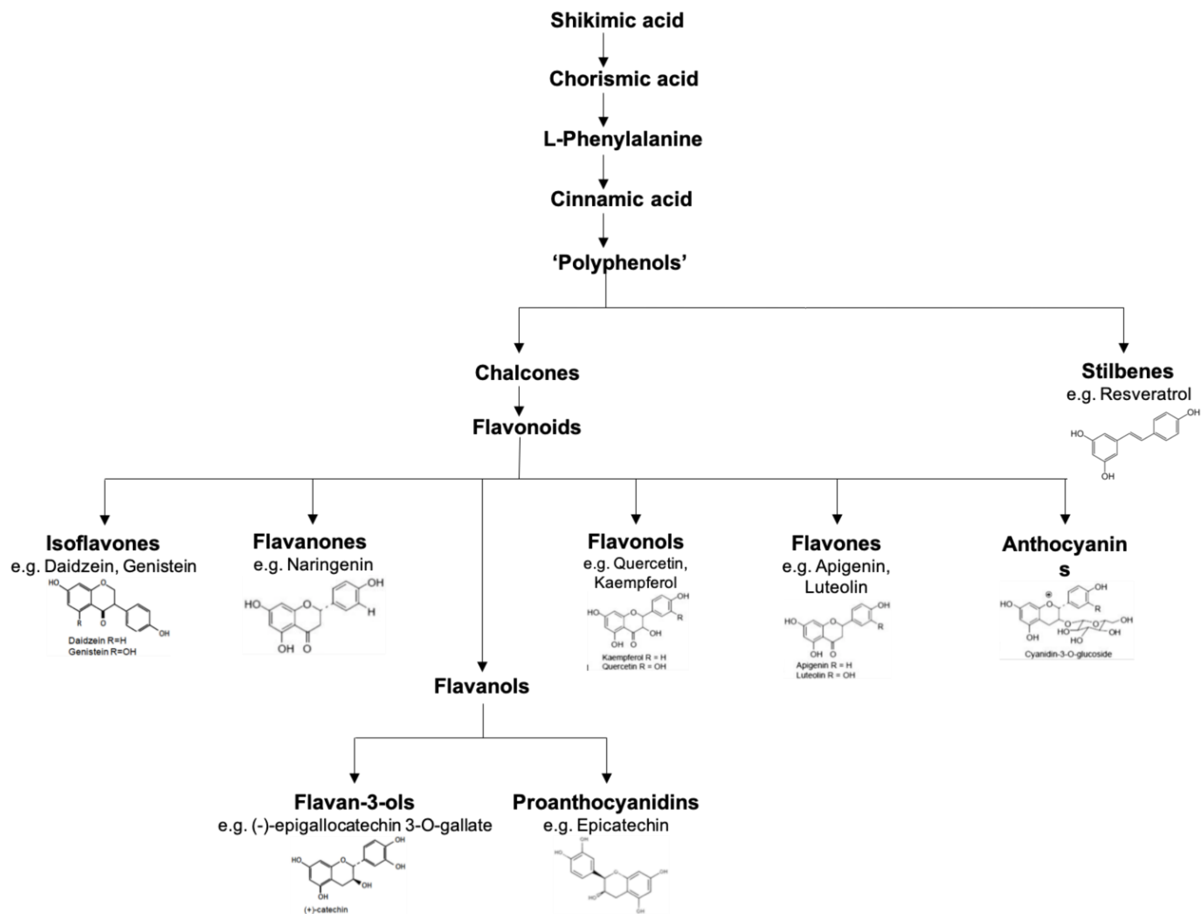

Figure 1.1 Synthesis and main sub-groups of polyphenols with examples. Chemical structures are adapted from Kennedy (2014a).

1.1.2. Dietary sources and consumption of polyphenols

Each of the aforementioned structural groups of polyphenols are present in plant-based foods. However, the concentrations differ significantly between food sources, with the highest phenolic concentrations observed in flavanols and anthocyanins in dietary elements that we tend to consume very little of; including herbs and spices and berries (Pérez-Jiménez, Neveu, Vos, & Scalbert, 2010). Whilst these foods contain the highest concentrations, it has been suggested that we consume the bulk of our daily phenolic intake through the plant-based products that we consume most often and in the greatest quantities, in particular tea and coffee, fruits and vegetables (Kennedy, 2014a).

Of these subgroups, flavonols including quercetin are found widely across the plant kingdom, with especially rich concentrations observed in yellow and red onions *(Allium cepa)* (Del Rio et al., 2013). Evidence has indicated that the flavonol content within fruits and vegetables differs greatly, potentially due to seasonal changes and differences in local growing conditions (Crozier, Lean, McDonald, & Black, 1997; Macready et al., 2009). Whilst flavones, such as apigenin, are structurally similar to flavonols, they are generally not observed as widely. However highest concentrations have been observed in celery, parsley and some additional herbs (Hostetler, Ralston, & Schwartz, 2017). Isoflavones, classified as phytoestrogens due to their structural similarity to oestrogen, like daidzein and glycitein, are found almost exclusively in leguminous plants with the highest amounts found in soybeans (El Gharras, 2009; Munro et al., 2003). Flavanones are present within a few aromatic plants, however they are observed in especially high amounts in the flavedo (coloured outer peel) of citrus fruits including grapefruit (Del Rio et al., 2013). Anthocyanins are polyphenolic pigments responsible for the differing colours ranging from blue-purple and orange-red, which are readily visible in flowers, fruits and leaves (Wallace & Giusti, 2015). Of these, the most commonly consumed anthocyanin is cyanidin, with high concentrations observed in fruits, particularly berries (Horbowicz, Kosson, Grzesiuk, & Dębski, 2008). Finally, flavanols are the most complex subclass of flavonoids, as they range from simple monomers to the oligomeric and polymeric proanthocyanidins (Pascual-Teresa, Moreno, & García-Viguera, 2010). Of them, catechin and epicatechin are the two most common, which are widely spread in nature. In terms of food sources, green tea contains very high levels of these as well as epigallocatechin, epigallocatechin-3-gallate (EGCG) and epicatechin-3-gallate (ECG) (Del Rio et al., 2013).

Belonging to the non-flavonoid group, stilbenes are a group of phytoalexins, with a C_6-C_2-C_6 structure which are produced by the plant in response to stress, injury and disease (Pryce Langcake & Pryce, 1977). The primary example is resveratrol (3,5,4'-trihydroxystilbene),

although other stilbenes include piceid, pinosylvin and pterostilbene (Chong, Poutaraud, & Hugueney, 2009). Sources include Japanese knotweed (*Polygonum cuspidatum),* grape (*vitis vinifera)* and peanut (*fabaceae)*, although these constitute very minor dietary components when compared to the levels of other polyphenolic components observed in food sources.

Due to the widespread presence in food components and food products, the consumption of polyphenols is considered unavoidable (Kennedy, 2014a). Perhaps because of this, it is difficult to calculate the actual amounts consumed by individuals from their regular diet. There are a number of methodological issues associated with this. Firstly, most often it requires participants to recall their dietary consumption using questionnaires. This may result in inaccurate dietary information, particularly as this method is likely prone to recall bias and individuals may also intentionally misreport their consumption of certain foods (Naska, Lagiou, & Lagiou, 2017). Secondly, these questionnaires assign individual phenolic values and calculate dietary intake based on the assumption that all products consumed globally will contain the same levels of polyphenols. However, these levels are likely variable based on growth location as well as transport, storage and cooking methods (Del Bo et al., 2019; Leclercq, Valsta, & Turrini, 2001).

The consumption of phenolics can also be highly variable depending on geographic location. This is due, in part, to differing eating habits, with more developing countries consuming a 'Westernised' diet which tends to consume fewer high-polyphenolic rich food components like fruits and vegetables (Moubarac, Parra, Cannon, & Monteiro, 2014). Nevertheless, several studies have attempted to calculate the average phenolic intake in various samples; with total flavonoid intake estimated at 189.7 mg/day in a US sample (Chun, Chung, & Song, 2007); whereas a Spanish cohort demonstrated a higher consumption of 313.26 mg/day (Zamora-Ros et al., 2010). A slightly more recent figure, calculated using data from adults from 21 surveys (~30,000 individuals) from 14 European countries, estimated flavonoid intake of 428 mg/day (Vogiatzoglou et al., 2015). To our knowledge, this is the most recent data available, however it is crucial to recognise that dietary patterns vary over time and therefore the current flavonoid intake could differ from these figures. In comparison, when looking at all polyphenols (not just flavonoids as the previous studies), the SU.VI.MAX trial, within French adults aged 40-60 years (n =4942), analysed 24-hour dietary data (collected 6+ times over a 2 year period (1994-1995)) and observed that a total of 337 individual polyphenols were consumed, with an average intake of 1193 mg/day (Perez-Jimenez et al., 2011). Most recently, a study compared the polyphenol intake of participants who either followed UK dietary guidelines or consumed a control diet that was nutritionally balanced and typical of a UK diet, for a period of 12 weeks (Castro-Acosta, Sanders, Reidlinger, Darzi, & Hall, 2019). Using food diaries and a food

frequency questionnaire (FFQ), they were able to estimate polyphenol dietary intake. They found that total polyphenol intake was significantly higher in the dietary guidelines group (1279 mg/day) in comparison with the control group (1084 mg/day). They attributed this difference to higher intake of anthocyanins, proanthocyanidins and hydroxycinnamic acids, due to greater consumption of fruits, cereal products, nuts and seeds. They further found that the dietary guidelines group had a greater intake of flavonoids. The authors conclude that the UK dietary guidelines increased total polyphenol intake by approximately 20%. As such, it is difficult to establish a concrete understanding of polyphenolic intake by the general population, especially when considering that the above data is from several cultures, where dietary patterns hugely vary. Moreover, an important consideration is that these trials used data from healthy volunteers. Where it has previously been suggested that these trials likely attract volunteers who are health conscious and potentially have a higher nutritional intake than the general population (Morris & Tangney, 2011; Young, Gauci, Scholey, White, & Pipingas, 2020). As such it is difficult to establish if these findings are representative of the general population. Nonetheless, these figures can be used as a general guidance.

1.1.3. Classification and sources of resveratrol

Resveratrol (3,4',5 trihydroxystilbene) is the most commonly referenced stilbene polyphenol; structurally it consists of two phenolic rings bonded together by a double styrene bond, which is responsible for the isometric *cis-* and *trans-*forms (Gambini et al., 2015). First identified in the roots of white hellebore (*Veratrum grandiflorum)* and Japanese Knotweed (*Fallopia japonica)*, resveratrol and its derivatives have been isolated and identified in over 70 plant species with dietary sources including berries, nuts and soy (Boocock et al., 2007; Gambini et al., 2015; Püssa, Floren, Kuldkepp, & Raal, 2006). However, the most notable source is in red grapes and consequently wine, particularly red wine (Burns, Yokota, Ashihara, Lean, & Crozier, 2002).

As shown in Figure 1.2, resveratrol exists in both *cis-* and *trans-*isomeric forms; with the *trans-*isoform more common and biologically active than *cis-* (King, Bomser, & Min, 2006; Mukherjee, Dudley, & Das, 2010). First detected in grapevines in 1976, it was found that the compound was synthesised by leaf tissues in response to fungal infection or exposure to stressors such as ultraviolet light (Langcake & Pryce, 1976). Interestingly the detection of resveratrol and other compounds present in grapevines was prompted by epidemiological studies showing a correlation between red wine consumption and incidence of cardiovascular disease in populations with high intakes of saturated fat (commonly referred to as the 'French paradox') (Frémont, 2000; Haseeb, Alexander, & Baranchuk, 2017; Siemann & Creasy, 1992).

In terms of concentrations of resveratrol within these dietary sources, it has been estimated that mean concentrations in wines are in the range of 0.01 to 3 mg/100ml (Rocha-González, Ambriz-Tututi, & Granados-Soto, 2008; Rothwell et al., 2013). Highest concentrations are observed in red wines, and lower quantities in rosé and white wines, although this varies largely between type, origin and colour of the grape. In other dietary sources, concentrations are estimated at 0.08 mg/100 g for peanuts, 0.15 mg/100g for black grapes and higher concentrations observed in lingonberries (3 mg/100 g) and cranberries (1.92 mg/100g) (Rothwell et al., 2013). Using a reasonable estimate of resveratrol content of 5mg/litre in red wine (Gescher & Steward, 2003), calculations show that assuming a consistent daily intake of 375 ml, a person weighing 70 kg would receive a dose of ~27 µg per kg each day (Baur & Sinclair, 2006). Preclinical literature indicates that resveratrol can have beneficial effects at very low doses (between 200 µg/kg and 2 mg/kg) which are sufficient to produce peak plasma concentrations of unmetabolised resveratrol of ~20 nM-2 µM and these levels are reported to exert beneficial chemoprotective effects (detailed in Gescher and Steward (2003)). It has been estimated that dietary intake of stilbenes in Europe is between 2 and 3 mg/day, with resveratrol and piceid representing about 50% of the total stilbene intake (Zamora-Ros et al., 2010).

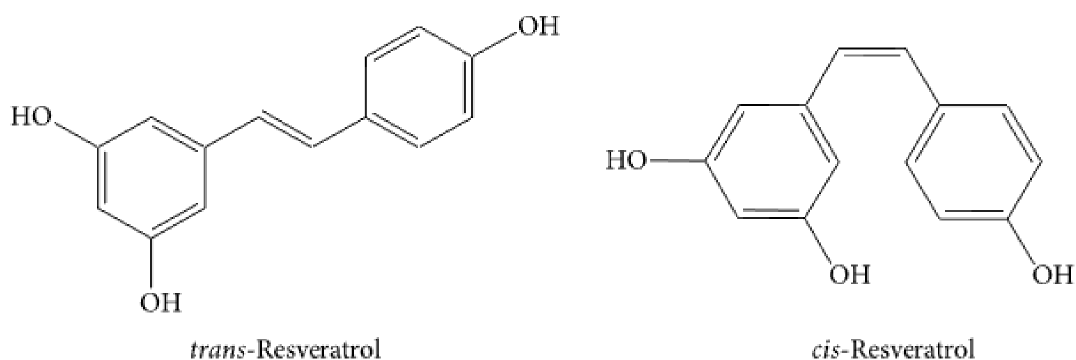

Figure 1.2 Chemical structures of *trans*-resveratrol and *cis*-resveratrol. Diagram obtained from Gambini et al. (2015).

1.2. Bioavailability and absorption of phenolic compounds

1.2.1. Bioavailability and absorption of polyphenols

The capacity of polyphenols to exert beneficial effects on the host is strictly related to the bioavailability and the products of their metabolism (Del Rio et al., 2013; Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004; Rodriguez-Mateos et al., 2014). Therefore, the study of the chemical transformations undergone by phenolic compounds after consumption is crucial to properly understand their biological effects (Manach et al., 2004; Selma, Espin, & Tomas-Barberan, 2009).

Once polyphenols have been consumed, their bioavailability strongly depends on their chemical structure. The majority of dietary polyphenols are present as esters, glycosides or polymers which cannot be absorbed in this native form (D'Archivio et al., 2007). The first metabolic process of these compounds begins in the mouth where hydrolytic enzymes release phenolic acid from glycoside conjugates (e.g. glucose, rhamnose, galactose, arabinose xylose and glucuronic acid) (Ginsburg, Koren, Shalish, Kanner, & Kohen, 2012). From there, it first reaches the stomach and then the small intestine, where these compounds follow the phase I metabolism with deglycosylation of phenolic acid; producing lower polar aglycones that become absorbable in the intestine (Ceppa, Mancini, & Tuohy, 2019). Polyphenols which are not absorbed by the small intestine, reach the large intestine where they are metabolised by the microbiota into a wide array of low molecular weight phenolic acids (Scalbert, Morand, Manach, & Rémésy, 2002). Once the final aglycone (or its derivative) has been absorbed, it then undergoes phase II metabolism at an enterocyte level (Marín, Miguélez, Villar, & Lombó, 2015). These resulting aglycones and polyphenol monomers are then transported via passive diffusion (Valdés et al., 2015) before metabolic detoxification processes take place in the liver involving methylation, sulfation and glucuronidation. This restricts the potential toxic effects of polyphenols and enables elimination through bile and urine (Barnes et al., 2011).

High molecular weight polyphenols are absorbed in the small intestine with great difficulty (with only ~5-10% of total ingested polyphenols absorbed) and reach the colon in almost unchanged form (Gowd, Karim, Shishir, Xie, & Chen, 2019). In the intestinal lumen area, colonic microbiota breaks down the original complex polyphenols into absorbable low molecular-weight phenolic metabolites (Guergoletto, Costabile, Flores, Garcia, & Gibson, 2016). These metabolites are more bioactive than their precursors and are able to substantially modulate gut microbial composition (Duda-Chodak, Tarko, Satora, & Sroka, 2015; Selma et al., 2009); representing the huge importance of the gut microbial community in the biotransformation of

polyphenols; as it is in this form that they reach blood, tissue and brain and can exert biological activities (Filosa, Di Meo, & Crispi, 2018).

The bioavailability of individual polyphenols varies greatly, potentially due to their glycosylation structure and degree of polymerization (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005; Pandareesh, Mythri, & Bharath, 2015), which impacts upon the rate and extent of absorption in the small intestine and colon (Cartea, Francisco, Soengas, & Velasco, 2011). As an example, a recent study aiming to expand knowledge on the metabolic fate of phenolic compounds, administered participants with a phenolic-rich drink made from grape pomace. They observed a high-inter-individual variability in both urine and plasma samples and different patterns of circulating metabolites (Castello et al., 2018); indicating that the bioavailability of polyphenols may further differ between individual participants. A potential explanation for this inter-individual difference is that the bioavailability and bioactivity of these compounds are mediated by many factors including the co-presence of other nutrients such as carbohydrates, lipids and proteins (Jakobek, 2015). The composition of the gut microbiota is also likely a contributing factor; where the overall microbial population, as well as abundance of specific bacterial strains can impact upon polyphenolic metabolism, bioavailability and bioactivity (Espín, González-Sarrías, & Tomás-Barberán, 2017; Stevens & Maier, 2016).

1.2.2. Bioavailability and absorption of resveratrol

The characteristics of the resveratrol compound, including lipophilicity, leads to a high absorption rate. As such, oral absorption of resveratrol in humans is relatively high, at an estimated level of ~70% in humans (Walle, 2011). Once ingested, resveratrol travels through the gastrointestinal tract, where it is estimated that around 70-80% of the intake of resveratrol is absorbed (Francioso, Mastromarino, Masci, d'Erme, & Mosca, 2014; Gambini et al., 2015). In the intestine, resveratrol absorption occurs by passive diffusion or by forming complexes with intestinal membrane transporters, including integrins (Chaplin, Carpéné, & Mercader, 2018). Resveratrol is rapidly metabolised in the liver, where it is taken up by the enterocytes and conjugated with glucuronic acid and sulfates, decreasing the circulating levels of free *trans*-resveratrol. Resveratrol metabolites including resveratrol monosulfate, monosulfate dihydroresveratrol and monoglucuronide dihydroresveratrol are also produced which can be detected in urine (Boocock et al., 2007; Menet et al., 2017; Rotches-Ribalta, Andres-Lacueva, Estruch, Escribano, & Urpi-Sarda, 2012).

From there, resveratrol can be found in three circulating forms: glucuronide (*trans*-resveratrol-3-glucoronide, *trans*-resveratrol-4'-glucuronide); sulfate (*trans*-resveratrol-3-sulfate, *trans*-

resveratrol-3,4'-disulfate, *trans*-resveratrol-3,5-disulfate) and free form (Gambini et al., 2015). Due to the extensive glucuronidation in the intestine and liver and sulfation in the liver, there exist very low levels of the free form resveratrol observed in the bloodstream (Walle, 2011). Despite this, the small quantities of free resveratrol that circulate can be bound to albumin and lipoproteins including low-density lipoprotein (LDL), allowing it to enter cells which have those receptors (Gambini et al., 2015). The amount of free resveratrol detected has also been found to increase with higher doses of resveratrol (Boocock et al., 2007). Evidence using a single dose of 25 mg resveratrol, assessed at 0.5-2 hours post dose, indicates that the detection of free resveratrol is difficult; with approximate calculations showing maximal concentrations of <10 ng/mL. In comparison, measuring plasma concentrations of free resveratrol, plus total metabolites, resulted in a much higher estimation of 400-500 mg/Ml; indicating a very low oral bioavailability of free resveratrol, but more pronounced levels of one of its metabolites (Goldberg, Yan, & Soleas, 2003; Walle, 2004).

The low bioavailability of resveratrol is well established, with evidence indicating that only ~1-8% of free resveratrol is found in serum (Walle, 2011), since the compound is rapidly metabolised by the microbiota and in the liver by the first pass metabolism. Despite this, there is evidence of efficacy *in vivo;* which can potentially be explained by the conversion to both sulfates and glucuronides. Additionally, the enterohepatic recirculation of resveratrol metabolites, followed by its deconjugation in the small intestine, aids its reabsorption (Marier et al., 2002; Wenzel & Somoza, 2005). It has been suggested that the bioavailability of resveratrol and its metabolites differs depending on the dosage and length of time of administration; with evidence indicating that dihydroresveratrol and free resveratrol are detected in tissues after sustained administration, whereas glucuronide and sulfate are detected following acute administration (Bresciani et al., 2014; Menet et al., 2017).

Due to the rapid biotransformation, research indicates that resveratrol concentrations have been identified following 30 minutes in humans, with peak plasma concentrations identified to occur between 30-90 minutes after oral administration in humans (Almeida et al., 2009; Boocock et al., 2007; Kennedy et al., 2010; Vaz-da-Silva et al., 2008; Walle, 2004). However, due to enterohepatic transport in bile, resveratrol re-cycles to the small intestine during metabolism; meaning that conjugated metabolites of resveratrol which are reabsorbed and synthesised by enzymes such as β-glucuronidase, could convert conjugates back to free resveratrol (Crozier, Jaganath, & Clifford, 2009; Gambini et al., 2015). This would account for those studies reporting final plasma peaks at ~6 hours post-consumption (Amri, Chaumeil, Sfar, & Charrueau, 2012).

1.2.3. Polyphenols and the blood brain barrier

The above evidence demonstrates that polyphenols and their metabolites can reach concentrations in the bloodstream which are sufficient to exert effects *in vivo* (Figueira et al., 2017). A key question though, is whether polyphenols (or, more likely, their metabolites) are able to reach the central nervous system (CNS) and to influence more complex processes influencing health and brain function. It is suggested that the mediating factor here is their ability to cross the blood-brain barrier (BBB), although it is important to note that some argue for the ability of polyphenols to exert these effects via indirect mechanisms without the need for direct CNS access.

The BBB regulates and limits molecular exchanges between the blood and neuronal tissue (and the brain interstitial fluid) and as such plays a crucial role in providing nutrients to the brain. It also controls the access of compounds, in this case polyphenols, to neuronal cells (Abbott, Rönnbäck, & Hansson, 2006; Cardoso, Brites, & Brito, 2010; Figueira, Menezes, Macedo, Costa, & Nunes dos Santos, 2017). In order to gain access to the brain, polyphenols or their metabolites must cross a tightly regulated, selectively permeable endothelial layer (Figueira et al., 2017). The capability of polyphenols to pass through the BBB is likely dependent upon its lipophilicity (its ability to dissolve in lipids), where evidence has indicated that less polar derivatives (for example methylated derivatives) are capable of higher brain uptake than more polar metabolites (including sulphated and glucuronides) (Youdim et al., 2003; Youdim, Qaiser, Begley, Rice-Evans, & Abbott, 2004). Evidence of the transport of different flavonoids (flavonols, flavan-3-ols and anthocyanins) and some of their methylated and glucuronidates metabolites has also been observed, where in most cases the metabolites exhibited a higher transport efficiency than their parent compounds (Faria et al., 2014). Additionally, the BBB permeability to polyphenols is also influenced by supplementary factors including the functionality and precise location of efflux transporters, solute carriers and organic anionic transporters (Watson, Preedy, & Zibadi, 2013). It is not yet entirely clear whether the primary route by which polyphenols cross the BBB is simple diffusion or carrier-mediated transport (Schaffer & Halliwell, 2012); however Figueira et al. (2017) state that the potential existence of different specialised transporters in the plasma membranes of luminal (blood) and abluminal (brain) sides of the endothelial cells should be considered.

Despite this, data from animal models revealed that various polyphenols and their metabolites can enter the brain at measurable levels (Chen et al., 2015; Gasperotti et al., 2015; Ho et al., 2013; Wu et al., 2012). Studies in humans have also successfully demonstrated that polyphenols can cross the BBB endothelium, with quercetin-3-*O*-glucuronide localised in

human brain tissue (Ishisaka, Mukai, Terao, Shibata, & Kawai, 2014). Figueira et al. (2017) used an *in vitro* model of the human BBB and evidenced that a 'plasma-bioavailable' polyphenol metabolite (previously identified in Pimpao, Ventura, Ferreira, Williamson, and Santos (2015)) could be transported across the BBB endothelium and, further that the endothelial cells transform these metabolites into novel components. Additionally, evidence has indicated that concentrations of polyphenols and their metabolites vary according to brain region (Janle et al., 2014), which may raise important questions about differential effects on brain function and behaviour.

1.3. The function of polyphenols and potential for improved human health

1.3.1. Polyphenols and human health – epidemiological and observational evidence

A relatively large body of research has considered the impact of habitual polyphenol consumption on human health. Epidemiological evidence consistently shows that cultures consuming polyphenolic-rich diets, such as the Mediterranean diet, are associated with protection against cardiovascular disease and mortality (Sánchez-Sánchez et al., 2020). In addition, observational studies and randomised clinical trials have associated the long-term consumption of polyphenols or polyphenol-rich diets with reduced risk of developing diseases including cancer, cardiovascular disease, type 2 diabetes and neurodegenerative disorders (Bonaccio et al., 2017; Fraga, Galleano, Verstraeten, & Oteiza, 2010; Fujiki, Sueoka, Watanabe, & Suganuma, 2015; Xiao & Hogger, 2015).

Much of this evidence has considered the consumption of the flavonoid class of polyphenols; which have been consistently associated with reduced mortality due to cardiovascular disease (CVD) (Bauer, Ding, & Smit, 2011; Sebastian, Wilkinson Enns, Goldman, & Moshfegh, 2017; Wang, Ouyang, Liu, & Zhao, 2014). It has also been shown that individuals who consume low quantities of flavonoids display a higher number of non-fatal cardiovascular events in comparison to those who consume high amounts of flavonoids (Ponzo et al., 2015). Several large longitudinal studies have further corroborated that individuals with a higher total flavonoid intake were significantly less likely to have died from CVD during the follow-up period of 7 years, as has been observed in a population of US older adults (McCullough et al., 2012). Similar effects have been observed in other populations. Most recently, an observational Danish study which followed participants for 23 years, found that a moderate habitual intake of flavonoids was inversely associated with all-cause cardiovascular- and cancer-related mortality. However, they suggest that the strong association plateaus at intakes of approximately 500 mg/day (Bondonno et al., 2019). These findings imply that regular

consumption of polyphenol-rich (specifically flavonoids) foods and beverages can lead to cardiovascular benefits, however this effect may be restricted to ~500 mg/day, where intake above this produces no greater beneficial effect.

Several recent meta-analyses and systematic reviews of the literature have supported this further. This includes work by Kim and Je (2017) who conducted a meta-analysis of 15 studies examining the association between flavonoid intake and mortality from CVD and all-causes. They found that all subclasses of flavonoids (with the exception of flavonols and isoflavones) showed significant inverse associations with mortality. Those with the highest flavonoid intake were associated with lower total-, CVD- and all cause- mortality when compared with those of the lowest flavonoid intake group. Importantly however, within the dose-response analysis they observed a non-linear association between flavonoid intake and CVD mortality; with the significant association only observed up to a daily flavonoid intake of 167.5 mg. This again indicates that the beneficial cardiovascular effects observed may have an 'upper limit' in terms of dose.

Most recently, a systematic review of 91 papers found an overall inverse association between total polyphenol intake and cardiovascular risk events and mortality (Del Bo et al., 2019). Again, they observed a dose-dependent effect depending on the level of polyphenol intake. Here they reported a lower risk of CV events for an intake of total flavonoids ranging from 115- to 944 mg/day and a low risk of mortality or cardiovascular events and all-cause mortality for the highest quantile of total flavonoid intake (ranging 360-800 mg/day). Similarly, Grosso et al. (2017) systematic review indicated that, when compared with lower consumption, high consumption of total flavonoids was associated with decreased risk of all-cause mortality. They evidenced that increased flavonoid intake by 100 mg/day led to a linear decreased risk of 6% and 4% of all-cause and CVD mortality respectively.

In attempting to find a consensus on the mechanisms underpinning these CVD effects, it becomes clear that this does not exist and this lack of agreement is likely indicative of a wider issue which blights the nutrition literature; including polyphenols. This is the problem of huge inter-trial variability; which makes comparisons across an area of literature very challenging. To use this CVD mechanisms area as an example, a recent meta-analysis reported that different polyphenol-based interventions were found to significantly reduce diastolic blood pressure and triglyceride levels, however no conclusive effects were observed on pro-inflammatory cytokines and oxidative stress markers (Marx et al., 2017). The lack of consistency between the study design in the 10 trials included might explain these null findings. Firstly, the studies included used various polyphenolic interventions (soy proteins,

grapes, turmeric, cocoa, and pomegranate) of differing doses. The sample sizes varied greatly (ranging between n = 22-101), as did the demographics of participants (in terms of age, gender, and existing health conditions). Lastly, they were conducted in different countries (including Iran, USA, and Taiwan). These factors, and more, may explain the variability in the findings and researchers like Espín et al. (2017) have drawn attention to the impact of substantial heterogeneity between study design amongst trials included in supplementary reviews and meta-analyses. As such, it is crucial to recognise the need for further investigation in the form of well-controlled and specifically targeted dietary intervention studies, not just for the CVD trials utilized in this example, but also the wider polyphenol literature where this variability also exists.

Despite these shortcomings in the literature, the general consensus is that consumption of polyphenols are associated with beneficial human health. This seems particularly apparent when multiple polyphenols are presented concurrently in the form of a phenolic-rich diet (such as the Mediterranean diet) (Sánchez-Sánchez et al., 2020).

1.3.2. Polyphenols and interaction with cellular signalling pathways

Several mechanisms have been proposed to explain these beneficial effects of polyphenols (Fraga et al., 2010). It was initially considered that the primary mechanism of action was due to the antioxidant potential of polyphenols. However, with further understanding it has been suggested that this cannot solely explain the beneficial effects. Indeed, evidence indicates that polyphenol metabolites do not scavenge substantial amounts of free radicals *in vivo* to reach concentrations in most tissues that are high enough to have a significant effect (Fraga, 2007; Fraga et al., 2010; Ruskovska, Maksimova, & Milenkovic, 2020). As such, a number of other molecular mechanisms have been identified, including interaction with cellular signalling pathways and modulation of inflammatory pathways.

Evidence has indicated that the biological activity of polyphenols is likely due to their binding to specific proteins and subsequent interaction with signalling pathways and cellular transduction (Kennedy, 2014a; Ruskovska et al., 2020). Polyphenols interact with these cellular responses to stressors by interacting with extracellular signalling molecules (such as cytokines, hormones and growth factors), causing the conformation of the receptor protein to be changed. This triggers a signalling cascade within the cell (Kennedy, 2014a). Of these, one of the most prominent is the mitogen-activated protein kinase (MAPK) pathway, where a series of protein kinases activate each other in turn, transmitting the signal within the cell. This, alongside interacting with signals from other signalling cascades, activates transcription

factors within the cell nucleus. There, they have the capability to change the cellular function, by removing inhibitory proteins from the transcription factors, which regulates the activity of the genes and subsequent synthesis of proteins.

Specifically, MAPK plays a key role in the modulation of transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF- kB) and cAMP response element-binding protein (CREB), which are involved in numerous cellular responses including the synthesis of growth factors, cell proliferation and inflammatory molecules. It has been shown that *in vitro,* flavanols can directly interact with NF-kB proteins, preventing their binding to DNA kB sites and ultimately inhibiting NF-kB activation (Fraga et al., 2010).

1.3.3. Polyphenols and modulation of inflammation

Inflammation is an adaptive physiological process of the immune system; a natural response to pathogens and injury which protects the organism against infection (Sarkar et al., 2018). One of the main biological changes associated with inflammation is the activity of cytokines; proteins that modulate inflammation (Turner, Nedjai, Hurst, & Pennington, 2014). Pro-inflammatory cytokines like interleukin-1, interleukin-12, interleukin 1 beta, tumour necrosis factor-α (TNF-α) and interleukin-γ promote the inflammatory process. Whereas anti-inflammatory cytokines reduce inflammation (these include interleukin-4, interleukin-10 and interleukin-13), some cytokines, such as interleukin-6, may have anti- or pro-inflammatory properties depending on the context in which they are secreted (Scheller, Chalaris, Schmidt-Arras, & Rose-John, 2011). It has been shown that overproduction of reactive oxygen species (ROS), triggers pyrin domain-containing 3 (NLRP3) inflammasome, that subsequently activates toll-like receptor (TLR) mediated inflammatory signalling and consequently NF-kB and MAPK signalling pathways which trigger pro-inflammatory signalling (Zhang & Tsao, 2016). Evidence has indicated that polyphenols have the ability to modulate the NLRP3 inflammasome and therefore exert protective effects against inflammation (detailed in Zhang and Tsao (2016)). This ability is likely due to polyphenol-induced antioxidant action towards ROS; where the ability of ROS to interact with NF-κβ and JNK signalling results in an anti-inflammatory response (Blaser, Dostert, Mak, & Brenner, 2016)

In addition, flavonoids have been shown to inhibit inflammasome mediated secretion of IL-1β in lipopolysaccharide (LPS)-induced human macrophages (Martinez-Micaelo, González-Abuín, Pinent, Ardévol, & Blay, 2015; X. Zhang, Wang, Gurley, & Zhou, 2014). Polyphenol compounds can also attenuate the pro-inflammatory cytokine-induced activation of NF-kB via various molecular mechanisms. Specifically, it has been identified that there is a cross-talk

between aryl hydrocarbon receptor (AhR) and transcription factors RelB/A, which control the activation of NF-kB (Vogel et al., 2014). It is known that flavonoids are involved in modulating AhR mediated signalling pathways (Köhle & Bock, 2006). Flavonoids in particular, have also been shown to regulate activation of peroxisome proliferator-activated receptor gamma (PPARγ), which exerts effects on inflammatory transcription factors, leading to suppression of inflammation (Wang et al., 2014; Zhang & Tsao, 2016). Specifically, quercetin supplementation has resulted in modulation of inflammation, through activation of PPARγ (Chuang et al., 2010).

It is further suggested that activation of PPARγ can affect sirtuin (SIRT)-1-regulated signalling transductions including the transcriptional factor NF-kB (Anastasiou & Krek, 2006; Davis, Murphy, Carmichael, & Davis, 2009). Evidence strongly supports that phenolics can act as regulatory molecules and have the ability to attenuate NF-kB mediated inflammatory signalling transduction (Bisht, Wagner, & Bulmer, 2010). Specifically, protocatechuic acid (a metabolite of anthocyanins) has the ability to reduce LPS-stimulated activation of NF-kB and MAPKs signalling pathways (Wang et al., 2015). Additionally, *in vitro* studies have indicated that dietary polyphenols including luteolin, cyanidin-3-glucodise and resveratrol, have the ability to inhibit cytokine-induced activation of other pro-inflammatory signalling pathways, namely the Janus kinase/signal transducer and activator of transcription (JAK/STAT) (Nunes, Almeida, Barbosa, & Laranjinha, 2017; Serra, Paixão, Nunes, Dinis, & Almeida, 2013; Serra, Rufino, Mendes, Almeida, & Dinis, 2014).

1.3.4. Resveratrol and modulation of inflammation

Resveratrol has long been illustrated to have anti-inflammatory effects and has the ability to inhibit pro-inflammatory signalling cascades, particularly the NF-kB, JAK/STAT and activator protein-1 (AP-1) pathways. Results include a decrease in the expression of pro-inflammatory and pro-oxidant markers, such as IL-6, IL-1α, IL-1β, IL-12 and TNF-α (Ma, Wang, Dong, Li, & Cai, 2015; Renaud & Martinoli, 2014; Spencer, Vafeiadou, Williams, & Vauzour, 2012). Studies have reported that resveratrol regulates inflammatory responses through a variety of signalling pathways such as the Arachidonic Acid (AA) pathway, NF-kB, MAPK and AP-1 (Adhami, Afaq, & Ahmad, 2003; X. Li et al., 2018; Manna, Mukhopadhyay, & Aggarwal, 2000; Pirola & Fröjdö, 2008); these are detailed further in a recent review paper (Meng et al., 2021).

Briefly, in terms of the AA pathway, resveratrol has been shown to inhibit the functions of cyclooxygenase-1 and -2 (COX-1 and COX-2), which metabolise AA, inhibiting the synthesis of prostaglandins (such as PGD2, PG12 and PGE2) (Calamini et al., 2010; Jang et al., 1997).

Specifically resveratrol induces a decrease in prostaglandins and COX-2 expression by reducing AA release (Martinez & Moreno, 2000). Indeed, in rats, resveratrol was shown to decrease the production of PGD2 and PGE2, showed lower COX-2 expression and a reduced degree of colonic injury in rats with inflamed colons (Martín, Villegas, La Casa, & de la Lastra, 2004; Zykova et al., 2008).

Much research focusses on the anti-inflammatory effect of resveratrol through its ability to inhibit the NF-kB signalling pathway, specifically via suppressing the activities of NF-kB and IκB kinase, and the phosphorylation of JAK/STAT pathways (Dvorakova & Landa, 2017; Holmes-McNary & Baldwin, 2000; Li et al., 2018; Ma et al., 2015). The activation of NF-kB can lead to the expression of inflammatory cytokines in LPS-stimulation cells (Wang et al., 2014). Resveratrol has also been shown to suppress TNF-induced NF-kB activation in a dose- and time-dependent manner in myeloid cells, lymphoid, and epithelial cells (Estrov et al., 2003; Manna et al., 2000). Another study showed that resveratrol treatment initiated substantial changes in protein acetylation and methylation patterns, which indicates deacetylase induction and demethylase reduction activities that primarily affect regulatory cascades NF-kB and JAK/STAT mediated pathways (Pinheiro et al., 2019). Additionally, the suppression of NF-kB correlated with inhibition of AP-1 (Manna et al., 2000) and resveratrol has been shown to have the ability to block TNF-induced activation of AP-1-mediated gene expression (Manna et al., 2000; Subbaramaiah et al., 1998). As such the anti-inflammatory effects of resveratrol can partly be explained due to blocking both NF-kB and AP-1 activation as well as related kinases (Manna et al., 2000).

In addition, resveratrol can activate SIRT-1, which operates by blocking the TLR-4/NF-kB/STAT pathway and consequently exerts anti-inflammatory effects (Saiko, Szakmary, Jaeger, & Szekeres, 2008; Wiciński et al., 2018). Specifically, SIRT-1 regulates NLRP3 expression; partially via NF-kB signalling (Fu et al., 2013; Misawa et al., 2015; Yang & Lim, 2014). Resveratrol-induced SIRT-1 activation also inhibits RelA acetylation which, in turn, lowers expression of genes including TNF-α, IL-1β, IL-6 induced by NF-kB (Gao, Kong, Kemp, Zhao, & Fang, 2012). Resveratrol has further been shown to interrupt an inflammatory amplification loop; inhibition of NF-kB pathways leads to a decreased secretion of IL-6, which results in suppressed signal transducer and activator of transcription 3 (STAT3) activation in macrophages. As STAT3 is responsible for the positive regulation of IL-6 secretion, the inhibition of STAT3 causes IL-6 levels to lower further (Limagne, Lançon, Delmas, Cherkaoui-Malki, & Latruffe, 2016). The suppression of IL-6 transcription and translation, results in attenuation of its secretion by macrophages (Ohtsu et al., 2017).

Many studies in disease models have observed the beneficial anti-inflammatory effects of resveratrol administration. In patients with stable angina pectoris, 60 day administration of a combination of resveratrol (20 mg/day) and calcium fructoborate (112 mg/day), was observed to significantly reduce high sensitivity C-reactive protein (hsCRP) (Militaru et al., 2013). In addition, resveratrol treatment (2 mg/kg/day for 4 weeks) has been shown to reduce serum levels of IL-1β, IL-17A and TNF-α and increase anti-inflammatory factors, in patients following oral implantology (BaGen, Liu, & Han, 2018). In patients with an inflammatory condition of the aorta and its branches (Takayasu arteritis), resveratrol supplementation (250 mg daily for 3 months) showed reductions in CRP and TNF-α, alongside a reduction in the Birmingham vascular activity score for vascular inflammation (Shi, Hua, Xu, & Ren, 2017). Comparable reductions in plasma inflammatory markers have been observed in patients with ulcerative colitis following 6 week supplementation of 500 mg daily (Samsami-Kor, Daryani, Asl, & Hekmatdoost, 2015), as well as in other clinical diseases such as type 2 diabetes and cardiovascular diseases (Tomé-Carneiro, Gonzálvez, et al., 2013; Tomé-Carneiro, Larrosa, et al., 2013; Tomé-Carneiro et al., 2012).

Despite these findings, some studies have not observed anti-inflammatory effects following resveratrol supplementation. For example, supplementation of 75 mg/day for 12 weeks in postmenopausal women, showed no change in inflammatory markers or other endpoints of metabolic function such as plasma lipids and insulin sensitivity (Yoshino et al., 2012). Another study supplementing metabolic syndrome patients with both high (1000 mg) and low (150 mg) doses of resveratrol were unable to change the inflammatory gene expression or CRP levels (Kjær et al., 2017). Likewise, a pilot study administered a single 5 g dose to healthy participants and reported a significant increase in *pro*-inflammatory plasma TNF-α and NF-kB activation (Gualdoni et al., 2014), suggesting a pro-inflammatory role of resveratrol at a high dose. These contrasting results may be due to the polarising low and high doses utilised in these studies. As previously, when anti-inflammatory effects have been observed, studies have used moderate doses (such as 250- or 500 mg), or a low dose presented as a combined supplement, over a sustained period. Therefore, it could be argued that those dosages may be more effective in reducing inflammation than a single higher dose or a low dose of resveratrol alone. Indeed, a recent meta-analysis of 33 studies indicated a significant reduction in 1L-6 reduction was observed only in patients receiving ≥500 mg/day (Omraninava et al., 2021). Similarly, Gorabi et al. (2021) indicated in a meta-analysis of 35 RCTs that resveratrol supplementation was capable of reducing levels of hs-CRP and CRP, with subgroup analysis indicating particular efficiency following ≥10 weeks supplementation (for hs-CRP and CRP) and with ≥500 mg/day (CRP levels only). Moreover, it is likely that anti-inflammatory effects may be more apparent within certain models or demographics; specifically in extreme disease

models (such as oral implantology) categorised by high inflammation. Here resveratrol may have a more effective anti-inflammatory response than in healthy individuals with a less pronounced inflammatory response.

1.3.5. Resveratrol and modulation of oxidative stress

In addition to modulating inflammation, resveratrol may exert beneficial health effects due to oxidative stress reduction. Oxidative stress is defined as an imbalance between the production of ROS and the ability to detoxify the reactive intermediates (Francisqueti et al., 2017; Ndisang, Vannacci, & Rastogi, 2014). Excessive ROS accumulation may induce the oxidative modification of cellular macromolecules such as proteins, lipids and nucleic acids (Seyyedebrahimi, Khodabandehloo, Esfahani, & Meshkani, 2018). Oxidative stress damages macromolecules and impairs their functions, which underlies many age-related diseases including chronic inflammation, cancer, diabetes, cardiovascular and neurodegenerative diseases (Liguori et al., 2018; Reuter, Gupta, Chaturvedi, & Aggarwal, 2010). Under oxidative stress conditions, the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), regulates the expression of antioxidant genes including superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase (Javkhedkar et al., 2015; Jiménez-Osorio et al., 2014). There are several potential biomarkers of oxidative stress, including malondialdehyde (MDA), ROS, total antioxidant capacity (TAC) and protein carbonyl and sulfhydryl contents (Tiwari, Pandey, Abidi, & Rizvi, 2013).

Resveratrol is thought to have the ability to reduce oxidative stress via numerous mechanisms including an increase in TAC and reduction in ROS (Babu et al., 2015; Gülçin, 2010). It also has the ability of free radical scavenging, where it inhibits oxygen free radical formation by preventing nicotinamide adenine dinucleotide phosphate oxidases and subsequent ROS production (Gerszon, Rodacka, & Puchała, 2014; Halliwell, 2007; Yousefian et al., 2019). Additionally, it may exert anti-inflammatory effects by activating protein kinases signalling pathways such as AMPK, MAPK, Nrf2 and SIRT (detailed further in Meng et al. (2021)), which act as an anti-oxidant and anti-inflammatory pathway (Santos et al., 2021). Resveratrol further upregulated the phosphatase and tensin homolog (PTEN), which decreased Akt phosphorylation, leading to the upregulation of antioxidant enzyme mRNA levels such as catalase (CAT) and SOD (Inglés et al., 2014). It can also improve the antioxidant defence system by modulating antioxidant enzymes through downregulation of extracellular signal-regulated kinase (ERK) activated by ROS (Singh & Vinayak, 2017). Resveratrol has the ability to reduce the ischemia-reperfusion injury-induced oxidative stress by inhibiting the activation of MAPK pathway, thus the levels of antioxidants like glutathione (GSH) increased, and free

radicals were directly scavenged (Fu et al., 2018). Generally, however, it is thought that resveratrol protects against oxidative stress by reducing ROS and reactive nitrogen species (RNS) generation, directly scavenging free radicals, improving antioxidant enzymes and by promoting antioxidant molecules through various signalling pathways (Meng et al., 2021).

In studies with cell cultures, resveratrol has been shown to prevent an increase in ROS production, alongside a decrease of mitochondrial membrane potential, which indicates a role in maintaining cellular redox homeostasis (Bobermin, Souza, Gonçalves, & Quincozes-Santos, 2018). These experiments on cell cultures are conducted by exposing cells to a high glucose concentration or to pro-inflammatory cytokines and results show a reduction in ROS levels in many cell types, including vascular endothelial cells (Chen et al., 2013; Ungvari et al., 2009), adipocytes (Yen, Chen, Chang, & Hsu, 2011), monocytes (Yun, Chien, Jialal, & Devaraj, 2012) and cardiomyocytes (Guo et al., 2015). In rats with high-fat diet-induced oxidative stress, resveratrol (400 mg/kg) supplementation was shown to have no effect on ROS levels (Yang et al., 2019). However, the authors observed significant reductions in MDA (which is indicative of biological membrane damage induced by ROS) in the liver and colon, but not in plasma. Additionally, in diabetic rats, resveratrol intake has been shown to normalise antioxidant status (Hussein & Mahfouz, 2016). Within animal models, resveratrol supplementation reduced production of ROS; elevated membrane potential and inhibition of cytochrome c release from the inner mitochondrial membrane (Zhang et al., 2019). Taken together, the research suggests that resveratrol supplementation can reduce oxidative stress in animal models.

In humans, the intake of resveratrol has been shown to reduce oxidative stress in healthy individuals and those with metabolic diseases (which are typically characterised by high oxidative stress). In healthy women, administration of a resveratrol-containing grape powder for 4 weeks, showed that whole-body oxidative stress (as measured by urinary F_2-isoprostanes) was significantly reduced (Zern et al., 2005). Similarly, resveratrol supplementation (150 mg daily for 4 weeks) was able to significantly increase TAC in healthy adults (Apostolidou, Adamopoulos, Iliadis, & Kourtidou-Papadeli, 2016). In another crossover study, healthy individuals were given a high-fat, high-carbohydrate meal, which is known to induce oxidative stress, as reflected by increased ROS levels. Participants received a nutraceutical supplement, containing 100 mg resveratrol and an additional 75 mg polyphenols from a grape extract. Results showed that markers of oxidative stress and several inflammatory biomarkers were suppressed when the meal was consumed with the supplement, when compared with consuming the same meal and a placebo (Ghanim et al., 2011). Most recently, resveratrol supplementation was observed to be able to reduce ROS in

healthy individuals, particularly in middle aged participants (aged 40-59 years old) when compared with older-elderly participants (aged 60-80 years old), although the decrease was significant for both groups (Santos et al., 2021).

Research in patients with metabolic disorders also shows benefit here. In type-2 diabetic patients, resveratrol supplementation (800 mg/day for 8 weeks) significantly increased TAC, reduced intracellular superoxide anion production and increased Nrf2 and SOD expressions (Seyyedebrahimi et al., 2018). Similarly, resveratrol-reduced markers of oxidative stress, alongside other related health improvements including blood pressure, insulin sensitivity and cardiovascular function, have been observed in type-2 diabetes patients (Brasnyó et al., 2011; Imamura et al., 2017). As such, the ability of resveratrol to reduce oxidative stress may be valuable in the prevention or treatment of metabolic diseases, including diabetes, obesity and cardiovascular diseases.

1.3.6. Cardioprotective effects of resveratrol

As previously mentioned, the discovery of the 'French paradox' is likely the impetus for research into the potential cardioprotective effects of resveratrol. The moderate intake of red wine in the French population (and other similarly located populations) was found to correlate with a decreased incidence of heart disease and obesity, despite their relatively high saturated fat diet (Kopp, 1998; Sun, Simonyi, & Sun, 2002). Whilst initially this paradox was attributed solely to the presence of resveratrol in red wine, at present it is thought to arise from the combination of resveratrol with other food components within the French diet, many of which have a high polyphenolic content and are typical of the Mediterranean diet (Singh et al., 2019). As resveratrol has the potential to modulate inflammation and oxidative stress, which both likely underpin several cardiovascular health risk factors (such as hypertension and hypertriglyceridemia) and the development of cardiovascular diseases, much research has considered resveratrol's cardioprotective role.

Cardiovascular diseases (CVD) are the leading cause of death worldwide, with CVD deaths accounting for 32% of all global deaths in 2019 (WHO, 2021). Cardiovascular disorders include any pathological condition of the blood vessels or heart, leading to the obstruction of continuous blood supply and nutrients to cardiac tissue and, therefore, to the entire body (Zhang, Syed, Liu, & Yu, 2017). Resveratrol has been demonstrated to have a therapeutic potential here and likely by protecting the cardiovascular system in a multidimensional way (Wu & Hsieh, 2011). For example, resveratrol has been demonstrated to exert improvements

in many CVD risk factors, including fasting blood glucose, triglycerides and heart rate (Zamora-Ros et al., 2012).

Inflammation has been established as a central driver of many cardiovascular disorders (Welsh, Grassia, Botha, Sattar, & Maffia, 2017). Significant evidence suggests that resveratrol's anti-inflammatory activity might underlie its protective mechanism against cardiovascular diseases (de Sá Coutinho, Pacheco, Frozza, & Bernardi, 2018). Indeed, several *in vitro* studies have revealed the anti-inflammatory effects of resveratrol in cardiac tissue, specifically evidenced by the inhibition of intercellular adhesion molecule 1 (ICAM-1), inducible nitric oxide synthase (iNOS) and IL-1β messenger RNA (mRNA) (Huang et al., 2017).

In addition, resveratrol may offer cardiovascular protective effects by improving endothelial dysfunction in patients with related metabolic disorders. Flow mediated dilation (FMD) can be utilised on the branchial artery as a marker of endothelial function and cardiovascular health (Grassi et al., 2010). Evidence has shown that resveratrol supplementation (100 mg for 3 months), improved endothelial function (as measured by FMD) in patients with metabolic syndrome and other associated cardiovascular risk factors (Fujitaka et al., 2011). This improvement was observed to return to baseline function following discontinuation of treatment, when participants were examined 3 months later, reinforcing the role of resveratrol here and suggesting that sustained treatment may be necessary. Similar beneficial endothelial function effects have been observed in obese subjects (Wong et al., 2013), patients with hypertension (Marques et al., 2018) and mild hypertension (Wong et al., 2011).

Many studies have shown the anti-hypertensive effects of resveratrol in preclinical models, which may be due to multiple mechanisms; including through the inhibition of vascular inflammation; the stimulation of endothelial nitric oxide (eNOS) production; SIRT1 activation; AMPK phosphorylation; decreased ROS production and through the prevention of platelet aggregation (Borghi & Cicero, 2017; Chaplin et al., 2018; Cicero, Fogacci, & Colletti, 2017; Li, Xia, & Förstermann, 2012; Yu et al., 2017). Each of these may promote blood pressure reductions and also improve blood pressure control in patients with hypertension; a chronic medical condition defined by sustained arterial blood pressure elevation (Cicero et al., 2017). Indeed, in animal models of hypertension, resveratrol has consistently been observed to reduce blood pressure (Cheng et al., 2014; Gordish & Beierwaltes, 2016; Mozafari, Nekooeian, Mashghoolozekr, & Panjeshahin, 2015).

Despite promising results from animal work, the ability of resveratrol to reduce blood pressure in humans seems less convincing. Reductions in systolic and diastolic blood pressure have been observed mostly in individuals with metabolic diseases including obesity, type-2 diabetes and non-alcoholic fatty liver disease (Bhatt, Thomas, & Nanjan, 2012; Heebøll et al., 2016; Imamura et al., 2017; Timmers et al., 2011). However, additional studies using similar populations have not observed these beneficial effects (Faghihzadeh, Adibi, & Hekmatdoost, 2015; Kjær et al., 2017; Poulsen et al., 2013; Wong et al., 2013; Zamora-Ros et al., 2012). In fact, one study in Iranian adults reported that participants in the highest quartile of stilbene intakes (>0.054 mg/day) was positively associated with high blood pressure (Sohrab et al., 2013). However, it should be noted that this study utilised FFQs to measure dietary polyphenol intake and they only consider total stilbene content (rather than resveratrol content); therefore, it is difficult to compare these findings with intervention trials which have observed beneficial or null findings.

Several meta-analyses have also cast doubt on the effects of resveratrol supplementation on systolic and diastolic blood pressure (Liu, Ma, Zhang, He, & Huang, 2015; Sahebkar et al., 2015). However, most recently, a meta-analysis including 17 studies with a total of 681 participants, observed favourable, but non-significant blood pressure lowering effects of resveratrol on systolic blood pressure, mean arterial pressure and pulse pressure and no effects were observed on diastolic blood pressure (Fogacci et al., 2019). Subgroup and meta-regression analyses within these studies, however, indicate that resveratrol supplementation reduces systolic and diastolic blood pressure at doses higher than 150-300 mg/day (Fogacci et al., 2019; Huang et al., 2016; Liu et al., 2015); which may explain previous null effects in studies utilising doses lower than this. Despite being non-significant, the authors report that the reduction of systolic blood pressure may still have important clinical implications; namely due to the National Institute for Health and Care Excellence (NICE) recommendation for systolic blood pressure to be below 140 mmHg in order to reduce hypertension-related morbidity and mortality (Antza, Doundoulakis, Stabouli, & Kotsis, 2018). In addition, this meta-analysis also showed a more pronounced blood pressure lowering effect in patients with high cardiovascular risk (including obese and diabetic patients) (Fogacci et al., 2019; Huang et al., 2016); which may indicate the importance of resveratrol's therapeutic effect in Westernised society, where these conditions are frequent. This again speaks to the more pronounced effect of resveratrol in models of damage, as opposed to a non-compromised state, and suggests that resveratrol might be of most benefit when under challenge.

Besides hypertension, another main contributor to CVD is atherosclerosis; a disorder associated with arterial inflammation, lipid accumulation in the vessel wall, plaque formation,

thrombosis and late mortal complications, such as myocardial infarction and ischemic stroke (Chistiakov, Grechko, Myasoedova, Melnichenko, & Orekhov, 2018). Resveratrol has been shown to block atherosclerotic plaque progression by acting against pro-atherogenic oxysterol signalling in M1 (inflammation-encouraging) and M2 (inflammation-decreasing) macrophages (Buttari et al., 2014). It also may ameliorate atherosclerosis partially through restoring intracellular glutathione (GSH) via AMPK-α activation, which results in inhibited monocyte differentiation and reduced pro-inflammatory cytokine production (Vasamsetti et al., 2016). Evidence in rhesus monkeys fed a high-fat and sucrose diet supports this further; here resveratrol supplementation prevented diet-induced arterial wall inflammation, and the accompanying increase in aortic pulse wave velocity, both of which are major risk factors in the development of CVD (Mattison et al., 2014).

In addition, the cytokines IL-8, vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM), together with passive lipid accumulation in the artery walls, are known to play an important role in the initiation of atherosclerosis (Luc et al., 2003). In a study where participants were supplemented with a resveratrol formulation (containing quercetin, grape skin extract and *trans*-resveratrol), a significant reduction in the expression of IL-8, VCAM and ICAM was found compared with baseline (Agarwal et al., 2013). The same study further observed an inverse relationship between the concentration of plasma resveratrol and the expression of those biomarkers, which supports a cardioprotective effect of the formulation of the supplement. Additionally, in cultured cells stimulated with LPS, resveratrol treatment suppressed the formation of foam cells (which are considered to trigger atherosclerosis). Alongside this, the expressions of SIRT1 and AMPK, which inhibit inflammation, were upregulated (Dong et al., 2014). As inflammatory responses play a crucial role in the development and progression of atherosclerosis, the anti-inflammatory activity of resveratrol could suggest a potential therapeutic intervention for the disease.

Similarly, resveratrol has been shown to have positive effects in patients with other CVDs. For example, supplementation with a combined resveratrol and calcium fructoborate intervention for 60 days significantly reduced weekly frequency of angina attacks and improved the quality of life of patients with stable angina pectoris (Militaru et al., 2013). Another randomised controlled trial investigated the cardioprotective effects of resveratrol in patients who had previously suffered from a heart attack. They found that 10 mg resveratrol supplementation, for 3 months, resulted in improved endothelial function, left ventricular diastolic function and lowered low-density lipoprotein (LDL) cholesterol (Magyar et al., 2012). In patients who were at high-risk of CVD and currently receiving statin treatment, supplementation with a resveratrol-enriched grape supplement for 1 year resulted in a significant decrease in CRP

and TNF-α, and an increase in the anti-inflammatory cytokine 1L-10 (Tomé-Carneiro et al., 2012). These results suggest that resveratrol may be used alongside statins in the prevention of cardiovascular disease. Similarly, a study in smokers, who are more likely to be at risk of developing CVD, supplementation with 500 mg resveratrol for 30 days, showed significant reductions in CRP, triacylglycerol concentrations and improvements in antioxidant status (Bo et al., 2013).

These trials suggest that resveratrol supplementation is likely to exert beneficial cardioprotective effects in healthy adults; those at high risk of cardiovascular disease and those currently suffering from CVD (when presented alongside regular medication). These effects are potentially mediated through the inhibition of inflammatory markers, reduction of blood pressure, improvement of atherogenic profiles and maintenance of endothelial function.

1.3.7. The potential for resveratrol to modulate health in obese populations and those with metabolic conditions

The wide biological properties of resveratrol; including antioxidant, anti-inflammatory, and cardiovascular protective effects, indicate that supplementation may provide beneficial health effects in humans. The above research findings provide sufficient support for effects in numerous disease models but specifically those that are likely triggered by chronic inflammation, including obesity, metabolic diseases and metabolic syndrome. As such, resveratrol may exert beneficial effects due to these biological properties or through additional mechanisms such as modulating cholesterol, glucose and insulin sensitivity and fat accumulation. The following sections will detail the current research into the health effects of resveratrol in obese populations and those with metabolic disorders. Some effects have been summarised within Figure 1.3.

Obesity is now categorised as a global public health epidemic, with latest reports by the World Health Organisation (WHO) indicating that, in 2016, more than 1.9 billion adults were overweight, with 650 million of these classified as obese worldwide (WHO, 2020). These rates are rising drastically worldwide and are likely to be triggered by the change in lifestyle habits in the past century; specifically living a more sedentary lifestyle and detrimental changes in dietary choices (Kopp, 2019). Whilst the development of obesity is multifactorial, with both genetic and environmental contributions interacting, chronic inflammation is considered a pathophysiological trigger in obesity and closely related metabolic disorders including type 2 diabetes, impaired glucose tolerance, insulin resistance and impaired fasting glucose (Esser, Legrand-Poels, Piette, Scheen, & Paquot, 2014; Monteiro & Azevedo, 2010; Shoelson, Lee, & Goldfine, 2006). Metabolic syndrome refers to a cluster of these cardiovascular risk factors

(Abete, Goyenechea, Zulet, & Martinez, 2011), and patients presenting with several of these conditions have a greater chance for future cardiovascular events than those with any one factor alone (Huang, 2009).

Whilst the current main options for obesity management are energy restriction and increasing physical activity, compliance with these are typically poor and therefore not particularly effective (Fernández-Quintela, Milton-Laskibar, González, & Portillo, 2017). As such, interest has been placed on naturally occurring active biomolecules which may be utilised in body weight management and the prevention or treatment of related conditions. Due to the potential anti-inflammatory role of resveratrol in these diseases, it may be a promising treatment in comorbidity or multimorbidity conditions. Indeed, a recent clinical trial in patients with metabolic syndrome observed a significant decrease in plasma ferritin levels and in CRP levels (which are both likely good biomarkers of inflammation), following 3-month supplementation with a combined resveratrol, piperine and alpha-tocopherol intervention (Pastor et al., 2020). They further saw decreases in the oxygen consumption and spontaneous chemiluminescence of polymorphonuclear cells; which might be indicative of a drop in the proinflammatory metabolism of these cells of the immune system and of decreased levels of ROS. A recent systematic review and meta-analysis including 16 studies (10 in humans and 6 in animals) evaluated the effect of resveratrol supplementation on metabolic syndrome components including body weight, waist circumference, systolic blood pressure, HDL, total cholesterol, triglyceride and glucose levels (Asgary, Karimi, Momtaz, Naseri, & Farzaei, 2019). They found that, in human studies, resveratrol supplementation had a significant impact on glucose level and waist circumference. In addition, the subgroup analysis indicated that these effects were significant at the >500 mg dose and, with long-term interventions, >10 weeks. Despite this, a review paper concludes that whilst there is evidence to suggest that resveratrol supplementation might improve health and potentially treat chronic diseases such as metabolic syndrome, the evidence to date in humans is less convincing than that in animals (Hou, Tain, Yu, & Huang, 2019). As discrepancies in study designs likely explain some of the variability between findings in humans (Singh et al., 2019); further studies are necessary to understand the physiological and health related responses to resveratrol supplementation in individuals with multiple co-occurring metabolic disorders. Additional work has considered how resveratrol supplementation may be beneficial in these diseases, as the following sections will detail.

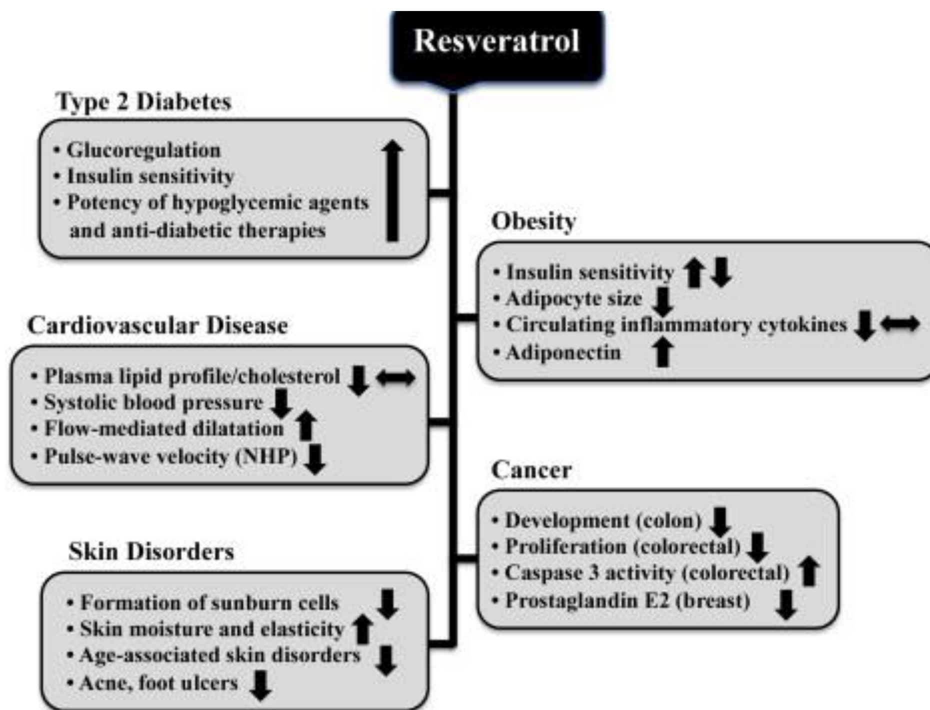

Figure 1.3 Summary of health effects of resveratrol in human clinical trials. When conducted in patients with those health conditions. The symbol ↔ denotes lack of effect, and ↑↓ opposite action in some trial. Diagram obtained from Novelle, Wahl, Dieguez, Bernier, and de Cabo (2015). Reproduced with permission from Elsevier.

1.3.7.1. Fat accumulation

As obesity is characterised by an abnormal or excessive fat accumulation (Malik, Willett, & Hu, 2013), the ability of resveratrol to alter fat accumulation has received considerable interest. The potential metabolic effect of resveratrol was initially observed in mice who were administered resveratrol (200 or 400 mg/kg/day) in either a chow diet or high fat diet for 15 weeks (Lagouge et al., 2006). They found that resveratrol treated mice gained significantly less weight when they consumed a high-fat diet. They were also found to weigh the same amount as chow-fed mice, which was accounted for by a decrease in fat and significant improvement in energy expenditure. Additional studies in obese models in rodents (induced by a high-fat diet) have consistently observed reductions in body fat accumulation (Aguirre, Fernández-Quintela, Arias, & Portillo, 2014; Cho, Jung, & Choi, 2012; Kim, Jin, Choi, & Park, 2011; Macarulla et al., 2009; Um et al., 2010).

In addition to modification in energy expenditure, resveratrol has further been shown to have the ability to reduce diet-induced obesity, by mimicking calorie restriction, through SIRT1 activation. SIRT1 activation deacetylates and activates PPARɣ coactivator 1 α (Lagouge et al., 2006), which controls mitochondrial biogenesis and function. It also triggers lipolysis and fat loss by repressing PPARγ in adipocytes (Picard et al., 2004). Other anti-lipogenic

mechanisms of action have been described, including the upregulation of certain microRNAs by resveratrol, which leads to the inhibition of lipogenesis in white adipose tissue (Gracia et al., 2016). Resveratrol can also act by inhibiting cAMP-specific phosphodiesterases, which leads to elevated cyclic adenosine monophosphate levels, which in turn activates the AMPK pathway (Park et al., 2012). As individual studies have detailed multiple mechanisms of action here, a recent review study aimed to provide an overarching view of the anti-obesity effects of resveratrol (Fernández-Quintela et al., 2017). They concluded that, in rodents, resveratrol has the ability to modify energy balance by increasing thermogenesis in brown adipose tissue, increase adipogenesis in white adipose tissue, increase lipogenesis in white adipose tissue and decreased lipid oxidation in skeletal muscle.

Research in humans, however, is less consistent, with most research to date showing a lack of effect of resveratrol supplementation on body weight and adiposity (Arzola-Paniagua, García-Salgado López, Calvo-Vargas, & Guevara-Cruz, 2016; Poulsen et al., 2013; Timmers et al., 2011). Although one study evidenced modification of adipose tissue by resveratrol intake, with adipocyte size reduced following 30 days supplementation with resveratrol (150 mg per day) in obese men (Konings et al., 2014). Again, when considering individuals with comorbid metabolic health conditions, studies have observed contradicting results. In individuals with metabolic syndrome, supplementation with 150 mg and 1000 mg of resveratrol for 16 weeks had no effect on body composition (Kjær et al., 2017). Whilst another study administering 1500 mg for 90 days observed significant differences in total weight, body mass index, fat mass and waist circumference (Méndez-del Villar, González-Ortiz, Martínez-Abundis, Pérez-Rubio, & Lizárraga-Valdez, 2014).

Looking to a recent systematic review and meta-analysis, comprising 12 studies investigating the effects of resveratrol on obesity in humans, for some clarity here, we see that only three studies showed a significant effect of resveratrol on weight loss, BMI or body fat (Delpino, Figueiredo, Caputo, Mintem, & Gigante, 2020). Of these, one study observed these effects when combining resveratrol with orlistat (a drug designed to treat obesity) (Arzola-Paniagua et al., 2016). They conclude that resveratrol had no positive effects on weight loss or BMI, which is corroborated by a previous systematic review (Christenson et al., 2016). Despite this, another recent meta-analysis did identify positive anti-obesity results, demonstrating that resveratrol was able to decrease body weight, BMI, waist circumference and body fat (Tabrizi et al., 2020). The discrepancies between these study findings are potentially due to the latter meta-analysis including more studies (36 in total) as well as those with supplements combining resveratrol with other substances. Additionally, methodological design differences including the form of supplementation and the demographics of the participants used, including utilising

participants with metabolic diseases, is likely to impact upon findings (Chaplin et al., 2018). As such, supplementary research is warranted to determine if resveratrol can modulate fat accumulation in obese patients and those with comorbid conditions.

1.3.7.2. Cholesterol & hypertriglyceridemia

High levels of total cholesterol, low-density lipoprotein cholesterol (LDL) and triacylglycerols, and low concentrations of high-density lipoprotein cholesterol (HDL) are consistently associated with higher risk of developing cardiovascular disease (Arsenault et al., 2009; Lamarche et al., 1996) and increasing cardiovascular mortality (Pirro et al., 2011; Simental-Mendía & Guerrero-Romero, 2019). Additionally, continuously heightened levels of blood cholesterol leads to hardened and narrowed arteries that further contributes to high blood pressure (Sakurai et al., 2011).

Resveratrol has the potential to provide benefit here by reducing cholesterol levels and this may be explained by its phenolic hydroxyls; which result in oxidation of unsaturated fatty acids and decrease in circulating cholesterol (Xie, Han, Chen, & He, 2014). Additionally, this may be underpinned by increasing the synthesis and efflux of bile acids, decreasing the synthesis of hepatic cholesterol and increasing the efflux of cholesterol (Berrougui, Grenier, Loued, Drouin, & Khalil, 2009; Shao et al., 2016). Support for this comes from studies in animals which have reported this lipid-lowering effect of resveratrol (Ren et al., 2011; Xie et al., 2014) including reduced triglyceridemia in diet-induced obese rodents (Andrade et al., 2014; Cho et al., 2012; Kim et al., 2011; Pan et al., 2015). One potential explanation for this, is the resveratrol-induced inhibition of hepatocyte fatty acid and triacylglycerol synthesis described in rat hepatocytes (Gnoni & Paglialonga, 2009).

In human research, when combined with additional compounds (e.g. a plant extract or when combined with a nutraceutical formula) resveratrol has also been shown to reduce triglyceridemia. Specifically, when combined with EGCG (Most et al., 2016); as a nutritional supplement with four other polyphenols (Qureshi, Khan, Mahjabeen, Papasian, & Qureshi, 2012) and when presented as a grape extract containing additional polyphenols (Tomé-Carneiro et al., 2012; Zern et al., 2005). Studies utilising just resveratrol are inconsistent and scarce. However, significant reductions in total cholesterol concentration have been observed following resveratrol supplementation in patients with type-2 diabetes (Bhatt et al., 2012). Another study found a significant reduction in plasma triacylglycerols after resveratrol supplementation in obese individuals (Timmers et al., 2011). Recently, a study in adults with a new diagnosis of dyslipidaemia but who were otherwise healthy, observed a significant

decrease in total cholesterol and triacylglycerol, following 2-month resveratrol supplementation (100 mg/day) (Simental-Mendía & Guerrero-Romero, 2019).

Whilst these previous studies have illustrated beneficial cholesterol lowering effects following resveratrol supplementation, many have reported no change. Several studies in overweight older adults, observed no change in markers relating to cardiovascular health (including HDL cholesterol, LDL cholesterol and triacylglycerol) following 4-week consumption of 150 mg resveratrol (van der Made, Plat, & Mensink, 2015). Likewise, resveratrol has been shown to have no effect on triglyceridemia in patients with hypertriglyceridemia (Dash, Xiao, Morgantini, Szeto, & Lewis, 2013) or those with type-2 diabetes (Javid et al., 2017). A meta-analysis of seven randomised controlled trials found no significant effect of resveratrol on lipid profile, however it is worth noting that the dosages utilised in many of these studies were relatively low (4 of the included studies used doses <100 mg/day) and a higher dose may be necessary to exert beneficial effects (Sahebkar, 2013).

Since then, an updated meta-analysis has been conducted which included 15 studies utilizing larger dosages and longer intervention periods of just resveratrol alone (no combinations with additional compounds) (Haghighatdoost & Hariri, 2018). Their findings showed that resveratrol supplementation had no effect on serum LDL or HDL but observed a significant increase in serum triglyceride. However, the authors report that removing one study from the analysis removed the significance of that finding. This extracted study investigated the effects of 4-week resveratrol supplementation (200 mg/day) in men diagnosed with schizophrenia (Zortea et al., 2016). Patients within this study consumed a low-fat diet, which has previously been shown to increase triglyceride concentrations (Parks, Krauss, Christiansen, Neese, & Hellerstein, 1999). This likely explains the discrepancy in findings and potentially may have masked any beneficial effect of resveratrol on triglycerides. A further potential explanation for the contradictory results observed, is the potential for differential effects between male and female participants. Haghighatdoost and Hariri (2018) report that the studies utilised in their meta-analysis used participants of both sexes and that the different sex hormones between males and females may affect lipid profile.

1.3.7.3. Glucose and insulin sensitivity

An imbalance between insulin and glucagon can contribute to impaired glucose tolerance and the development of type-2 diabetes (Ahren & Larsson, 2001). Diabetes is one of the most prevalent metabolic diseases worldwide (Whiting, Guariguata, Weil, & Shaw, 2011) and

usually presents in comorbidity, with patients suffering from a variety of secondary clinical conditions (Struijs, Baan, Schellevis, Westert, & Van Den Bos, 2006).

It has been demonstrated that resveratrol has the ability to improve insulin sensitivity, by activating AMPK (Lagouge et al., 2006) and also the insulin-signalling components, insulin receptor substrate-1 and Akt (Kang, Heng, Yuan, Baolin, & Fang, 2010; Kang et al., 2012). Resveratrol further reduces the expression of adipokines that influence insulin sensitivity, including adiponectin (dos Santos Costa et al., 2011), resistin and retinol-binding protein 4 (Mercader, Palou, & Bonet, 2011). It may also improve insulin sensitivity indirectly by enhancing endothelial function, increasing liver fatty acid oxidation and decreasing oxidative stress (Bakker et al., 2010; Brasnyó et al., 2011). Additionally, resveratrol could relieve diabetes via increasing insulin action and glucose utilisation due to SIRT1 activation and glucose transporter modulation (Gencoglu, Tuzcu, Hayirli, & Sahin, 2015).

Studies utilising cultured cells and animal work have developed an understanding of the mechanism of action of resveratrol on insulin resistance and glycaemic control. In cultured cells, resveratrol has been shown to improve insulin-stimulated glucose uptake (Fischer-Posovszky et al., 2010); whilst in rodents it has been shown to reduce glycemia and improve insulin resistance in diet-induced insulin-resistant mice (Bagul et al., 2012; Kang et al., 2012). Resveratrol is also reported to reduce the glycaemic index in a rodent model in response to upregulated glucagon like peptide-1 (GLP-1), which is known to stimulate insulin and suppress glucagon production (Dao et al., 2011). In diabetic mice, resveratrol has been shown to significantly reduce blood glucose levels, plasma lipids, and free fatty acids and prevent the expression of inflammatory mediators, by inhibiting the NF-kB pathway (Guo et al., 2014).

In terms of human research, the findings to date are mixed. A study in male patients with type-2 diabetes, observed improvements in insulin resistance following 10 mg supplementation of resveratrol, daily for 4 weeks (Brasnyó et al., 2011). The authors observed that the improvement in insulin sensitivity was correlated with a decrease in the oxidative stress marker and an increase in protein kinase B phosphorylation (which is a key factor in insulin signalling). Further evidence in humans with type-2 diabetes showed key antidiabetic effects, including decreased fasting blood glucose, haemoglobin A1c, insulin and insulin resistance, and a significant rise in HDL cholesterol following supplementation with 1 g resveratrol for 45 days (Movahed et al., 2013). Additional beneficial effects have been observed in other studies utilising patients with type-2 diabetes (Bhatt et al., 2012; Crandall et al., 2012; Javid et al., 2017). Despite these positive findings, supplementation of 1500 mg resveratrol for 4 weeks in type-2 diabetic patients, had no effect on various outcome measures including insulin

sensitivity and glucose production (Poulsen et al., 2013). Similarly, additional studies have reported no effect on circulating levels of glucose and insulin, as well as glucose tolerance and insulin sensitivity in type 2 diabetic patients (Bo et al., 2016; Pollack et al., 2017; Thazhath et al., 2015). These contradicting findings may again potentially be explained due to vastly differing study designs. Indeed, many of the aforementioned studies which reported beneficial effects, have been criticised due to small sample sizes and short follow-up periods (Tomé-Carneiro et al., 2013). They also employed differing dosages and often beneficial effects were observed in studies that utilised younger participants. A recent systematic review and meta-analysis observed significant effects on the reduction of insulin resistance and glycated haemoglobin in type 2 diabetic patients; indicating that resveratrol supplementation has a protective effect on some diabetes parameters (Delpino & Figueiredo, 2021). The literature to date suggests that lower doses of resveratrol may be more beneficial for diabetic patients than higher doses, however further investigations are needed to understand the apparent discrepancies of resveratrol's dose response effects in diabetic patients.

When considering non-diabetic adults, the results of several studies indicate that resveratrol supplementation has no effect on insulin sensitivity and insulin and glucose levels (Dash et al., 2013; Poulsen et al., 2013; van der Made et al., 2015; Yoshino et al., 2012). Most recently, a study in overweight adults supplemented with 150 mg/day of resveratrol for 6 months, also observed no effect on insulin sensitivity when compared with placebo (de Ligt et al., 2020). However, in other studies with non-diabetic individuals, resveratrol supplementation has been observed to decrease circulating glucose and evince an improved homeostatic model assessment-insulin resistance (HOMA-IR) score. A suppression in postprandial glucagon response was observed following supplementation of resveratrol for 30 days with a 150 mg dose, in obese non-diabetic individuals (Knop et al., 2013). Additionally, overweight adults with impaired glucose tolerance were administered 1-, 1.5- or 2 g of resveratrol for 4 weeks and showed that insulin sensitivity and postprandial glucose levels were improved by resveratrol intake (Crandall et al., 2012). It is worth noting, however that both studies were pilot studies, utilising just 10 participants each.

In an attempt to cut through some of this confusion, several meta-analyses have concluded that, in non-diabetic individuals, resveratrol supplementation did not significantly affect fasting glucose and insulin concentrations (Liu et al., 2015). This finding was further corroborated with another meta-analysis which indicated that resveratrol can improve glucose control and insulin sensitivity in diabetic patients, but not in healthy individuals (Liu, Zhou, Wang, & Mi, 2014). They further observed that resveratrol may be more efficient in controlling diabetes when administered at low doses. When looking further at the effects in diabetic patients, a meta-

analysis of 6 studies identified beneficial effects on haemoglobin A1c, but no effects were observed on insulin, fasting glucose or HOMA-IR (Hausenblas, Schoulda, & Smoliga, 2015). Whereas a more recent meta-analysis including nine studies showed beneficial effects of resveratrol supplementation on fasting plasma glucose, insulin levels and HOMA-IR and this was particularly favourable in doses over 100 mg per day (Zhu, Wu, Qiu, Yuan, & Li, 2017). Despite these mixed findings, the authors report that resveratrol may be a potential therapeutic treatment to be used for diabetes, alone or in combination with current anti-diabetic treatments (Chaplin et al., 2018; Öztürk, Arslan, Yerer, & Bishayee, 2017). Further, it may simply be the case that, whilst resveratrol may be beneficial in various disease states, this may be the result of interacting with just some of its pathways, not all of them, and that combinations of resveratrol and other phenolics, alongside prescribed medications, may offer a multi-treatment option where each bridges the gaps of the others.

1.4. The behavioural effects of polyphenols

Due to the previously observed beneficial health effects of polyphenols and diets high in polyphenols, a reasonably large body of research has been conducted to explore the impact of polyphenols (and specifically resveratrol) on brain function, behaviour, and mood. The capacity for polyphenols to exert effects on the central nervous system is likely due to their ability to interact and modulate numerous cellular signalling cascades (Vauzour, 2017). Polyphenols, including resveratrol, may have the ability to improve long-term brain functioning due to interaction with inflammatory cascades, the consequent reduction in pro-inflammatory cytokines and modulation of neuroinflammation; thus protecting the brain from injury and potentially improving cognitive functioning. In other models; i.e. inducing acute boosts to brain function, the ability of polyphenols to interact with nitric oxide expression and subsequent modulation of peripheral and cerebral blood flow, may be important mechanisms of action. The following sections will therefore review the current literature surrounding the behavioural effects of both polyphenols in general and with a specific focus on resveratrol.

1.4.1. Polyphenols and cognitive performance

Whilst the mechanisms behind this are yet to be fully elucidated, consumption of polyphenol-rich food sources and polyphenolic-rich diets (such as the Mediterranean diet) have been demonstrated to have a beneficial effect on cognitive health and performance (Angelino et al., 2019; Dinu, Pagliai, Casini, & Sofi, 2018). Research utilising the Prevención con Dieta Mediterránea (PREDIMED) study population found that higher intakes of high-phenolic foods were associated with better memory function and global cognition (Valls-Pedret et al., 2012). They also observed that total urinary polyphenol excretion was associated with better human

memory function. Similarly, a recent observational study in Italian adults, used dietary data collected using FFQs to calculate an estimate of dietary flavonoid intake (Godos et al., 2020). Their results showed that higher dietary intake of flavonoids was associated with better cognitive health, measured using the Short Portable Mental Status Questionnaire (SPMSQ). Evidence has also indicated that these beneficial effects on cognitive function have the potential to be long lasting. A good example here is the Supplementation en Vitamines et Mineraux Antioxydants (SU.VI.MAX) trial, which included a total of 12,741 French adults for a planned follow-up of 8 years. Within this study, participants completed 24 hour dietary records every 2 months for a total of 6 records each year, the Phenol-Explorer database was used to compute polyphenol intake. In a follow up of 13 years, 2574 participants were reassessed using four neuropsychological tests (Kesse-Guyot et al., 2012). Their findings showed that high total polyphenol intake, and also specifically intake of catechins, theaflavins and flavonols, was associated with better language and verbal memory, especially episodic memory. In contrast, no benefit was observed for total polyphenol intake and executive functioning performance. In fact, they observed negative associations between scores on executive functioning and intake of catechins, proanthocyanidins and flavonols. Whilst the authors are unable to explain any molecular mechanisms to account for this negative association; this evidence suggests that polyphenol intake may exert beneficial effects on certain aspects of cognition and this may be as a result of site-specific effects within brain regions; resulting in differential effects to specific cognitive function domains (Shukitt-Hale, Carey, Jenkins, Rabin, & Joseph, 2007). In support of this, a review of the literature concluded that polyphenols can lead to cognitive benefits, although these effects are likely to be small and specific to certain cognitive domains and polyphenol source (reviewed in Lamport, Dye, Wightman, and Lawton (2012)).

As well as large scale epidemiological correlations between polyphenol consumption and cognition, randomised controlled trials (RCTs) have also observed direct effects of supplemented polyphenols on cognitive function in a lab-based setting. Several studies have indicated positive enhancements in cognitive performance following acute supplementation with cocoa flavanols. Notably, significant improvements are reported in working memory performance following supplementation of 520 mg and 994 mg cocoa flavanols in healthy adults when utilising a highly-demanding cognitive test battery (Scholey et al., 2010). Similar beneficial effects were observed following 773 mg cocoa flavanols; showing enhanced performance in accuracy of spatial working memory and various other areas of cognitive functioning (Field, Williams, & Butler, 2011). Additionally, beneficial cognitive effects have been observed following acute cocoa flavanol supplement in humans (Grassi et al., 2016; Massee et al., 2015). However, contemporary studies have not observed any differences in

cognitive performance between placebo and treatment groups (Decroix et al., 2016; Pase et al., 2013) and the lack of beneficial findings in the latter may be due to the cognitive tasks utilised being too short (5-30 minutes) to be challenging in the young, healthy population utilised. This argument is supported by the longer cognitive batteries (45-60 minutes) utilised in the studies of Field et al. (2011) and Scholey et al. (2010) where potential ceiling effects were mitigated by the increased mental fatigue ratings and challenged cognitive ability in young subjects. Within this setting, potential beneficial effects of cocoa flavanols are likely to have been easier to detect; again supporting the role of resveratrol in models of challenge.

In support of this, evidence from RCTs in older-elderly adults have indicated that chronic supplementation of cocoa flavanols can improve aspects of cognitive performance. Specifically, the administration of 520 mg and 993 mg cocoa flavanols presented as a drink, daily over an 8-week period, was associated with improvements in processing speed, executive function and working memory in elderly participants with mild cognitive impairment (Desideri et al., 2012). Similar effects have also been observed in elderly individuals without clinical evidence of cognitive dysfunction, following 8-week supplementation of the same dosages (Mastroiacovo et al., 2015). However additional studies, utilising much lower flavanol contents have not observed beneficial cognitive effects (Crews Jr, Harrison, & Wright, 2008; Francis, Head, Morris, & Macdonald, 2006). Collectively, these findings seem to support that regular cocoa flavanol intake possess the potential to improve cognitive performance, particularly in aged populations (Vauzour, 2012). A recent systematic review of 12 studies supports this with memory and executive function showing the most significant effects following doses of cocoa flavanols 500 – 750 mg/day (Barrera-Reyes, de Lara, González-Soto, & Tejero, 2020).

When considering the effect of different polyphenols on cognitive performance, supplementary work has investigated effects of single doses of fruit flavanones. Improvements in performance have been observed on a digit symbol substitution task in healthy, young adults following supplementation with a flavanone-rich orange juice (Lamport et al., 2016). Another recent study observed beneficial cognitive effects following a single dose of 300 mg mango leaf extract (containing high levels of the polyphenol mangiferin), with specific enhancements observed in global accuracy of performance, accuracy of attention and episodic memory (Wightman et al., 2020). Most recently, a review paper aimed to provide an accurate reflection of the current evidence regarding polyphenols and cognition (Lamport & Williams, 2021). They included four meta-analyses and thirteen systematic reviews (published between 2017 and 2020) assessing the impact of polyphenols (subclasses, specific polyphenol-rich foods and all polyphenols) on cognitive outcomes in humans. They concluded that whilst each of the

systematic reviews indicated some beneficial effects of polyphenols on cognition; these conclusions should be treated with caution. The authors particularly highlight that the literature may be over-interpreting the strength of beneficial findings and Ammar et al. (2020) highlight one such example here where studies will utilise one positive finding from a wider battery of cognitive tasks to demonstrate beneficial cognitive effects. They evidenced that, of the thirteen studies included in their meta-analysis, just three showed significant improvements on two or more outcomes whereas, a further six reporting beneficial effects, showed enhanced performance on only one of the outcome measures. As such, caution should be used when interpreting positive findings. In addition, as detailed within Table 1.1 the distinct methodological disparity of studies included within the meta-analyses and systematic reviews further restricts the scope of analysis and conclusions that can be drawn.

Table 1.1. Summary of studies investigating the effects of polyphenols on cognitive performance in humans. Including methodological limitations that are aimed to be addressed within the studies that comprise this thesis.

Reference	Demographic	n	Intervention	Duration	Cognitive tasks	Key findings	Methodological Limitations
Kesse-Guyot et al. (2012)	French adults as part of SU.VI.MAX trial	2574	Observational trial using dietary data to assess polyphenol intake	13 year follow up	Episodic memory RI48 Verbal fluency tasks Forward and backward digit span Delis-Kaplan TMT	High polyphenol intake = ↑ Language and verbal memory ↑ Episodic memory	No baseline cognitive data. Potential inaccuracies in polyphenol intake assessment (total polyphenols and individual classes) using FFQs. Difficulty in conclusively associating polyphenol classes with cognitive findings.
Scholey et al. (2010)	Healthy young adults	30	Cocoa flavanols (520 mg and 994 mg)	90 minute post dose	Cognitive demand battery (CDB) (SS3, SS7, RVIP) x 6	↑ SS3 performance ↑ RVIP RT (994mg dose) ↓ More SS7 errors (994 mg)	Limited to three cognitive tasks Acute design, with one post-dose assessment Healthy, young demographic
Field et al. (2011)	Healthy, young adults	30	720 mg cocoa flavanols	2 hours post dose	Visual spatial working memory Choice reaction time	↑ Spatial memory ↑ CRT	Single-blind design No baseline cognitive assessment Non-matched 'placebo' condition Limited to two cognitive tasks Healthy, young demographic
Massee et al. (2015)	Healthy, young adults	40	250 mg cocoa flavanols	Acute – 2 hours post dose and Chronic – 4 weeks	Simple reaction time Choice reaction time Stroop Picture recognition CDB x 3	↑ Impr. Mental fatigue (acute) ↑ SS7 performance	Very limited training on cognitive tasks – potential practise effects Relatively short chronic intervention

							Healthy, young demographic
Decroix et al. (2016)	Healthy male adults	12	903 mg cocoa flavanols	100 min post dose	Stroop	No effect	Limited to just one cognitive task Healthy, young demographic Only male participants Small sample size Acute design, with one post-dose assessment
Pase et al. (2013)	Healthy middle aged (40-65 yrs) adults	72	250 mg and 500 mg cocoa polyphenols	Acute – 1, 2.5 and 4 hours post dose. Chronic – 30 day	Cognitive Drug Research – Word recall, SRT, Digit vigilance, CRT, NWM, Picture recognition Bond-Lader Mood	↑ Calmness (30 day) ↑ Contentedness (30 day)	Relatively short chronic intervention
Desideri et al. (2012)	Elderly adults with mild cognitive impairment	90	900 mg, 520 mg, 45 mg cocoa flavanols	8 weeks	Mini mental state examination Trail making tests Verbal fluency test	↑ Quicker trail making (high and medium dose) ↑ Verbal fluency score (high)	Limited cognitive tasks included, which may not be appropriate in those with mild cognitive impairment
Mastroiacovo et al. (2015)	Healthy elderly adults	90	993 mg, 520 mg, 48 mg cocoa flavanols	8 weeks	Mini mental state examination Trail making tests Verbal fluency test	↑ Quicker trail making (high and medium dose) ↑ Verbal fluency score	No acute timepoint Relatively limited cognitive battery
Crews Jr et al. (2008)	Healthy older (>60 years) adults	101	37 g dark chocolate and 237 mL cocoa beverage	6 weeks	Selective reminding test Wechsler memory scale Trail making test Stroop	No effects	Relatively limited cognitive battery Relatively short chronic intervention period
Francis et al. (2006)	Healthy, young adults	16	150 mg cocoa flavanols	5 days	Letter judgement task Number judgement task	No effects	Healthy, young demographic Small sample size Limited to just two cognitive tasks

Lamport et al. (2016)	Healthy, young adults	24	High flavanone (70.5 mg) citrus juice	2 hour post dose	Freiburg vision test Word recall Logical memory Sequence learning task Digit symbol substitution task Stroop Letter memory test Go-NoGo task	↑ Digit symbol substitution	Healthy, young demographic Acute design, with one post-dose assessment Relatively small sample size
Wightman et al. (2020)	Healthy adults	70	300 mg mango leaf extract	30 min, 3 hr and 5 hr post dose	Word and picture recall NWM, CRT, Digit vigilance, SRT, Corsi blocks, CDB x3, Peg and Ball	↑ Accuracy of attention domain ↑ Episodic memory domain ↑ SS3 ↑ SS7 ↑ RVIP	Acute design only Healthy, young demographic

1.4.2. Polyphenols and neuroinflammation

The ability of polyphenols to exert beneficial cognitive effects may be attributed to an attenuation of inflammatory pathways which provide neuroprotective properties to the brain. The activation of pro-inflammatory signalling pathways, and subsequent production of cytokines, are able to communicate with the central nervous system via a number of different routes including via the vagus nerve or by direct or indirect transport of cytokines across the blood-brain barrier (Perry, 2004). In the short-term, inflammation of brain tissue can be beneficial; providing a defence against injury to the tissue. However, sustained neuro-inflammation results in the exacerbated production of pro-inflammatory cytokines, alongside ROS and RNS, which is consistently associated with the pathogenesis of neurodegenerative conditions including Alzheimer's disease (AD) and Parkinson's disease, alongside negatively impacting cognitive processing (Banks, Farr, & Morley, 2002; Sochocka, Diniz, & Leszek, 2017).

Given the potential anti-inflammatory properties of polyphenols, recent research has considered the impact of phenolic supplementation on reducing neuroinflammation and consequently the prevention or treatment of said neurological and neurodegenerative disorders. Indeed, evidence indicates that flavonoids have the ability to inhibit each stage of the inflammatory signalling process, resulting in suppression of neuroinflammation (Kennedy, 2014a). Evidence indicates that the structure of the polyphenol molecule impacts upon the ability to interact and disrupt the signalling pathways. Therefore, different polyphenols will exert different cellular effects (Williams & Spencer, 2012). A review by Spencer et al. (2012) details the potential of various flavonoids (including quercetin, luteolin and daidzein) to reduce neuroinflammation in *in vitro* and *in vivo* models. More recently, evidence has further indicated the neuroprotective effects of other polyphenols including curcumin, apigenin and resveratrol, where anti-inflammatory effects on the central nervous system have been observed (Renaud & Martinoli, 2014; Spencer et al., 2012; Venigalla, Gyengesi, & Münch, 2015). Subsequent work in humans has supported these findings and provides promise for the potential use of polyphenols, such as resveratrol, in the treatment of neurodegenerative disorders, via reduction in pro-inflammatory cytokines (Moussa et al., 2017).

1.4.3. Vasodilatory and blood flow effects of polyphenols

It has been hypothesised that another potential mechanism behind the cognitive-enhancing effect of polyphenols, is their ability to induce endothelial vasodilation. Evidence indicates that polyphenol interactions within the phosphoinositide 3-kinase (PI-3K/Akt) pathway, modulates intracellular calcium ions (Ca^{2+}), leading to increased expression of endothelial nitric oxide

synthase (eNOS), resulting in nitric oxide (NO) synthesis (Kennedy, 2014a; Vauzour, Rodriguez-Mateos, Corona, Oruna-Concha, & Spencer, 2010). The modulation of nitric oxide controls the vasodilatory response and consequently improves endothelial dysfunction, blood pressure and blood flow (Spencer, 2009; Williams & Spencer, 2012; Williamson, 2017). Indeed, much evidence confirms that supplementation of polyphenols has the ability to increase NO production (Fisher, Hughes, Gerhard-Herman, & Hollenberg, 2003; Fraga et al., 2010; Kim et al., 2007).

The polyphenolic interaction with eNOS, resulting in enhancing endothelial functioning and vasodilation, has been consistently supported by controlled intervention trials with polyphenolic-rich foods. Much of the early work here considered the impact of flavonoids on peripheral blood flow, with particular interest placed on cocoa-flavanols. Initial work in healthy adults showed that following a 5-day consumption of flavanol-rich cocoa (821 mg/day) resulted in a significant increase in peripheral vasodilation, as measured by flow-mediated dilation (FMD) (Fisher et al., 2003). Since then, several meta-analyses and systematic reviews have consistently shown that FMD can be improved following acute and chronic supplementation with cocoa-flavanols (Hooper et al., 2012; Sun, Zimmermann, De Castro, & Actis-Goretta, 2019). Most recently, a systematic review and meta-analysis of research to date, comprising 22 studies (n=794), assessed the effects of dark chocolate and flavonoids on FMD (Ebaditabar, Djafarian, Saeidifard, & Shab-Bidar, 2020). The results concluded that acute and chronic consumption had a protective effect on FMD, with greater improvement observed following higher doses (>20g/day) of chocolate consumed over a chronic period (>1 month). In addition, similar effects have also been observed with other polyphenols, including tea polyphenols, which have been shown to improve FMD following acute (single dose, assessed after 2 hours) and chronic (4 week) supplementation (Duffy et al., 2001; Grassi et al., 2008; Hodgson, Puddey, Burke, Watts, & Beilin, 2002).

Of particular interest here is the research indicating that NO production is reduced in obese individuals and that they are more likely to have endothelial dysfunction (Westerbacka et al., 1999). It has been hypothesised that obesity-related oxidative stress reduces the bioavailability of NO (Higashi et al., 2001; Huang et al., 2001) resulting in impaired endothelium-dependent vasodilation. As an example, it has been demonstrated that the increase in blood flow into the leg following methacholine (a muscarinic agent) is blunted in obese humans, with the degree of dilation being inversely proportional to the degree of obesity (Steinberg et al., 1996). Similar blunted vasodilatory responses in obese individuals has been observed in the forearm in response to insulin (Tack, Ong, Lutterman, & Smits, 1998). It has been suggested that within obese cohorts with endothelial dysfunction, polyphenolic-induced

increase in blood flow via increased NO signalling may be particularly effective. Indeed, in healthy overweight and obese adults, a 4-week consumption of cocoa and dark chocolate (total dose of natural cocoa 22 g/day) was associated with significant increases in basal and peak arterial diameter and increased arterial blood flow through the arterial stiffness (West et al., 2014).

Given these findings of polyphenolic-induced improvements in peripheral blood flow, much of the recent work here has considered if these effects may extend to blood flow within the brain. Here it is thought that, due to greater bioavailability of NO and subsequent improvements in blood flow, phenolics may confer a neuroprotective effect (Aliev et al., 2009). Improvements in brain blood flow induces neurogenesis in the denate gyrus of the hippocampus, promoting nerve cell growth and leading to changes in neuronal morphology (Pereira et al., 2007; Rees, Dodd, & Spencer, 2018; Spencer, 2010). Cerebral blood flow (CBF) is essential for the normal functioning of the brain, furnishing it with a constant supply of blood and oxygen, where an insufficient supply of energy will result in neuronal damage. Cardiovascular risk factors, such as obesity, have been shown to lead to reductions in CBF, particularly areas like the hippocampus and anterior cingulate cortex, which are associated with the pathogenesis of neurodegenerative diseases such as AD (de La Torre, 2012; Di Marco et al., 2015).

A number of studies have observed a beneficial effect of polyphenol supplementation on CBF. For example, acute consumption of a single high flavanol (516 mg) cocoa drink resulted in an increase in CBF when compared with a low flavanol (39 mg) drink, in young healthy adults (Francis et al., 2006). Likewise, another study observed a significant increase in CBF, particularly within the anterior cingulate cortex and central opercular cortex of the parietal lobe, when a high-flavanol (494 mg) cocoa drink was consumed compared with a low-flavanol (23 mg) drink (Lamport et al., 2015). Several studies have observed an increase in CBF when supplemented with flavanol-rich cocoa drinks over a longer period of time; with mean blood flow velocity in the middle cerebral artery increased following one- and two-week supplementation compared with flavanol-poor cocoa consumption in healthy elderly adults (Sorond, Lipsitz, Hollenberg, & Fisher, 2008). An enhancement in denate gyrus function, measured using fMRI, was observed following supplementation of 900 mg cocoa flavanols per day for three months, in healthy older adults (Brickman et al., 2014). Studies utilising other flavonoid-rich foods have observed comparable effects, with supplementation of a single flavonoid-rich blueberry drink resulting in increased CBF in the precentral and middle frontal gyrus of the frontal lobe and the angular gyrus of the parietal lobe in young, healthy adults one hour post-dose (Dodd, 2012). Increases in regional perfusion, specifically in the parietal and occipital lobes, have been observed following 12-week supplementation of a blueberry

concentrate (Bowtell, Aboo-Bakkar, Conway, Adlam, & Fulford, 2017), and similar effects have been observed in the interior and middle right front gyrus following consumption of a citrus drink containing 70.5 mg flavanones (Lamport et al., 2016). A recent review concluded that research to date suggests positive effects of flavonoid intake on CBF; however supplementary work is necessary to understand the impact on specific regions of the brain Rees et al. (2018).

Despite much of the work indicating positive CBF effects, some studies have demonstrated contradictory polyphenolic-induced results. For example, a randomised controlled intervention trial observed a significantly lower CBF response during cognitive tasks following consumption of dark (394 mg total polyphenols) and milk (200 mg) chocolate compared with white chocolate (34 mg). This reduction in CBF however, had no impact upon cognitive ability (Marsh et al., 2017). These results indicate that high concentrations of cocoa may enhance NO production and modify cerebral metabolism, consequently decreasing oxygen demand in active brain regions. Additionally, a significant reduction in oxygenated and total haemoglobin in the frontal cortex has been observed following a 135 mg dose of EGCG, again with no improvements observed in cognitive performance (Wightman, Haskell, Forster, Veasey, & Kennedy, 2012). The authors suggest that EGCG may have the ability to improve other aspects of brain function, reducing the need for blood flow in the frontal cortex.

Based on current evidence, it seems that polyphenol (particularly flavonoid) supplementation can modulate CBF, with region specific enhancements observed. However, further research is necessary to understand why the results indicate contradictory enhancements and attenuations following polyphenolic intervention; as well as understanding which specific brain regions are affected and how this modulation impacts upon cognitive performance. In order to do this, it is also important to understand what specific modulations in CFB mean in terms of the beneficial outcomes, particularly when related to enhancements in cognitive performance. When neural demand increases, so too does the necessity for metabolic resources, specifically oxygen and glucose (Denfield, Fahey, Reimer, & Tolias, 2016). This alteration in CBF due to neuronal demand is known as neurovascular coupling. Neuroimaging techniques like NIRS, exploit said neurovascular coupling and indirectly assess neuronal activity by measuring changes in oxygenated-haemoglobin (oxy-Hb) and deoxygenated-haemoglobin (deoxy-Hb) (Jackson & Kennedy, 2013). Local CBF is related to the metabolic activity of specific brain regions and, as such, these measures show the oxygen consumption of neuronal cells. NIRS outcomes therefore may be used to infer local neural activation. Typically, a response during local neural activity is expected to be observed as an increase in oxy-Hb, paired with a simultaneous decrease in deoxy-Hb (Obrig & Villringer, 2003). As blood flow increases in active tissue, a sudden influx in oxy-Hb is observed and a resulting decrease

in deoxy-Hb. Increased oxy-Hb is usually observed throughout the period of sustained activation, as delivery of oxygenated blood to active areas typically exceeds local oxygen utilisation. Similarly, the total concentration of haemoglobin (the sum of oxy-Hb and deoxy-Hb) typically follows this pattern, due to the “overshoot” in cerebral oxygenation (Obrig et al., 1997). Despite this typical response that would be expected when measuring CBF during cognitive demand, some studies have observed improvements in cognitive performance alongside reductions in oxy-Hb (Wightman, Haskell-Ramsay, Thompson, et al., 2015). And as such, further work is necessary to understand the specific relationship between modulation of CBF parameters and cognitive performance following polyphenolic supplementation. In addition, much of this research has considered only acute or relatively short chronic effects, therefore future work assessing long-term chronic consumption would be useful.

1.4.4. Polyphenols, cerebral blood flow and cognition

Recent work has investigated the effect of polyphenolic supplementation on CBF and cognitive performance concurrently. However, much of this work has not demonstrated simultaneous improvements in these two areas (Lamport et al., 2016; Sorond et al., 2008). Indeed, whilst previous work indicates cognitive improvements following cocoa-flavanol administration, several studies have observed no cognitive benefits despite observing significant increases in CBF (Decroix et al., 2016; Francis et al., 2006). A review of the cocoa-flavanol literature observed that, whilst consistent improvements in cerebral and peripheral blood flow have been observed, these are seldom found in conjunction with cognitive findings (Socci, Tempesta, Desideri, De Gennaro, & Ferrara, 2017).

It has been theorised that in order to observe the subtle behavioural effects of polyphenols, particularly when utilising a young, healthy population, it may be most effective when participants are subjected to a highly demanding cognitive context. As such, one study mimicked this by investigating the effects of flavanol-rich chocolate administration in participants who had been deprived of sleep (a condition which is known to impair cognitive function). Whilst direct CBF was not measured, these studies indicated improved endothelial function (measured by FMD) and concurrent improvements in cognitive performance; indicating that supplementation with cocoa flavonols could exert beneficial effects on cognitive performance potentially through acute changes in peripheral and central blood flow (Grassi et al., 2016).

Much of the work here utilises cognitively demanding tasks, administered over a sustained time period, rather than sleep deprivation, to disrupt cognitive function. For example, recent

work investigating the co-supplementation of phenolic-rich apple, blueberry and coffee berry extracts with extracts of beetroot, ginseng and sage in the form of a drink, assessed the effect on cognition, mood and CBF (Jackson et al., 2020). They observed that consumption of these products led to changes in subjective mood state, with reductions in mental fatigue and additional beneficial effects on mood observed. In terms of CBF, all drinks resulted in an increase in oxygen saturation within the frontal cortex, and the flavonol-rich apple drink resulted in increased levels of total haemoglobin. They did not however, observe any beneficial cognitive effects, despite clear enhancements in CBF. The authors note that a possible explanation to this is that CBF parameters were too small to elicit effects in the young, healthy population that was utilised in this study. A more compromised sample population, for example older adults or those who are obese, may be a more sensitive cohort to investigate; as they are at risk of cerebrovascular and metabolic dysfunction. It's important to note here that, despite the trial design including 4 drinks, each contained the 'base' drink (comprising water, sucralose, preservatives and artificial flavourings), making it impossible to disentangle the effects of the phenolics from the base ingredients.

Supporting this, studies within an older population, who are likely suffering from age-related cognitive deficits, have observed some concurrent improvements in CBF and cognitive performance. For example, supplementation of a flavonoid-rich blueberry concentrate for 12 weeks in healthy older adults, resulted in significant increases in brain activation responses in a number of task-associated regions (specifically in the parietal and occipital lobes). Alongside this, there was some weak evidence of improvement in the 2-back version of the n-back test only, with no other significant differences between treatment groups (Bowtell et al., 2017). Similarly, a 4 week supplementation of pomegranate juice in older adults was shown to increase fMRI activity during verbal and visual memory tasks, alongside a signficiant improvement in performance on the former task (Bookheimer et al., 2013). Likewise, following 16-weeks supplementation of Concord grape juice in older adults (Krikorian et al., 2012), increased activation on fMRI in the right anterior and posterior cortical regions was observed during performance of the *n*-back working memory task. Whilst no accuracy improvements were observed in cognitive performance, those who consumed the grape juice showed reduced semantic interference on memory tasks; meaning that although they acquired new information at the same level as those who consumed placebo, they were better able to suppress interference of extraneous material. These results may indicate that, particularly in a compromised sample population, polyphenol supplementation might have some concurrent benefits on CBF and cognitive performance.

Despite this, to date much of the published work identifying cognitive benefits of polyphenols are done so without simultaneous measurement of CBF; moreover those studies simultaneously measuring these employ considerably small sample sizes. This is evident when considering the intervention studies including within Table 1.1. and 1.2. where average sample size of studies just measuring cognitive performance is n = 52, whereas in comparison those simultaneously measuring CBF and cognitive performance in n = 24. Therefore, it is necessary for future research to monitor cerebral haemodynamic in conjunction with cognitive performance to determine the efficacy of this proposed mechanism of cognitive enhancement (Bell, Lamport, Butler, & Williams, 2015). In addition, the differential effects observed in the research summarised in this section, are likely to be due to the various polyphenols (and indeed the use of single phenolic extracts and whole extracts) utilised in the study design. As different phenolics are likely to exert disparate effects on both cognitive performance and CBF.

Table 1.2. Summary of studies investigating the effects of polyphenols on cognitive performance and cerebral blood flow in humans.

Reference	Demographic	n	Intervention	Duration	Methodology	Key findings
Lamport et al. (2016)	Healthy, young adults	16 in CBF imaging arm, 24 cognition	High flavanone citrus juice	Acute – 2- and 5-hours PD	Cognitive performance and CBF (fMRI) measured in isolation. 45 min cog battery of 11 individual tasks	↑ Regional perfusion (inferior and middle right frontal gyrus) ↑ Digit Symbol Substitution Test
Sorond et al. (2008)	Healthy, elderly adults	34	Flavanol-rich cocoa	2 weeks	TCD measuring MFV in the middle cerebral artery No measure of cognition	↑ Mean blood flow
Decroix et al. (2016)	Healthy men	12	903 mg cocoa flavanol	Acute - 100 min PD	Prefrontal NIRS during cognitive assessment Stroop task only	↑ OxyHb during rest
Francis et al. (2006)	Healthy, young female adults	16	150 mg cocoa flavanols	5 days	fMRI BOLD response 2 cognitive tasks – letter and word judgement tasks	↑ BOLD signal intensity
Grassi et al. (2016)	Healthy, young adults	32	100 g flavanol-rich dark chocolate	Acute – 90 min PD	Sleep deprivation condition FMD of branchial artery 2 cognitive tasks – psychomotor vigilance task and 2-back task	↓ Blood pressure ↑ Countered impaired flow-mediated dilation caused by sleep deprivation ↑ Working memory accuracy after sleep deprivation
Jackson et al. (2020)	Healthy adults	32	Phenolic rich drinks	Acute – 60-, 180- and 360-min PD	60 min cog battery – 4 rounds of CDB, with additional 5 tasks QNIRS measured throughout each cog assessment	↑ Alertness (apple extract) ↓ Mental fatigue (apple & coffeeberry extract) ↓ POMS – anger, confusion, depression, fatigue, TMD ↑ Total haemoglobin ↑ Oxygen saturation
Bowtell et al. (2017)	Healthy, older adults	26	30 mL blueberry concentrate (387 mg anthocyanidins)	12 weeks	35 min cog battery with 7 individual tasks fMRI with stroop task completed concurrently	↑ fMRI brain activation ↑ 2-back test performance
Bookheimer et al. (2013)	Middle-aged & older adults	28	Pomegranate juice	4 weeks	fMRI during cognitive tasks 2 cognitive tasks	↑ Verbal memory task performance

	with memory complaints					↑ fMRI activity during verbal and visual memory tasks
Krikorian et al. (2012)	Older adults with mild cog impairment	21	Concord grape juice	16 weeks	fMRI during n-back memory task California Verbal Learning Test	↑ Activation in anterior and posterior regions in right hemisphere ↓ Semantic interference on memory tasks

1.4.5. Polyphenols and mood

Polyphenol consumption may also have indirect effects on neuropsychological function by supporting mental health and well-being. Epidemiological data shows that consumption of fruit and vegetables predicts a lower incidence of depression in later life (Mihrshahi, Dobson, & Mishra, 2015). A large prospective study examined whether the long-term dietary intake of flavonoids was related to lower incidence of depression in 82,643 middle aged and older adult women without a previous history of depression (Chang et al., 2016). They calculated intake of subclasses and total flavonoids using FFQ data collected every 2-4 years and assessed depression based on medical diagnosis and use of medication. They observed that the highest intakes of flavonols, flavones and flavanones were significantly associated with a 7-10% lower risk of depression compared with the lowest intakes during a 10-year follow up.

In terms of intervention studies of polyphenols, consumption of a single administration of a cocoa flavanol-rich drink (520 mg) was shown to reduce self-reported levels of mental fatigue following completion of a cognitively demanding battery of tasks (Scholey et al., 2010). Likewise, 30 days supplementation with a dark chocolate drink containing 500 mg cocoa flavanols, showed improvements in self-reported ratings of calmness and contentedness (Pase et al., 2013). Similar positive mood effects have been observed in children (aged 7-10 years old) and young adults (aged 18-21 years old) supplemented with a flavonoid-rich wild blueberry drink (Khalid et al., 2017); where the authors observed increases in Positive Affect but not Negative Affect, using the Positive and Negative Affect-Schedule-NOW (PANAS-NOW) self-report questionnaire. Despite this, a recent systematic review of blueberry polyphenol interventions on cognition and mood (comprising 5 trials); showed that only the aforementioned work by Khalid et al. (2017) observed improvements in mood and the remaining reported no improvements (Travica et al., 2020). It is, however, worth reiterating the frequently mentioned observation that the studies included differed vastly in study design; with positive mood effects observed in children and young adults and the other studies utilizing an older or elderly population. Alongside study population, differences in dosages and anthocyanin content were apparent between these studies, which could explain the discrepant results.

When considering other polyphenols, acute administration of Concord grape juice has been shown to result in an increase in self-reported ratings of calmness in healthy, young adults (Haskell-Ramsay, Stuart, Okello, & Watson, 2017). Another study in healthy older adults observed significantly lower levels of fatigue following 4 weeks supplementation with solid lipid curcumin (Cox, Pipingas, & Scholey, 2015). They also observed a decrease in fatigue induced

by the mental challenge, alongside a smaller reduction in calmness and contentedness when compared with placebo; suggesting that in young, healthy adults, polyphenol supplementation may provide resilience to the detrimental impact of cognitive stress on mood.

Based on this evidence, it seems that the ability of polyphenols to influence mood is most visible in the areas of fatigue, depression, calmness and contentedness. Whilst the mechanisms of action behind these mood effects are unknown there have been several tentative explanations provided. This includes the role of polyphenols in inhibiting monoamine oxidase (MOA) and, as a consequence, regulating neurotransmitters associated with anxiety and fatigue (Nemeroff et al., 2003; Pathak, Agrawal, & Dhir, 2013). More recently, it has been suggested that the positive mood effects may be due to interaction between polyphenols and the gut microbiota via the so-called gut-brain axis (reviewed in Westfall and Pasinetti (2019)).

1.4.6. Resveratrol and neuroprotection

A probable mechanism behind the beneficial effects of polyphenols on cognitive function, is the ability to exert neuroprotective effects. Much work has considered the potential neuroprotective effects that resveratrol may exert. Much of this work considers the effects of supplementation in neurodegenerative disorders including Alzheimer’s disease and dementia. Global data indicates that mild cognitive problems disturbs 5.5-7.7% of individuals over 60 years old and 22% of those over 70 (Apostolo et al., 2016). Considering this, it is essential to explore new tools that can downgrade dementia advance and prevent cognitive decline. Evidence suggests that resveratrol may be a potential therapeutic agent, as it likely possesses neuroprotective actions and could have positive effects against the deterioration of human cognition (Ranney & Petro, 2009).

Indeed, resveratrol has been shown to reduce neurodegeneration in the murine cerebral cortex and enhance memory recovery after exposure to fluoride (Sharma, Suhalka, & Bhatnagar, 2018). Similarly, neuroprotective properties of resveratrol have been suggested in studies of its effects in intracerebral haemorrhage (Bonsack, Alleyne Jr, & Sukumari-Ramesh, 2017), cerebral neuro-damage (Nalagoni & Karnati, 2016) and central nervous system injuries such as stroke (Lopez, Dempsey, & Vemuganti, 2015). As the pathogenesis of cognitive decline and AD is associated with numerous genes and pathways, including oxidative stress and mitochondrial damage (Hung, Chen, Hsieh, Chiou, & Kao, 2010; Z. Liu, Zhou, Ziegler, Dimitrion, & Zuo, 2017) resveratrol may be of benefit here by interacting with these. It is thought that resveratrol plays a major role in neuroprotection by reducing oxidative damage, mitochondrial dysfunction and chronic inflammation (Molino et al., 2016; Ramassamy, 2006).

A wealth of research conducted in various animal models demonstrates that resveratrol protects against cognitive decline through the activation of SIRT1 (Du et al., 2014) or promoting SIRT1 expression (Li et al., 2014; Zhang et al., 2017; Zhao et al., 2013). Some of this research in animals uses isoflurane anaesthesia to induce inflammation. Indeed, research indicates that in the hippocampus, the isoflurane anaesthesia-induced over-production of pro-inflammatory cytokines impairs synaptic plasticity leading to neurodegeneration, with a consequent deficit in cognitive function (Pugh, Fleshner, Watkins, Maier, & Rudy, 2001; Terrando et al., 2010). Research in aged mice indicated that 7-day administration of 100 mg/kg of resveratrol was able to attenuate the isoflurane anaesthesia cognitive impairment, via anti-inflammatory and anti-apoptosis effects (Li et al., 2014). Resveratrol's ability to modulate SIRT1 expression and activity is a likely mechanism behind this effect. Specifically, resveratrol may enhance neural malleability and promote neurite outgrowth in the hippocampus area (Hasegawa & Yoshikawa, 2008). Considerable importance has been placed on this pathway. A study in a rat model of streptozotocin-induced AD, showed that 8 weeks administration of 30 mg/kg/day resveratrol, resulted in activation of SIRT1 (Du et al., 2014). This activation was also presented with attenuation of the hyperphosphorylation of tau protein and improvements in the Morris Water Maze (MWM), which the authors conclude confirms resveratrols role in protecting hippocampal neurons from tau hyperphosphorylation and preventing cognitive impairment. Similarly, a recent study in a rat model of combined diabetes and AD showed that resveratrol administration (25 mg/kg/day for 4 weeks) significantly increased SIRT1 expression and also inhibited memory impairment on the Morris Water Maze (MWM) task (Ma et al., 2020).

Additionally, SIRT1 regulates brain-derived neurotropic factor (BDNF) (Cao, Dou, & Li, 2018); a potent modulator involved in the control of neuronal survival, synapse formation and synaptic plasticity (Leal, Afonso, Salazar, & Duarte, 2015). Specifically, SIRT1 binds to the transcription factor YY1 complex to limit the expression of miR-134, which affects the CREB-BDNF axis and promotes BDNF transcription (Cao et al., 2018). Sequentially, BDNF release may activate the mTOR signalling pathway and negatively regulate the activity of miR-134 while repressing Lim-domain-containing protein kinase 1 (Limk1) translation and promoting dendritic development. SIRT1 overexpression in the hippocampal neuron may also deacetylate methyl-CpG binding protein 2 (MeCP2) and thus promote BDNF transcription (Leal et al., 2015; Ng, Wijaya, & Tang, 2015; Tao, Finkbeiner, Arnold, Shaywitz, & Greenberg, 1998). Each of these pathways increase BDNF levels via SIRT1 and as such, resveratrol administration could enhance BDNF via these pathways.

The evidence of a neuroprotective effect of resveratrol has been further corroborated in *in vitro* rat hippocampal H19-7 neuronal cells in which a 2-hour pre-treatment with resveratrol attenuated the Aβ-induced oxidative damage and the decrease of proteins essential for synaptic maturity and plasticity (Rege, Geetha, Broderick, & Ramesh Babu, 2015). Similarly, in streptozotocin-induced diabetic rats, resveratrol normalised the hippocampal expression of genes implicated in neurogenesis and synaptic plasticity (Hdac4, Hat1, Wnt7a, ApoE) and reduced that of Jak-Stat pro-inflammatory signalling (IL-15, IL-22, Socs2 and Socs5) (Thomas, Garg, & Smith, 2014). In addition, in a rodent model of vascular dementia, resveratrol supplementation induced hippocampal nerve growth factor expression, attenuating pyramidal cell death in the CA1 hippocampal sub-region and improving spatial working memory (Anastácio et al., 2014). The use of resveratrol led to the expression of the hippocampal nerve growing component, decreased pyramidal cell mortality in the hippocampus CA1 region and increased spatial working memory in a vascular dementia rat model (Anastácio et al., 2014). In another model of vascular dementia (permanent bilateral common carotid artery occlusion), the daily administration of resveratrol improved learning and memory ability as evaluated by the MWM test. Also, following resveratrol administration, levels of malondialdehyde; a key marker of oxidative stress in neurodegenerative disease, were decreased in the cerebral cortex and hippocampus and resveratrol resulted in increased SOD activity and glutathione levels (Ma et al., 2013).

Cognitive deficits are associated with higher levels of ROS and nitrogen species and, as such, oxidative stress seems to precede the formation of senile plaques (Wahlster et al., 2013). Therefore, the potent antioxidant activity of resveratrol is likely to play a role in the prevention of neurodegeneration in AD (Kim, Lee, & Lee, 2010). Particularly, resveratrol scavenges free radicals, protects neurons and microglia (Candelario-Jalil et al., 2007; Zhuang, Kim, Koehler, & Doré, 2003) and attenuates Aβ-induced intracellular ROS accumulation (Jang & Surh, 2003). The treatment of a hippocampal cell line with resveratrol attenuated ROS production and mitochondrial membrane-potential disruption. Moreover, it restored the normal levels of glutathione depleted by Aβ1-42 (Kwon, Kim, Shin, & Han, 2010). Resveratrol may also attenuate Aβ-induced intracellular ROS accumulation (Koukoulitsa et al., 2016) and induce the up-regulation of cellular antioxidants (e.g. glutathione) and the gene expression of phase 2 enzymes; thus protecting against oxidative and electrophilic injury (Cao & Li, 2004).

Whilst the effect of resveratrol on cognitive function is likely magnified in extreme models such as AD and dementia, there is evidence to suggest that resveratrol is also effective in murine models of mild stress. In rats exposed to chronic unpredictable mild stress, able to induce cognitive deficits, the chronic administration of resveratrol significantly attenuates the deficit in emotional learning and spatial memory (Yazir, Utkan, Gacar, & Aricioglu, 2015). This study

further observed that in the hippocampus, when compared to stressed rats, resveratrol administration prevented the decrease of BDNF observed in the stressor condition. Resveratrol has also been shown to significantly improve sleep deprivation-induced contextual memory deficits, through the activation of cAMP-response element-binding protein and mammalian target of rapamycin (mTOR) signalling pathways (Zhao et al., 2015). Additionally, data on rats in late middle-age highlighted how the effect of resveratrol in improving memory and mood function is mainly due to the modulation of hippocampal plasticity and the suppression of chronic low-level inflammation (Kodali et al., 2015).

As inflammation may be a factor in the pathogenesis of neurodegenerative disorders, the anti-inflammatory activities of resveratrol may provide a further protective role. Studies indicate that markers of inflammation such as TNF-alpha, IL-1BETA, IL-16 are increased in the brain, cerebrospinal fluid and plasma of AD patients (Alcolea et al., 2014; Heneka et al., 2015; Perry, Nicoll, & Holmes, 2010; Swardfager et al., 2010). In AD, neuroinflammation triggers synaptic pruning, contributes to neuronal damage and prompts Aβ and tau pathologies (Heneka et al., 2015; Hong, Dissing-Olesen, & Stevens, 2016; Sadigh-Eteghad, Majdi, Mahmoudi, Golzari, & Talebi, 2016). Taken together, these alterations contribute to impaired synapse function (Wang, Tan, Yu, & Tan, 2015) resulting in memory dysfunction; the main characteristic of this disease. Resveratrol has been shown to have the ability to protect organotypic hippocampal cultures from Aβ-induced toxicity through decreasing TNF-α, IL-1β, and IL-6 levels and increased IL-10 cytokine levels (Frozza, Bernardi, Hoppe, Meneghetti, Battastini, et al., 2013). Corroborating data appear in a recent study showing that resveratrol inhibits neuroinflammation triggered by Aβ in cultured astrocytes and microglia (Zhao et al., 2018).

Resveratrol also interferes with the neuroinflammatory process (Venigalla, Sonego, Gyengesi, Sharman, & Münch, 2016) by suppressing the activation of astrocytes, microglia (Bi et al., 2005; Wang et al., 2002), TNF-α and NO production by inhibiting NF-kB activation and p38 mitogen-activated protein kinase phosphorylation (Cheng, Wang, Li, & Zhao, 2015). Resveratrol treatment has also been shown to reverse the Aβ-induced iNOS overexpression (Huang, Lu, Wo, Wu, & Yang, 2011) and exert anti-inflammatory effects as it inhibits TNF-α, IL-1β, and IL-6 expression (Yao et al., 2015). Attenuating neuroinflammation is also a therapeutic strategy for treating ischemic stroke, and several *in vivo* studies have shown that resveratrol effectively reduces the increased expression of proinflammatory cytokines, inhibits NF-kB, reduces the phosphorylation of p38-MAPK and JNK activation via decreased COX-2 and iNOS expression and inhibits astroglial and microglial activation induced by ischemia/reperfusion (Bureau, Longpré, & Martinoli, 2008; Inoue et al., 2003; Shin et al., 2010; Simão, Matté, Pagnussat, Netto, & Salbego, 2012; Wang et al., 2002). These findings suggest

that the suppression of inflammation is associated with the neuroprotective effects of resveratrol and that it may be a promising therapeutic candidate for stroke.

TNF-α-dependent mechanisms appear to drive memory defects (Lourenco et al., 2013); thereby indicating a causal role of inflammation in the deleterious processes linked to AD. A study indicated that chronic administration of resveratrol blocked cognitive impairment in an animal model of AD, and this effect seemed to be related to the inhibition of synaptic dysfunction, and microglial and astroglial activation triggered by Aβ (Frozza, Bernardi, Hoppe, Meneghetti, Matté, et al., 2013). Resveratrol treatment also modulated important cell signalling pathways, such as the JNK, GSK-3β, and β-catenin pathways, which might be involved in neuroinflammation, cell metabolism and survival. These observations are consistent with the idea that resveratrol can modulate several signalling pathways involved in neuroinflammation.

Microglial activation may contribute to neuronal death during brain damage by releasing neurotoxic pro-inflammatory molecules (Perry et al., 2010). Resveratrol inhibits the pro-inflammatory molecules known as cyclooxygenases, particularly cyclooxygenase-1 (COX-1), an enzyme involved in the production of pro-inflammatory molecules known as cytokines. Resveratrol is also able to reduce the release of pro-inflammatory factors through the inhibition of cellular cascade signalling pathways involving NF-kB and activator protein-1 (AP-1) (Das & Das, 2007). Using rat primary microglia cultures exposed to LPS it has been reported that resveratrol reduced the production of prostaglandins, NO, and TNF-alpha, as well as the expression of COX1 and activation of NF-kB (Bi et al., 2005; Candelario-Jalil et al., 2007; Kim, Kim, Park, & Choi, 2007; Meng et al., 2008). Figure 1.4 provides a summary of the discussed potential neuroprotective actions of resveratrol.

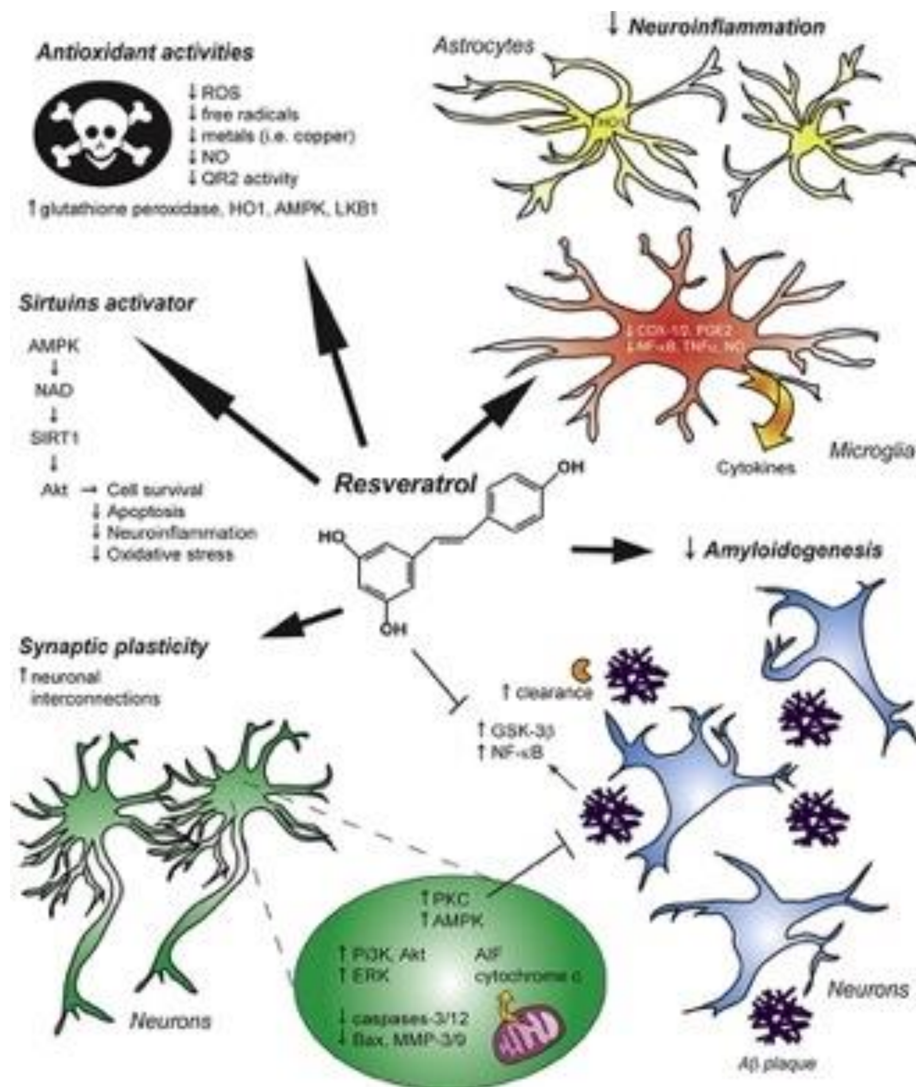

Figure 1.4. Summary of the neuroprotective action of resveratrol. Resveratrol displays potent antioxidant activity by scavenging free radicals, protecting against NO toxicity and upregulating endogenous enzymes such as glutathione peroxidase. It also inhibits pro-inflammatory enzyme expression, reduces NF-kB activation and cytokine release. Treatment with resveratrol can also affect multiple signalling pathways involved in cell survival, programmed cell death and synaptic plasticity. Diagram obtained from (Bastianetto, Ménard, & Quirion, 2015), reproduced with permission of Elsevier.

1.4.7. Resveratrol, cognition, cerebral blood flow and mood

The above sections detail the myriad pathways that resveratrol is able to interact with and, given the nature of some of these pathways in the brain, it is perhaps not surprising that investigations into the potential cognitive effects of resveratrol have flourished; especially those targeting the cerebral blood flow pathway. The following section will detail this previous work. Owing to the ease of access of the sample, much of this initial work has been conducted in young and healthy individuals, without cognitive compromise.

The first study to investigate the acute effects of resveratrol administration on cognitive performance and CBF, utilised a crossover design in 24 young, healthy adults (aged 18-25 years) (Kennedy et al., 2010). Participants received three single dose treatments (placebo, 250 mg and 500 mg), consumed on visits 1 week apart. The researchers simultaneously measured cerebral blood flow in the prefrontal cortex (using functional NIRS) and cognitive performance. The latter was assessed via 3 cognitively demanding tasks, which had previously been shown to activate the prefrontal cortex (Drummond et al., 1999). Following a 45-minute absorption period, participants completed this cognitively demanding battery of tasks 4 times (totalling 36 minutes of continuous performance). The results indicated clear dose-dependent modulation in CBF during task performance, with significantly higher total haemoglobin observed during each task period when compared with placebo, following the higher dose (500 mg). Similarly, higher total haemoglobin was observed during several time-points following the lower dose (250 mg). In addition, significant higher deoxygenated-haemoglobin concentrations were observed following both dosages during both rest and task performance, which suggested resveratrol enhanced oxygen extraction and utilisation. However, despite these clear changes in CBF, the authors did not observe any significant differences in cognitive task performance or ratings of mental fatigue between the treatment groups. The authors suggest that the lack of findings are potentially due to the low bioavailability of resveratrol.

As such, supplementary work by the same research team aimed to improve bioavailability by supplementing participants with a combination of resveratrol and piperine (a pepper derived alkaloid) (Wightman et al., 2014). Previous research indicated that co-supplementation enhanced resveratrol levels in mice (Johnson et al., 2011). The authors therefore aimed to investigate the effects of 250 mg resveratrol alone and in co-supplementation with 20 mg of piperine, in 23 young, healthy adults (aged 19-34 years). In order to maximise the cerebral activity-induced modulation of blood flow, the authors carried out a pilot study, to ascertain the most 'mentally demanding' and 'difficult' tasks from a battery of eleven tasks. From that, the five tasks rated as both the most 'demanding' and 'difficult' were used in this study. The study design was similar to that of the previous study (Kennedy et al., 2010), where NIRS data was captured throughout the session, a 40-minute absorption period was employed and participants completed four repetitions of the demand battery following treatment. The results showed that supplementation with resveratrol alone failed to modulate CBF. However, when co-supplemented with piperine, both total-Hb and oxy-Hb increased during most epochs. The authors suggest that piperine likely amplifies the capacity of resveratrol to modulate CBF. Yet, despite this piperine-mediated enhancement in CBF, the authors did not observe any significant effect on cognitive outcomes or self-reported ratings of mood. The research team

suggest that taking these results together with the previous study, it appears that acute increases in CBF are not sufficient to alter cognitive function in young, healthy adults without cognitive deficits. They hypothesised that longer-term daily supplementation may increase plasma bioavailability levels and therefore may enhance CBF and cognitive performance effects.

Consequently, a following study investigated the effects of 4-week supplementation of 500 mg resveratrol in 60 young, healthy adults (18-30 years) (Wightman, Haskell-Ramsay, Reay, et al., 2015). This study measured cognitive performance on Day 1 and Day 28 of supplementation, alongside employing NIRS to measure CBF and transcranial doppler (TCD) to measure cerebral blood velocity (CBV). On each of the two testing visits, participants completed self-report mood questionnaires, followed by a 5-minute baseline reading of TCD. The NIRS headband was fitted, participants completed two repetitions of the cognitive battery and then consumed the treatment. This was followed by a 40-minute absorption period. They then completed 4 further repetitions of the cognitive battery (equalling to 36 minutes of continuous performance). A subsample of participants also provided blood samples (n=15) or blood pressure readings (n= 26) pre- and post-treatment administration, on both visits. The acute blood flow results were consistent with previous work, showing total-Hb and oxy-Hb were significantly higher following resveratrol, across several time-points in the absorption and post-dose task period. However, no significant differences were observed on Day 28 for CBF or on either day for TCD CBV. In terms of cognitive results, acute and chronic supplementation with resveratrol resulted in reduced number of errors on the Serial 7 subtraction task and the Serial 17 subtraction task, respectively. However, chronic resveratrol supplementation also resulted in reduced correct responses of Serial 17s. In addition, supplementation with resveratrol improved performance on the 3-back task following 28 days of supplementation. Taken together these findings indicate that acute supplementation with 500 mg resveratrol can modulate CBF however, prolonged supplementation does not result in any clear improvements in cognitive function. Despite this, using the bioavailability data, they were able to demonstrate a novel finding; that chronic resveratrol administration resulted in accumulating levels of resveratrol metabolites, as measured in plasma. This suggests that, even when plasma levels of resveratrol are sufficient to induce mechanisms like CBF upregulation, which would be anticipated to improve cognitive function, this may not be possible in a sample of young, healthy adults, who are likely at the peak of their cognitive abilities (Rönnlund, Nyberg, Bäckman, & Nilsson, 2005).

Based on the previous results of increased levels of oxygen extraction (shown by increased deoxy-Hb concentrations), it was hypothesised that resveratrol supplementation may also

influence substrate oxidation and cerebral metabolic rate during cognitive performance. Therefore, additional work by the same research group aimed to examine changes in metabolic rate during high cognitive demand following acute supplementation with resveratrol (Eschle, Goodall, Kennedy, & Wightman, 2020). This study employed indirect calorimetry (ICa) to assess changes in metabolic rate in 27 young, healthy adults (mean age= 22 years) following supplementation with 250 mg and 500 mg resveratrol. Participants completed a baseline ICa measurement and cognitive battery, consumed treatment and, following a 45-minute absorption period, completed the post-dose cognitive battery a further three times, with continuous ICa measurement throughout. The results showed that both doses resulted in significant increases in respiratory exchange ratio (RER), with the 500 mg dose demonstrating consistently higher RER across all tasks and repetitions. Again, however no effects of resveratrol were observed on cognitive performance or mood. The authors indicate that a higher RER is indicative of carbohydrate being the predominant fuel source. They suggest that these findings, indicating an increased excretion of carbon dioxide, may provide a proxy for increased exertion during cognitive demand. However, without concurrent improvements in cognitive performance, it is difficult to determine if the increase in RER has been beneficial. The authors propose that the lack of energy expenditure modulation could suggest that, as with cognitive performance in previous work, the young, healthy sample employed are unlikely to benefit from resveratrol supplementation.

Consequent work aimed to investigate resveratrol supplementation on CBF and cognitive function in an experimental model of human ageing (Eschle, 2017). It is proposed that the observable reductions in CBF associated with ageing (Aanerud et al., 2012), may explain detriments in cognitive performance (Duschek & Schandry, 2007). As the brain is particularly susceptible to reductions in O_2 supply (Larson, Drew, Folkow, Milton, & Park, 2014), it was proposed that disrupting O_2 availability (via an experimental hypoxia model) in young, healthy samples may mimic the cognitive detriments associated with ageing. As such, resveratrol administration may be more beneficial in this compromised sample. In this study, comprising 24 young, healthy adults (18-34 years old), researchers utilised an environmental chamber to create a hypoxic condition (imitating 2134 m above sea level) and a normoxic condition (imitating sea level). Participants received 500 mg resveratrol and completed three repetitions of a cognitively demanding battery, with CBF measured throughout the session. When considering the results of this study, the authors report these in two ways. Firstly, the effects of hypoxia on cognition and CBF and, secondly, the efficacy of resveratrol in increasing CBF and cognitive performance induced by hypoxia. Their results indicate that the hypoxia condition resulted in significantly lower oxy-Hb and higher deoxy-Hb concentrations; indicating that hypoxia was capable of reducing cerebral oxygenation and potentially signifying an

increase in O_2 extraction. However, the hypoxic condition failed to induce any significant reductions in cognitive performance. The authors suggest that these results are indicative of the hypoxic level used within this study being insufficient to disrupt the neurocognitive efficacy of the young, healthy cohort. When considering the resveratrol-mediated differences, the 500 mg dose resulted in significantly higher deoxy-Hb concentrations during the absorption period; indicating an amplification in the hypoxia-induced increases in O_2 utilisation. However, no significant modulation of CBF was observed during post dose task performance during hypoxia. In addition, this is the first study to tentatively demonstrate enhancements in cognitive performance following acute resveratrol administration in young, healthy adults, as the results indicate that resveratrol supplementation resulted in a significantly reduced number of errors on the Serial 3 and Serial 7 subtraction tasks. Despite these apparent improvements in cognitive performance and in contrast to previous findings, no resveratrol-mediated changes in CBF were observed. As such, the authors report the mechanism behind this improvement is unclear, with a potential explanation being due to anxiolytic effects of resveratrol. To summarise, they confirmed that the hypoxic condition employed was capable of reducing cerebral oxygenation however, it did not induce significant reductions in cognitive performance to mimic that of an ageing brain. They proposed that a higher level of hypoxia may be required to induce the cognitive deficits observed in ageing.

Therefore, a follow up study by the same research group aimed to build upon these findings by employing a more severe level of hypoxia (imitating 4000 m above sea level) to model age-related cognitive impairments (Eschle, 2017). Using the same study methodological design as the previous (however with two supplementary cognitive tasks), this study recruited 24 young, healthy participants (aged 19-33 years old). Results indicated that the hypoxic condition significantly increased CBF in the prefrontal cortex, as indicated by significantly higher concentrations of total-Hb and oxy-Hb across the absorption and post-dose task period. They also observed significant decrements in cognitive performance. Here, reductions in the number of correct and incorrect responses in the Serial 3 subtraction task, and a reduction in correctly identified words on the delayed word recall task, were observed in response to hypoxia. Additionally, overall accuracy was found to significantly decrease across task performance, which the authors report provides firm evidence of the overall success of hypoxia as an experimental model of the cognitive ageing process. In terms of the treatment effects, no clear effects on cognitive performance were observed. However, within the normoxic condition, consistent with earlier work, resveratrol supplementation resulted in increased total-Hb and oxy-Hb concentrations in comparison with placebo, across the post-dose task period. But, resveratrol was unable to modulate CBF during hypoxia; suggesting that the adaptive CBF reflex to hypoxia may have masked any potential CBF benefit of resveratrol. As hypoxia

naturally induces increases in CBF, which cannot be increased further by resveratrol, this has hindered the exact mechanism by which resveratrol is hypothesised to improve cognitive performance. Additionally, the authors suggest it could be argued that assessing the ability of resveratrol to increase CBF further in an already augmented CBF response, is not representative of the ageing population that the model was intended to mimic. As such, it is necessary for future research to directly target ageing cohorts, or those otherwise compromised.

Despite the previous studies not observing any clear cognitive benefits following resveratrol supplementation, it has been argued that this lack of cognitive findings may be due to potentially underpowered studies. The above studies were conducted with relatively small sample sizes, but these were calculated adequately based on primary outcome measures and specifically the number of repetitions within the cognitive battery. However, with such variability in human response to polyphenol intervention, it could be argued that whilst these sample sizes may be adequate to detect changes in CBF, they appear to be underpowered when aiming to detect cognitive changes in a young, healthy population. As such, a recent review paper aimed to overcome this by combining 6 of these aforementioned placebo-controlled studies in healthy samples; equating to 166 participants aged 18-35 years old (Wightman, Eschle, & Kennedy, 2019). Each of these studies were crossover in design, used the same cognitive tasks and participant demographics and post-dose testing timeframe were consistent. As an aside, the deliberate consistency in trial methodology across these resveratrol intervention studies demonstrates how relatively clear an overall picture of a research area can be when combining their findings.

Returning to the findings of Wightman et al. (2019), their results confirm that in young, healthy adults, acute supplementation of 500 mg resveratrol is not capable of producing cognitive improvements. The authors in fact suggest that the few cognitive effects observed in three of the six studies are more likely to be due to type 1 errors, rather than being underpowered. This conclusion is consistent with another recent systematic review of ten resveratrol supplementation (acute and chronic & co-supplementation) studies in adults who concluded that, to date, the data does not support the use of resveratrol supplementation in improving cognitive performance (Marx et al., 2018). Although some studies have been shown to enhance elements of cognitive performance, there is very limited consistency between studies. Taken together, this further suggests that resveratrol is likely to have limited or no beneficial effect in young, healthy individuals with high cognitive ability. It’s suggested that resveratrol may have the greatest beneficial effect in compromised individuals including older participants, those with oestrogen depletion or pathology-related deficits (such as obesity or

metabolic syndrome). In these cohorts, the biological abilities of resveratrol, such as anti-inflammatory and antioxidant effects, may provide more convincing mechanisms in improving cognitive function.

Therefore, supplementary work by the same research team aimed to measure 500 mg resveratrol mediated CBF and cognitive effects in twenty-four healthy older adults (aged 50-69 years) (Eschle, 2017). In contrast to previous findings no significant effects were observed on any of the NIRS outcome measures. In addition, the authors found some treatment related cognitive performance effects. Here, resveratrol supplementation led to a less accurate and slower performance on the Rapid Visual Information Processing (RVIP) task, no other effects were observed. A potential explanation for the lack of CBF findings in this study is not only the difference in demographic variables when compared to previous research, but also a difference in neuroimaging equipment. Where previous work used continuous wave NIRS systems, the present study employed a frequency domain NIRS system which provides quantitative values of haemoglobin within the prefrontal cortex. However, without observing any effect on CBF, the authors suggest that the cognitive findings may indicate that a single dose of resveratrol may be detrimental in older adults.

There are few additional studies that have considered the effect of resveratrol in older adults. One investigated the cognitive-beneficial effects of 90-day supplementation of a high (1000 mg) and low (300 mg) resveratrol dose in sedentary, overweight older adults (aged 65-93 years) (Anton et al., 2018). The results showed that 1000 mg supplementation resulted in improved psychomotor processing speed, as measured in the Trail Making Task. However, no significant effects were observed on any other cognitive tasks. Therefore, the findings of this pilot study suggests that chronic, high dose resveratrol supplementation has the potential to enhance selective aspects of cognitive functioning in overweight, older adults.

Two additional studies have investigated resveratrol when co-supplemented with additional polyphenols. The first of which assessed the effect of 26 weeks co-supplementation of resveratrol (200 mg) and quercetin (320 mg) in 46 overweight, older adults (aged 50-80 years) (Witte, Kerti, Margulies, & Flöel, 2014). Their results showed a significant increase in delayed retention of words, following resveratrol supplementation, compared with placebo. Alongside cognition, they also used neuroimaging to assess changes in the hippocampus. Results showed that resveratrol supplementation significantly increased functional connectivity of the hippocampus, which they suggest may reflect improvements in the integrity and functionality of the hippocampus. They also observed an improvement in glucose metabolism, which they suggest may be a potential underlying mechanism.

A second study aimed to investigate the acute effects of resveratrol-enriched red wine (200 mg resveratrol) in 16 older adults (65-78 years) (Scholey, Benson, Stough, & Stockley, 2014). Participants completed a cognitively demanding battery of tasks continuously for 1 hour. Their findings showed that when compared with red wine alone, resveratrol-enriched red wine resulted in a significant improvement in Serial 7 task performance. However, Serial 3 task performance was improved in the red wine alone condition. It should be noted that these findings must be interpreted with caution, as no inert control condition was employed. It may be worth noting that two of these aforementioned cohorts were overweight older adults; whereas Eschle (2017) employed healthy weight participants. Additional differences in study design including chronic supplementation, and co-supplementation with additional polyphenols, which are also potential explanations for the differences in cognitive findings. As such, it could be argued that resveratrol supplementation may be most effective when presented over a sustained period in those with declinations in cognitive functioning due to ageing but also those pathologically compromised by obesity.

Based on these findings it seems plausible to suggest that resveratrol supplementation may be most beneficial in cohorts with compromised cognitive ability (as a consequence of ageing). As such, it may be more likely that we observe benefits in other compromised cohorts, such as those with diabetes or obesity. Indeed, in a study in 36 older (aged 40-80 years) adults with type 2 diabetes (Wong, Raederstorff, & Howe, 2016), participants were supplemented with either 75 mg, 150 mg or 300 mg resveratrol. A TCD was used to measure blood flow velocity (BFV) and the cognitive battery consisted of dual- and multi-tasking tests that required attention on multiple tasks simultaneously. The results indicated that the 75 mg dose of resveratrol elicited a 35% greater increase in BFV during the cognitive task performance. However, no significant changes in cognitive test performance or overall cognitive performance were observed. They did however observe a trend towards an improvement on the dual-tasking test following 75 mg and 300 mg of resveratrol. They suggest that this potential cognitive benefit warrants future research in a chronic supplementation trial which uses complex cognitive tasks. Additionally, they did not find any correlation between plasma resveratrol concentrations and overall cognitive performance. One limitation of this study paradigm is the limited use of cognitive tasks (just 3) which assessed limited cognitive domains and were not representative of global cognitive function. They suggest that regular supplementation of resveratrol would enhance cerebrovascular function which may in turn improve cognitive function in type 2 diabetes patients.

When considering other compromised populations, evidence has indicated that resveratrol supplementation might also be beneficial in obese populations. A crossover-study investigated the effect of chronic (6 week) supplementation with 75 mg resveratrol on FMD and cognitive performance in twenty-eight obese, healthy adults (Wong et al., 2013). Following an initial assessment at weeks 6 and 12 (where participants had consumed their last supplement 18 hours previously), participants consumed a single additional dose of the supplement and were re-assessed 1 hour later. Their results indicated that chronic resveratrol supplementation resulted in a 23% increase in FMD and, in addition, administration of a single dose following chronic supplementation resulted in a 35% greater acute FMD response. Despite this, no effects were observed on cognitive performance, however it should be noted that only the Stroop task was employed in the paradigm. Therefore, any improvement in other cognitive domains was not measured.

Arguably, one of the areas of compromise which has received the greatest attention is the effect of resveratrol supplementation in post-menopausal women. Considerable evidence indicates that the loss of oestrogen following menopause may lead to accelerated deficits in brain function and reductions in peripheral and cerebral blood flow (Evans, Howe, & Wong, 2016; Genazzani, Pluchino, Luisi, & Luisi, 2007). Oestrogen likely provides a protective cognitive effect by modulating NO-mediated vasodilation, through oestrogen binding to oestrogen receptors (ER) and activating eNOS. This likely translates to protecting brain function as ER-β are abundantly expressed in the hippocampus and ER-α in the basal forebrain, which are associated with learning, memory, information processing and attention, respectively (Genazzani et al., 2007). As such, oestrogen reduction following menopause may promote cognitive decline, particularly memory and executive functioning (Genazzani et al., 2005). It has been suggested that resveratrol may act similarly to oestrogen and provide neurovascular protection by regulating eNOS activity and enhancing NO bioavailability (Li et al., 2012). Additionally, as resveratrol is structurally similar and mimics the activity of oestradiol, it has been suggested that it may enhance cognitive performance by stimulating ERs (Bowers, Tyulmenkov, Jernigan, & Klinge, 2000).

Based on this rationale, several studies have assessed the potential cognitive enhancing effects of resveratrol in post-menopausal women. The first of these assessed the effects of 14 week supplementation with 150 mg resveratrol in 80 healthy, post-menopausal women (mean age 61 years) (Evans, Howe, & Wong, 2017). Participants were fitted with a TCD ultrasound head piece, which assessed basal cerebral haemodynamics and CVR in the middle cerebral artery (MCA) during both cognitive and hypercapnic stimuli. Cognitive performance was assessed using a neuropsychological test battery which consisted of four tests targeting

semantic-, verbal- and visual spatial working memory and executive function. The tasks were selected as they reflect the ability to perform everyday tasks, and a decline indicated a loss of independence in the participants. The results indicated that, following resveratrol supplementation, participants performed better in all the individual tasks. Significant improvements were observed in overall cognitive performance and the semantic and verbal memory cognitive domains. However, post-hoc analysis indicated that age, years of education and depressive symptoms score (as measured by the Centre for Epidemiologic Studies Depression scale (CES-D)) all interacted with cognitive performance and so these variables were used as covariates in the analysis. As such, following controlling for depressive symptoms, only the verbal memory and overall cognitive performance domain remained significantly improved by resveratrol. They further observed that anxiety (as measured by the Profile of Mood States (POMS) questionnaire) was significantly reduced by resveratrol supplementation, which may tentatively suggest improvements in quality of life. In terms of CBV findings, they showed that resveratrol significantly improved cerebrovascular responsiveness (CVR) during both hypercapnic provocation and neuronal activity, which suggests resveratrol can modulate CBF. They conclude by noting the potential for resveratrol to be used as a strategy to improve cognitive functioning, specifically memory function, in post-menopausal women. However, this study did not collect blood samples to assess biomarkers which might reflect potential mechanisms of action of resveratrol. Specifically, the ability of resveratrol to up-regulate anti-inflammatory cytokines have been linked to accelerated memory decline (Wang et al., 2015). As such, follow up work was conducted to determine these findings and included additional clinical outcomes including quality of life and everyday functioning, when supplemented over a longer period of time.

With the aim of assessing if these cognitive and cerebrovascular improvements could be sustained over a longer time period, the same research team followed this up with a larger, chronic study. They assessed the effects of 150 mg Veri-te™ resveratrol supplementation in 146 post-menopausal women (average age 64 years) over a 24 month crossover trial, with results also presented at the interim 12 month point (Zaw, Howe, & Wong, 2020b). In a similar design to the previous work, cerebrovascular function was assessed with TCD. The cognitive test battery consisted of ten cognitive tests tailored to measure cognitive domains including processing speed, working-, episodic- and verbal- memory. The cognitive battery was completed at baseline and at 12 months. In addition, overnight fasted blood samples were collected to measure fasting serum glucose, insulin, lipids (total cholesterol, triglycerides, HDL and LDL-cholesterol) and hs-CRP. Findings following 12 months supplementation indicated that resveratrol supplementation resulted in improved performance on the pattern comparison speed test, however no other significant changes were observed on individual tasks. When

considering cognitive domains, both processing speed and cognitive flexibility were significantly improved following resveratrol, which resulted in a small increase in overall cognitive performance. They further observed that resveratrol significantly improved basal blood flow velocity, which may underpin the cognitive enhancement. However, no significant differences were observed on any cardiometabolic biomarkers or blood pressure. These findings confirmed those of the previous pilot study and demonstrated a sustained benefit of resveratrol supplementation on cognitive performance.

Following the analysis at the end of the study, the authors report that their findings confirmed those interim findings, specifically the sustained benefit of resveratrol supplementation on cognitive performance and cerebrovascular function (Zaw, Howe, & Wong, 2020a). Analysis of the cognitive task data indicated that overall cognitive performance was significantly improved by resveratrol, with small (but not significant) improvements observed in each of the cognitive domains. The authors acknowledge that although the effect size is small, it is likely to be clinically important in this cohort, as it may delay the progression of cognitive impairment. These improvements in cognitive functioning were observed concurrently with increases in several cerebrovascular functioning outcomes, suggesting a correlation between these two factors. However, one still cannot establish if CBF is the single mechanism underpinning said cognitive benefits, although it is tempting to do so when this is the mechanism that the study has measured. In addition, the subgroup analysis indicated that resveratrol supplementation was more beneficial in women aged 65+, in terms of improved verbal memory; which also correlated with improvements in overall neurovascular coupling. Further, the biomarker analysis indicated that resveratrol supplementation reduced fasting insulin and improved insulin sensitivity. This study has numerous strengths, particularly in the sense that it was the first clinical trial to examine the long-term effects of resveratrol supplementation in post-menopausal women, with a relatively low drop-out rate (14%). Nevertheless, there are some limitations that must be acknowledged; namely the crossover design utilised, as no washout period was employed between treatment periods. As previous work has evidenced an accumulation of resveratrol metabolites following supplementation of just 1 month (Wightman, Haskell-Ramsay, Reay, et al., 2015), it could be argued that this design is more prone to carry-over effects. Particularly as the pharmacokinetic profile following such a long chronic supplementation period is not known and, therefore, creates the potential for resveratrol to continue exerting effects into the start of the placebo trial phase for those participants with this treatment order. However, as this study did not reassess baseline performance at the 12-month point, it seems unlikely that any crossover effects would have impacted upon performance at the 24-month point.

1.4.8. Summary of behavioural effects

In summary, the research to date indicates that, whilst resveratrol supplementation may have the ability to modulate CBF, these effects do not seem to enhance cognitive performance in young, healthy adults. Promising research indicates that resveratrol may be more beneficial when supplemented in compromised groups, with potential detriments in cognitive function due to ageing or pathologies such as obesity, as indicated within Table 1.3. It may also be more beneficial when administrated over a chronic, sustained period of time, rather than an acute single dose or short chronic regiments.

In addition, a recent paradigm shift in the nutrition research field has begun to consider the potential interactions between polyphenols and resveratrol on the gut microbiota; specifically, how modulation of microbiota may impact upon health and brain function via the gut-brain axis. This will be detailed further in Chapter 3.

Table 1.3. Summary of studies investigating the effects of resveratrol on cognitive performance and cerebral blood flow in humans. Including methodological limitations that are aimed to be addressed within the studies that comprise this thesis.

Reference	Demographic	n	Dosage	Duration	Methodology	Key findings	Methodological Limitations
Kennedy et al. (2010)	Healthy, young adults	22	250 and 500 mg resveratrol	Acute – 45 min PD	CW NIRS measured during cog tasks Cog battery 3x CDB	↑ Total haemoglobin ↑ Deoxy Hb	Healthy, young demographic Acute design, with one post-dose assessment Relatively small sample size Limited to three cognitive tasks
Wightman et al. (2014)	Healthy, young adults	23	250 mg resveratrol with and without 20 mg piperine	Acute – 40 min PD	CW NIRS measured during cog tasks Cog battery – SS7, 13 and 17s, RVIP, n-back	↑ Total Hb (resv & piperine) ↑ Oxy Hb (resv & piperine)	Healthy, young demographic Acute design, with one post-dose assessment Relatively small sample size Quite limited cognitive battery
Wightman, Haskell-Ramsay, Reay, et al. (2015)	Healthy, young adults	60	500 mg resveratrol	4 week	CW NIRS measured during cog tasks TCD resting Cognitive tasks as in Wightman et al. (2014)	↑ 3-back task accuracy ↓ SS7 and SS17 incorrect ↓ SS17 Correct ↑ Total Hb (acute) ↑ Oxy Hb (acute)	Healthy, young demographic Relatively short chronic intervention period CW NIRS better suited to acute study designs Quite limited cognitive battery
Eschle et al. (2020)	Healthy, young adults	27	250 and 500 mg resveratrol	Acute – 45 min, 2- and 3- hour PD	Indirect calorimetry Cognitive task battery – SS3, 7 and 17s.	↑ Respiratory exchange ratio	Healthy, young demographic Acute design only Relatively small sample size Limited to three cognitive tasks
Eschle (2017)	Healthy, young adults	24	500 mg resveratrol	Acute – 45 min PD	Environmental chamber hypoxic condition (2134m above sea level) Cog battery – 3 x CDB CW NIRS measured during cog tasks	↑ Deoxy Hb (rest) ↓ SS3 and SS7 errors	Healthy, young demographic with hypoxia to mimic ageing Acute design, with one post-dose assessment Relatively small sample size Limited to three cognitive tasks
Eschle (2017)	Healthy, young adults	24	500 mg resveratrol	Acute – 45 min PD	Environmental chamber hypoxic condition (4000m above sea level) Cog battery – 3 x CDB & CRT and Stroop	↑ Total Hb (normoxic) ↑ Oxy Hb (normoxic)	Healthy, young demographic with hypoxia to mimic ageing Acute design, with one post-dose assessment Relatively small sample size

					CW NIRS measured during cog tasks		Quite limited cognitive battery
Eschle (2017)	Healthy, older adults	24	500 mg resveratrol	Acute – 45 min PD	Cog battery – 3 x CDB & CRT and Stroop QNIRS measured during cog tasks	↓ RVIP Accuracy ↑ RVIP RT	Acute design, with one post-dose assessment Relatively small sample size Quite limited cognitive battery
Witte et al. (2014)	Overweight, older adults	46	200 mg resveratrol with 320 mg quercetin	26 weeks	Auditory Verbal Learning Test MRI measurement	↑ Delayed word retention ↑ Functional connectivity of the hippocampus	Resveratrol co-supplemented with quercetin Limited to one cognitive task assessing memory performance
Scholey et al. (2014)	Older adults	16	Resveratrol -enriched red wine (200 mg resv)	Acute – 60 min PD	1 hour cognitive battery – 6 x CDB	↑ SS7 accuracy ↓ SS3 accuracy (better in red wine only condition)	Acute design, with one post-dose assessment Relatively small sample size Limited to three cognitive tasks No inert control used (red wine alone condition)
Wong et al. (2016)	Older adults with type 2 diabetes	36	75, 150 and 300 mg resveratrol	Acute – 75 min PD	Multitasking paradigm Dual tasking battery TCD measure of BFV during task performance	↑ Neurovascular coupling capacity (75 mg) ↑ Multi-tasking performance (75 and 300 mg)	Acute design, with one post-dose assessment Relatively small sample size
Wong et al. (2013)	Healthy, obese adults	28	75 mg resveratrol	6 week	FMD Cognitive performance – Stroop task only	↑ 23% increase in FMD (chronic) ↑ 35% increase in FMD (chronic + acute dose)	Relatively small sample size Limited to just one cognitive task
Evans et al. (2017)	Post-menopausal women	80	150 mg resveratrol	14 week	TCD assessing basal cerebral haemodynamics and CVR in MCA Cognitive battery of 4 individual tasks	↑ All cognitive tasks ↑ Overall cog performance ↑ Verbal memory ↓ POMs Anxiety ↑ Cerebrovascular responsiveness	Limited to a battery of 4 cognitive tasks No blood biomarker assessment
Anton et al. (2018)	Overweight older adults	32	High (1000 mg) and	90 days	Cognitive battery of 6 individual tasks	↓ Psychomotor processing speed (Trail making task)	Relatively small sample size Supplement also included grape polyphenols

			low (300 mg) dose				
Zaw et al. (2020a, 2020b)	Post-menopausal women	146	150 mg resveratrol	12 months	TCD assessing basal cerebral haemodynamics and CVR in MCA Cognitive battery of 10 individual tasks	↑ Pattern comparison speed test ↑ Processing speed and cognitive flexibility ↑ Overall cognitive performance ↑ Cerebrovascular responsiveness	Crossover design but no washout period between treatments No reassessment of baseline performance at 12-month point, despite ageing population

1.5 Rationale and aims of the thesis

The preceding literature illustrates the involvement of polyphenols, focusing on resveratrol, in a number of physiological processes which would be anticipated to modulate cognitive performance. A significant proportion of these RCTs have focussed on the cognitive and cerebral blood flow enhancing effects of resveratrol. However, these are limited by methodological issues such as sample size, sample population and duration of supplementation. Further, it could be argued that they have not considered potentially important variables such as lifestyle factors, gut microbial composition and inflammatory status. The evidence detailed within this literature review highlights that resveratrol supplementation may be largely ineffective in young, healthy populations that are at the peak of their cognitive performance. Whereas, it has been indicated that there is a need to consider resveratrol supplementation in 'compromised' demographics, specifically those who may have compromised cognitive function due to obesity and/or inflammatory status. To date, few studies on prolonged resveratrol supplementation exist within this population. In particular, little data exists in this population on the effects of resveratrol supplementation on cognitive function, cerebral blood flow, inflammation and gut microbiota and how these factors may be interlinked and have an underlying effect on the efficiency of supplementation.
As such, the primary purpose of this thesis is to investigate the effects of chronic resveratrol supplementation and the interrelations between inflammatory levels, cerebral blood flow, cognitive function and gut microbiota in adults of varying weight ranges.

Overall, this thesis intends to address the following aims:

1. Investigate the effects of resveratrol supplementation on cognition, subjective mood and inflammation in healthy adults of varying weight ranges (healthy weight, overweight and obese) (Chapter 2)
2. Investigate the effects of resveratrol supplementation on cognition, cerebral blood flow, subjective mood, inflammation and gut microbiota in overweight and obese adults (Chapter 4)

The experimental studies included within this thesis will include the first investigations into the simultaneous assessment of resveratrol on cognitive performance, cerebral blood flow, systemic inflammation and gut microbiota. Additionally, these effects will be assessed in populations of overweight and obese adults which is novel to the research area; as well as to the area of nutritional supplementation in general.

CHAPTER 2

THE ACUTE AND CHRONIC EFFECTS OF RESVERATROL SUPPLEMENTATION ON INFLAMMATION, COGNITIVE PERFORMANCE AND SUBJECTIVE MOOD IN HEALTHY WEIGHT, OVERWEIGHT AND OBESE ADULTS

2.1 Introduction

Reference to previous trials investigating the potentially cognitive enhancing effects of resveratrol supplementation reveals that many have employed a participant demographic of young, healthy adults, typically university students, who are readily accessible and arguably of above-average cognitive ability (Eschle, 2017; Kennedy et al., 2010; Wightman, Haskell-Ramsay, Reay, et al., 2015; Wightman et al., 2014). Despite consistent improvements in cerebral blood flow, studies in this demographic have failed to observe clear beneficial effects on cognitive performance.

Whilst previously it has been argued that under-powered studies likely explain this lack of findings, a recent review discounts this and concludes that acute resveratrol supplementation (500 mg) is not capable of producing cognitive improvements in this demographic group (Wightman et al., 2019). Indeed, it has been suggested that those within this demographic, at the peak of their cognitive ability (Rönnlund et al., 2005), are unlikely to benefit from resveratrol supplementation; whereas it may have the greatest beneficial ability in older participants who have some element of cognitive compromise, either through age or disease.

In support of this, the limited data from older and overweight populations appears to produce more positive findings for resveratrol supplementation when administered alone (Anton et al., 2018) and in combination with quercetin (Witte et al., 2014). It must however be noted that these studies employed a chronic design; whereas acute studies within this demographic have also shown no beneficial effects (Eschle, 2017). Nevertheless, it seems plausible that resveratrol supplementation may be most effective when supplemented in an older cohort of participants or those who are likely cognitively compromised due to obesity status and relatedly, inflammatory status.

It could be argued that inflammatory status, irrespective of obesity status and age, may negatively impact cognitive performance and accelerate the natural age-related decrements in cognition; which is suggested to deplete in a linear fashion, beginning in early adulthood (Salthouse, 2009, 2019). As such, interventions targeting reducing inflammation in younger adults may have a beneficial effect on cognitive performance and slowing cognitive decline.

A Western diet, classified by being high in saturated fatty acids, sugar and protein (Tengeler, Kozicz, & Kiliaan, 2018), has become the dominant dietary pattern in the modern Westernised world. Which, combined with an increasingly sedentary lifestyle, has been continuously associated with detriments to overall health. This includes an increased risk of developing cardiometabolic diseases such as obesity, type 2 diabetes and cardiovascular disease and consequently high blood triglycerides, altered cholesterol levels, glucose intolerance and hypertension (Chaplin et al., 2018; Telle-Hansen, Holven, & Ulven, 2018). Of importance here, exposure to a high-fat diet has detrimental effects on brain function including an increase in anxiety- and depression-like behaviour, alongside impairments in cognition in young mice (Gainey et al., 2016; Jørgensen et al., 2014). Whilst the underlying mechanisms here are still unclear, a growing body of research indicates that inflammation, particularly over a sustained period, is inextricably linked to the aforementioned health complications associated with a high-fat diet. Indeed, research suggests that long-term systemic inflammation contributes to changes in brain morphology, with the hippocampus suggested to be particularly susceptible to the effects of diet. As an example, memory deficits are evident after just 1 week of a high fat and sugar dietary intervention in rats (Beilharz, Maniam, & Morris, 2016). Similarly, low-grade systemic inflammation has been observed as having a role in cognitive and mood dysfunction in animal models (Misiak, Leszek, & Kiejna, 2012; Young, Bruno, & Pomara, 2014).

Whilst a short-term inflammatory response is considered beneficial and has a number of protective roles; including restoring homeostasis, repairing tissue and defending against infection (Medzhitov, 2008), the continuous activation of inflammatory pathways consistently observed in those consuming a high fat diet are not known to have a beneficial physiological purpose. Instead, chronic inflammation has been shown to have detrimental health effects, including the pathogenesis of previously aforementioned metabolic disorders (Cani, Neyrinck, et al., 2007). Of importance here, the activation of an inflammatory response and subsequent production of pro-inflammatory mediators, including cytokines, may communicate with the brain via numerous routes including the blood-brain barrier, the vagus nerve and systemic circulation (de Theije et al., 2011; Perry, 2004). Studies have indicated that brain function is particularly sensitive to inflammatory pathway activation (Pistell et al., 2010) and that prolonged activation may promote neuroinflammation (Serra, Almeida, & Dinis, 2018). This has been observed in patients with neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (Heneka et al., 2015; Hirsch & Hunot, 2009). Neuroinflammation is characterised by chronic activation of microglia and astrocytes, leading to the over production of pro-inflammatory cytokines, reactive oxygen and nitrogen species (ROS/RNS) (Serra et al.,

2018). Evidence suggests that this may promote apoptosis of neurons and glial cells, and increase blood-brain barrier permeability, leading to brain damage (Sochocka et al., 2017).

Considering the widespread negative impact of prolonged inflammation; increasing interest has been placed on developing therapeutic interventions. Whilst the importance of diet on physical health has long been established, growing evidence indicates the significance of an 'anti-inflammatory diet', whereby specific foods exert strong effects on inflammatory pathways in the body (Ricker & Haas, 2017). The anti-inflammatory diet emphasises the importance of consuming large quantities of vegetables and fruit, alongside smaller amounts of plant based protein, fish, lean meat and wholegrains, whilst avoiding pro-inflammatory foods such as processed meats, saturated fats and refined sugars (Casas & Estruch, 2016; Galland, 2010). This dietary pattern has been employed as an approach in the treatment of many metabolic disorders, mood and brain disorders (Georgousopoulou et al., 2016; Sears, 2009; Tolkien, Bradburn, & Murgatroyd, 2019).

Whilst the Western diet is characterised by consumption of pro-inflammatory foods, the Mediterranean diet (MD) has been identified as a dietary pattern which more closely aligns with this anti-inflammatory diet (Esposito et al., 2004). The Mediterranean diet typically involves a high consumption of fruits and vegetables, alongside polyunsaturated fatty acids, nuts and seeds; moderate intake of dairy products and fish; and low intakes of red meat (Chiva-Blanch, Badimon, & Estruch, 2014). Many components of this diet are associated with improved cardiovascular health and cognitive function (Chiva-Blanch et al., 2014; Galbete et al., 2015; Valls-Pedret et al., 2015). Of particular interest, the consumption of foods that are abundant in phenolic phytochemicals (such as fruits and vegetables) are consistently associated with lower incidence of metabolic diseases (Bauer et al., 2013). These also have well documented anti-inflammatory properties (González et al., 2011; Zhang & Tsao, 2016) and have been shown to potentially reduce neuroinflammation and oxidative stress, as well as improve memory, cognitive function and overall health (Anhê et al., 2013; Kennedy, 2014b; Sandhu et al., 2017; Spencer, 2009). Moreover, consumption of polyphenols may also offer a protective role against insults to host health induced by obesity or high fat diets. In support of this theory, consumption of orange juice prevented meal-induced oxidative and inflammatory stress following a high fat, high calorie meal (900 kcal, 51 g fat), in healthy normal-weight adults (Ghanim et al., 2011).

Resveratrol is found in relative abundance in many of the above-mentioned food sources associated with the Mediterranean diet, including fruits and red wine (Chaplin et al., 2018). Its anti-inflammatory properties are well documented and have been shown to inhibit crucial pro-

inflammatory signalling cascades, specifically the NF-κβ, JAK-STAT and AP-1 pathways (Serra et al., 2018), therefore decreasing the expression of pro-inflammatory and pro-oxidant mediators and markers, such as IL-6, IL-1α, IL-1β and TNF-α. Consequently, this has been shown to counteract neuroinflammation in a large number of *in vitro* and *in vivo* models (Renaud & Martinoli, 2014; Spencer et al., 2012) along with ensuing benefits on a number of health parameters. Much of the work in humans has shown the anti-inflammatory effects of resveratrol in those with chronic diseases such as diabetes, ulcerative colitis and cardiovascular disease, by decreasing circulating inflammatory markers including CRP, IL-1β and IL-6 (Kumar & Sharma, 2010; Samsami-Kor et al., 2015; Wahab et al., 2017). Specifically, research shows that six- to twelve- month administration of 350 mg resveratrol per day, decreases the production of pro-inflammatory cytokines IL-6, IL-10 and TNF-α in patients with high cardiovascular risk (Tomé-Carneiro et al., 2012; Tomé-Carneiro, Larrosa, et al., 2013). In similar patients, the serum levels of high sensitivity CRP were also lowered (Militaru et al., 2013) and levels of anti-inflammatory adipokines increased (Tomé-Carneiro, Gonzálvez, et al., 2013).

It is well established that chronic inflammation is a characteristic feature of obesity, where the excess of macronutrients in adipose tissue stimulates the release of proinflammatory mediators (Ellulu, Patimah, Khaza'ai, Rahmat, & Abed, 2017). As such, much of the research investigating resveratrol supplementation on inflammation, and related outcome measures, have utilised obese subjects who are expected to have elevated levels. Indeed, improvements in plasma triglyceride concentration and lower circulating cytokine levels have been observed in participants who are obese, but otherwise healthy (Bo et al., 2013; Timmers et al., 2011). Similarly, the intake of a single grape extract reduces plasma IL-1β levels induced by a high fat and high-carbohydrate meal, 1-, 3- and 5-hours post meal (Ghanim et al., 2011). Further, a meta-analysis of randomised controlled trials indicates that resveratrol treatment reduces the levels of CRP and TNF-α among obese subjects (Haghighatdoost & Hariri, 2018).

However, a recent pilot study in obese men with insulin resistance, found no differences between groups following a 30-day supplementation period of 2 g of resveratrol daily, for a number of end measures, including factors contributing to metabolic syndrome (Walker et al., 2019). Similar inconclusive results have been found in diabetic patients (Thazhath et al., 2015) as well as with smaller doses in obese subjects (Kjær et al., 2017; Poulsen et al., 2013). These disparities in findings are potentially due to vastly differing study designs, including dosages ranging from 10 mg-2 g per day over supplementation periods of 4-16 weeks. It may be that there is an optimal dosage needed to observe beneficial effects in obese patients that this

range of studies are simply yet to fully identify. It has further been suggested that the individual participants' metabolic status and indeed dietary choices, may dictate the effectiveness of the resveratrol treatment (Novelle et al., 2015).

Research to date has perhaps used too blunt of an assumption that only those who are categorised as 'overweight' or 'obese' are likely to have chronic inflammation and related health issues, without considering additional factors including lifestyle and diet, as well as those of differing weight categories. Whilst still the most utilised measurement of body mass in the industry, there are many shortfalls with only using BMI to classify participants by height and weight (Nuttall, 2015). The most well-voiced argument here is that it cannot differentiate between body fat mass and lean body mass; therefore an individual can be categorised as 'obese', with a high BMI, due to high lean body mass and low fat mass (and vice versa in lower BMI categories) (Flegal et al., 2009; Wellens et al., 1996). Additionally, important variables including gender, ethnic group and age are not considered when utilising BMI to categorise individuals (Deurenberg, Yap, & Van Staveren, 1998; Romero-Corral et al., 2008). It is therefore likely that individuals are incorrectly classified, when based on body weight alone. Indeed, when considering individual profiles, it is to be expected that some individuals classified as 'obese' do not have high body fat levels and therefore may not have high levels of inflammation.

In contrast, evidence indicates that some individuals identified as 'normal weight', may be considered normal weight obese (NWO); where they have a BMI of below 25, but have a surprisingly high level of body fat and low muscle mass (De Lorenzo, Martinoli, Vaia, & Di Renzo, 2006; Ruderman, Chisholm, Pi-Sunyer, & Schneider, 1998). Whilst it is clear that not all of these individuals will have metabolic abnormalities, a subset of the NWO group are also classified as metabolically obese, whereby those with increased body fat, not detected by BMI, are at an increased risk for metabolic dysregulation, systemic inflammation and mortality (Ding, Chan, & Magkos, 2016; Oliveros, Somers, Sochor, Goel, & Lopez-Jimenez, 2014). Although there is potentially a role of genetic factors (Oliveros et al., 2014), it is likely that environmental factors are contributing to NWO, including reduced physical activity, poor sleep quality and nutrition habits (Foulis, Hughes, & Friedl, 2020). Due to the inherent flaws when using BMI in isolation, those who are at risk of chronic inflammation and related health conditions, may not be accurately identified. Future work should include additional techniques such as waist-to-hip ratio, which provides a more informative approach to assessing the distribution of fat in the body, where abdominal obesity may be a more accurate indicator of inflammatory risk, irrespective of BMI status (Després, 2012).

As the link between chronic inflammation and cognitive deficits is well documented, it is theorised that supplementation with resveratrol may both reduce inflammation and have the potential to enhance cognitive performance. As detailed previously, research to date has failed to show cognitive enhancing effects of resveratrol. Alongside the small sample size design in these studies, many also targeted young, healthy populations, with no data collected regarding inflammatory levels, and BMI was employed in isolation. Previous work has suggested that resveratrol is most effective in those whose health is comprised, at least in terms of inflammatory effects, therefore it is expected that the greatest effects would be observed in those with chronic inflammation, regardless of BMI classification. As such, individuals with chronic inflammation, and also lacking in protective factors in their diet, are likely to respond most beneficially to resveratrol supplementation, both in terms of reducing inflammation and subsequently improving cognitive performance.

The present study therefore employs a more diverse demographic than previous work, with the inclusion of older participants and a more varied weight range, where we are likely to observe a range of inflammatory statuses. Whilst previous studies have indicated clear and consistent modulation of cerebrovascular parameters, the logistical limitations that this imposes whilst concurrently measuring cognitive performance and cerebral blood flow meant that in order to achieve a large sample size, adequate enough to detect any potential improvements in cognitive function, for this study cerebral blood flow was not measured. In keeping with previous work, this study utilised the dosage of 500 mg resveratrol daily. Despite previous null findings on cognitive performance with this dose, recent meta-analysis work indicates that resveratrol supplementation has most significant effects when presented at >500 mg daily (Asgary et al., 2019). Moreover, here the decision to employ this dosage, was guided by previous resveratrol work within the Brain, Performance and Nutrition Research Centre, notably the trials by Wightman, Kennedy and Eschle outlined in Section 1.4.7, which primarily utilised 500 mg supplementation. A key strength of this consistent methodological approach has meant that data from previous trials have been pooled and analysed as one larger trial with greater statistical power (Wightman et al., 2019). The decision here, within this PhD thesis to continue with 500 mg resveratrol supplementation, allows potential for a similar meta-analysis approach to be performed in the future, combining this work with that of previous trials from the same research centre. Providing more clarity and certainty on the effectiveness of resveratrol supplementation on cognitive performance.

Whilst several previous trials have been limited to acute design with often just one post dose assessment of cognitive performance, the current trial employs a chronic (4 week) design, alongside acute measurements of cognitive performance assessed 40 minutes post dose.

Furthermore, to obtain additional cognitive performance data during the supplementation period, this trial incorporates the novel Cognim^{app} program, which allows participants to complete cognitive paradigms on their own mobile phone, away from the research centre. One key benefit to using Cognim^{app} software here, is that it allows regular cognitive assessments during the supplementation period, without requiring participants to attend full assessment sessions within the research centre and as such is far less arduous and intrusive for the individual during the trial. Practically, both may improve interest, compliance, and retention of participants throughout intervention trials, such as this one, which typically require high face-to-face engagement from participants. Within this trial, participants will complete five short (10 minute) assessments at home, the first prior to their first testing visit (Day -1) and at intervals of 7 days throughout the supplementation period (Days 7, 14, 21 and 28). The chronic design of the trial, alongside the additional interim data will contribute to greater understanding of the effects of chronic resveratrol supplementation on cognitive performance, periodically throughout the supplementation period, rather than just at the end of the supplementation period, as in previous chronic intervention trials (Witte et al., 2014; Wong et al., 2013; Evans et al., 2017; Anton et al., 2018; Zaw et al., 2020a, 2020b).

This study therefore aims to address the following research questions relating to the effects of 500 mg resveratrol supplementation in healthy weight, overweight and obese adults:

1. What are the acute (40-minute post dose) and chronic (28-day supplementation) effects of resveratrol supplementation on cognitive performance. Specifically, here, the effect on the performance on the following cognitive tasks:
    a. Immediate word recall
    b. Corsi blocks
    c. Serial subtraction of threes
    d. Serial subtraction of sevens
    e. Rapid Visual Information Processing
    f. Stroop
    g. Delayed word recall
    h. Delayed word recognition
2. What are the acute (40-minute post dose) and chronic (28-day supplementation) effects of resveratrol supplementation on cognitive performance. Specifically, here, the effect on the performance on the following cognitive domains, with scores calculated using individual task performance:
    a. Episodic Memory
    b. Overall Accuracy

    c. Overall Speed

3. What are the acute (40-minute post dose) and chronic (28-day supplementation) effects of resveratrol supplementation on subjective mood. Additionally, the chronic (following 7-day, 14-day, 21-day and 28-day supplementation) effects on subjective mood, assessed during interim Cognim^{app} assessments. Specifically, here, as assessed with Visual Analogue Mood Scales (VAMS), with the following outcome measures:
    a. Alertness
    b. Stress
    c. Tranquillity
4. What are the chronic (28-day supplementation) effects of resveratrol supplementation on subjective mood, as assessed with Profile of Mood States (POMs).
5. What are the chronic (following 7-, 14-, 21- and 28-day supplementation) effects of resveratrol supplementation on cognitive performance. As measured at home via Cognim^{app} on participants mobile phones, completing the following individual tasks:
    a. Numeric working memory
    b. Choice reaction time
    c. Stroop
    d. Delayed picture recognition
6. What are the chronic (following 7-, 14-, 21- and 28-day supplementation) effects of resveratrol supplementation on cognitive performance. Specifically, here, the effect on the performance on the following cognitive domains, with scores calculated using individual task performance, from interim Cognim^{app} assessments:
    a. Overall Accuracy
    b. Overall Speed
7. What are the acute (70-minute post dose) and chronic (28-day supplementation) effects of resveratrol supplementation on the following blood biomarkers, related to inflammation, cholesterol and resveratrol supplementation:
    a. Total cholesterol
    b. C-Reactive Protein (CRP)
    c. Ferric reducing antioxidant power (FRAP)
    d. Glucose
    e. High-density lipoprotein (HDL)
    f. Interleukin-6 (IL-6)
    g. Low-density lipoprotein (LDL)
    h. Resveratrol-3-O-D-glucoside
    i. Resveratrol

j. Resveratrol-3-O-sulfate
k. Resveratrol-4-O-D-glucoronide
l. Triglycerides

8. What are the acute (70-minute post dose) and chronic (28-day supplementation) effects of resveratrol supplementation on blood pressure and heart rate.
9. What are the chronic (28 day supplementation) effects of resveratrol supplementation on body weight and Body Mass Index (BMI)

Based on the previous literature and above aims, it is hypothesised that acute (40 minutes post dose) and chronic (measured every 7 days throughout a 28-day period) supplementation with 500 mg resveratrol will improve performance on cognitive tasks (with improvements measured as increased accuracy and/or decreased reaction time on individual tasks and cognitive domains), in healthy adults (of a more diverse demographic range than previous trials). Moreover, given resveratrol's ability to interact with numerous biological systems; it is hypothesised that, in comparison to placebo, resveratrol supplementation will have a beneficial impact on host health, namely here by reducing inflammatory biomarkers, modulating cholesterol, blood pressure and participant body weight.

2.2. Materials and Methods

2.2.1. Study design and ethics

This study employed a double-blind, parallel groups, placebo-controlled design, where participants were randomised to one of two treatment groups – placebo or 500 mg Veri-te™ resveratrol for a supplementation period of 28-days. As shown within Figure 2.1. participants were required to initially attend a training and screening visit, followed by two assessment visits on the mornings of Day 1 and Day 28. These assessment visits comprised of provision of a blood sample and completion of a baseline cognitive assessment, consumption of treatment, completion of a second cognitive assessment (40 minutes post dose) and blood sample. With 500 mg resveratrol or placebo consumed daily at home, during the supplementation period. Interim cognitive assessments were also completed on Days -1, 7, 14, 21 and 28 at home, via Cognim^app. Further details of the procedure is detailed within Section 2.2.6, with the timeline of testing visits detailed further in Figure 2.5 within this section.

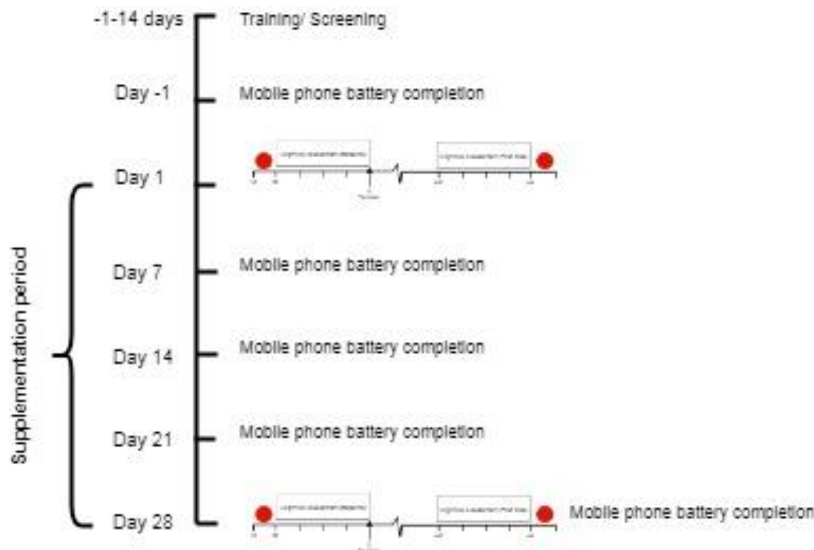

Figure 2.1. Overview of trial procedure. The figure depicts the overview of the trial. With participants assessed on the first (Day 1) and final (Day 28) day of their supplementation period, following a training session conducted prior to the initial, acute session. Cognim^{app} assessments are also completed throughout the supplementation period.

Ethical approval was gained from Northumbria University's Psychology Department (submission reference: 11882) and was conducted according to the Declaration of Helsinki (1964). The study was registered on www.clinicaltrials.gov under the identifier NCT04314739.

2.2.2. Participants

One hundred and nine males and females aged 18-55 were recruited. Of these, one hundred were enrolled and randomised into the study. Participants all lived in or in the surrounding areas of Newcastle upon Tyne and were recruited using various methods, as detailed in section 3.2.2. All participants provided blood samples; subject to physiological allowance. Six participants withdrew from the study following randomisation due to time commitments and the data from a further four participants were removed from analysis: 1 due to medication use during the supplementation period and a further 3 due to low treatment compliance. This resulted in ninety participants completing the study and inclusion in the analysis as planned. Participant disposition through the trial is displayed in Figure 2.2 and demographic data in Table 2.1.

Participants were self-reported as being in good health, which was defined as not meeting any of the following criteria. Participants were excluded from the study if they had a BMI out of the range of 18.5-42 kg/m^2; smoked or used nicotine replacement products; had a pre-existing medical condition or took prescription medications that would contraindicate with the study; had any food allergies or intolerances; had taken antibiotics, prebiotics or probiotics in the

preceding 8 weeks; had Type I or Type II diabetes; had a visual impairment that could not be corrected with glasses (including colour blindness); had any learning difficulties; suffered from frequent migraines (defined as >1 per month); had high blood pressure (defined as systolic >159 mmHg or diastolic >99 mmHg); had a recent history of alcohol or drug abuse; were pregnant, seeking to become pregnant or breastfeeding; had consumed nutritional supplements within the previous 4 weeks; had an excessive daily intake of caffeine (>500 mg per day); had any sleep disturbances or used sleep aid medication. Additionally, due to the inclusion of blood samples, participants must also have not met any of the following criteria: have any known active infections; have or be at high risk of having syphilis, hepatitis or HIV; have had breast cancer and/or a mastectomy; have haemophilia or a similar blood clotting disorder. To achieve equal age split across the cohort, participants were recruited in four age categories: 18-25-, 26-35-, 36-45- and 46–55-year-olds, with 25 participants in each group.

The sample size for this study was calculated based on a small effect size (d = 0.25); an a priori calculation of the size of sample required in order to detect a significant difference between the groups given 70% power and an alpha level of 0.05, is 101 participants. Power calculations were made using GPower 3.1.

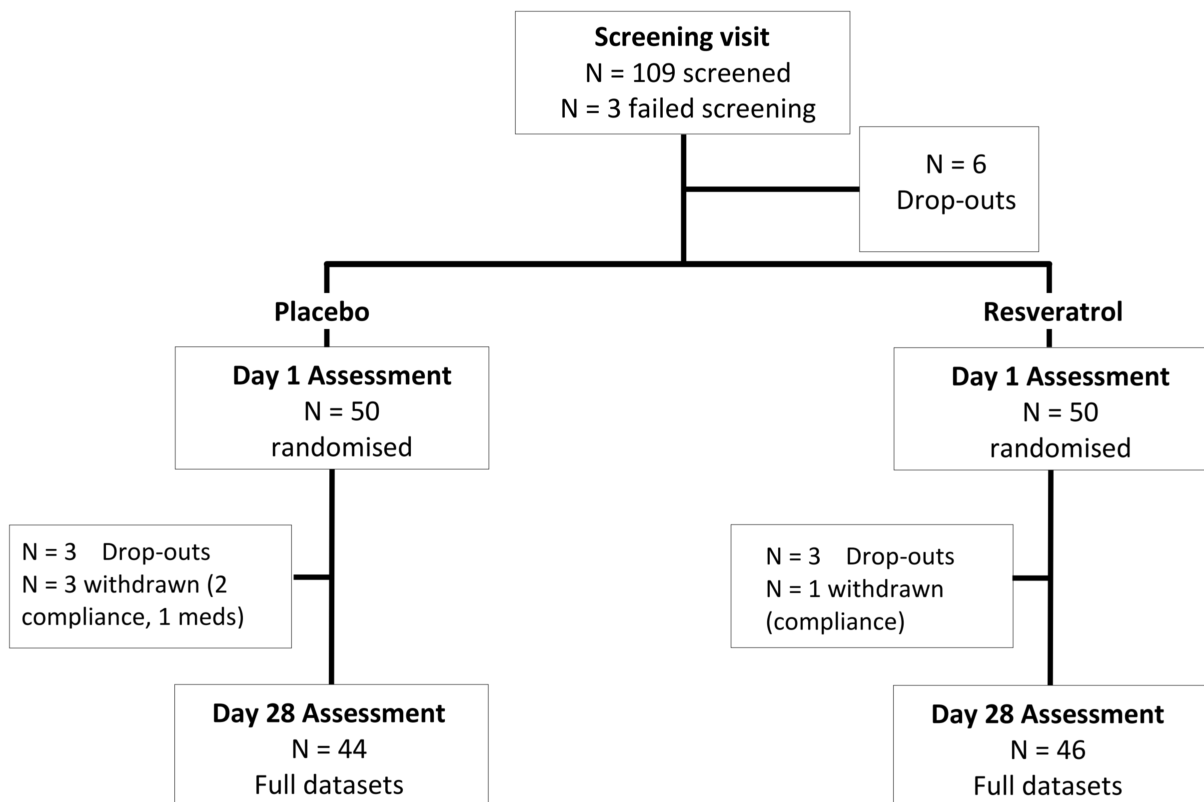

Figure 2.2. Participant disposition through the trial. The figure depicts the disposition of participants throughout the study, culminating in N=90 of the 100 who were randomised.

Table 2.1. Participant demographic information and characteristics. Means and Standard Deviation (SD) are presented where appropriate, with F and p values of the main effects from the one-way ANOVAs conducted on the baseline data by treatment group.

		Baseline		Main effects	
		Mean	SD	*F*	*P*
Age	Placebo	35.60	11.06	.01	.93
	Resveratrol	35.42	11.42		
Sex (Male/Female)	Placebo	11/39	-		
	Resveratrol	13/37	-		
Years in Education	Placebo	17.36	2.71	.00	.92
	Resveratrol	17.31	2.85		
Fruit and Vegetable (portions per day)	Placebo	3.93	1.34	.03	.84
	Resveratrol	3.99	1.70		
Alcohol (Units per day)	Placebo	0.77	0.90	.01	.90
	Resveratrol	0.79	1.00		
Caffeine consumption (mg/day)	Placebo	179.78	107.73	.04	.84
	Resveratrol	184.04	103.10		
Systolic blood pressure (mmHg)	Placebo	119.13	13.18	.19	.66
	Resveratrol	118.04	11.53		
Diastolic blood pressure (mmHg)	Placebo	77.39	9.99	.00	.99
	Resveratrol	77.40	8.70		
Heart Rate (BPM)	Placebo	73.31	10.51	2.09	.15
	Resveratrol	70.20	10.98		
BMI (kg/m^2)	Placebo	25.71	5.08	.74	.39
	Resveratrol	24.98	3.29		
Waist to hip ratio	Placebo	.84	.07	.36	.54
	Resveratrol	.85	.08		

2.2.3. Treatments

Participants were randomly assigned via Latin square into one of two treatment conditions, which each involved the consumption of two capsules daily.

1. 500 mg Veri-te™ resveratrol
2. Placebo (cellulose microcrystalline)

Each resveratrol (Veri-te™) capsule contained 250 mg of >98% pure synthetic trans-resveratrol and placebo capsules comprised cellulose microcrystalline. The Veri-te™ resveratrol capsules were manufactured under current Good Manufacturing Practise (cGMP) and Hazard Analysis and Critical Control Points (HACCP) based food safety conditions. The manufacturer (Evolva SA – Basel, Switzerland) provided the treatments, which were identical in appearance (white vegetarian capsules) to ensure the research team and participants remained blind to the treatment randomisation. The lead researcher reconstituted both treatments into identical white bottles containing 60 capsules in each. To ensure blinding was maintained throughout the trial, a third-party researcher coded the treatments as A and B and created a stratified randomisation schedule. Treatment bottles were labelled with a treatment randomisation number, assigning each participant to an A or B treatment. Treatment bottles were assigned to participants in a sequential order. Upon completion of all data analysis, the lead researcher and principal supervisor were unblinded to allow for interpretation of results.

Participants consumed their first (acute: Day 1) and final (chronic: Day 28 +/- 2 days) treatments in the lab (consuming both capsules approximately 30 minutes into the testing session). During the supplementation period, participants consumed one capsule in the morning and one in the evening, these were advised to be consumed 30 minutes after their breakfast and evening meals, respectively. Previous research has indicated that repeated resveratrol administration increases the half-life from to 1-3 hours from 2-5 hours (Cottart, Nivet-Antoine, Laguillier-Morizot, & Beaudeux, 2010). It has previously been suggested that splitting the resveratrol dose into several smaller doses throughout the day may produce the same effect (Timmers, Hesselink, & Schrauwen, 2013), as well as improve tolerability (Chachay et al., 2011).

Compliance was primarily measured by a count of the returned capsules and a treatment diary (Appendix II) was used as a second compliance measure. A treatment compliance percentage was calculated to measure adherence to the study protocol and adequate consumption of the investigational product. The treatment compliance percentage was calculated by comparing

the number of treatment that were returned by the participant at the end of the study, with the number of treatments that should have been returned. With compliance percentage calculated as the following:

$$\text{Treatment Compliance (\%)} = \frac{\text{Number of treatments returned (not consumed)}}{\substack{\text{Number of treatments that should have been consumed} \\ \text{per protocol} \\ (28 \text{ days x } 2 \text{ capsules} = 56)}} \text{ x } 100$$

Adequate compliance was assessed as >80% and <120% of the required supplementation as per the protocol (28 days supplementation). For this trial, a compliance of <80% would equate to missing >9 capsules during the supplementation period; whereas a value of >100% would be achieved if participants were in the trial >28 days (for example due to rescheduling testing visit 2). Here, if participants did not return any treatment, therefore consuming all 60 given capsules, their compliance would equate to 107%, to achieve a compliance score greater than this participants would need to be provided with additional capsules to those initially provided, for example if all 60 capsules were consumed during the supplementation period at home, an additional 2 capsules would be provided at testing visit 2 to consume within the laboratory.

2.2.4. Physiological measures

2.2.4.1. Computerised cognitive assessments

2.2.4.1.1. Study visits assessments

All cognitive assessments completed during the testing visits were delivered using the Computerised Mental Performance Assessment System (COMPASS; BPNRC, Newcastle upon Tyne, UK). This testing system has been used within a large number of nutritional trials over the previous 12 years, including those investigating resveratrol. The program allows the researcher to create a customised configuration of a set of cognitive tasks, during which fully randomised parallel versions of each task are presented for each assessment and participant.

These tasks were presented on a laptop PC with responses registered via a Cedrus RB-530 five-button response pad, mouse and cursor or the keyboard’s linear number pad. Participants used pen and paper to respond to the immediate and delayed recall of words tasks.

Due to limited evidence of domain specific cognitive effects of resveratrol, the selection of cognitive tasks were chosen to provide a broad assessment across all cognitive domains; including episodic memory, working memory, attention and executive function (Figure 2.2 below details a breakdown of the order of tasks within the configuration and the cognitive domain each task loads upon). Similar selections of tasks have previously been shown to be sensitive to a number of nutritional interventions in work from the Brain, Performance and Nutrition Research Centre (Jackson et al., 2021; Kennedy, Wightman, Khan, Grothe, & Jackson, 2019; Patan et al., 2021; Wightman et al., 2020; Wightman et al., 2018; Wightman et al., 2021). Within each of the cognitive assessments, participants completed one round of the 10-minute battery of the "Cognitive Demand Battery". This computerised battery comprises four components: the serial three subtraction task, serial seven subtraction task, rapid visual information processing (RVIP) task and a "mental fatigue" visual analogue scale. These tasks are detailed below. The Cognitive Demand Battery has previously been used effectively to investigate the effects of many different nutritional interventions, on cognitive function and mental fatigue; including within the studies referenced above which also encompass previous resveratrol studies (Wightman et al., 2019).

The tasks and other components of each assessment are described below in order of completion. The timelines of each assessment and the cognitive domains that individual tasks load upon are shown in Figure 2.3. The cognitive assessment lasted 25 minutes in total and was completed twice during the testing visit, pre-dose and 40 minutes post dose.

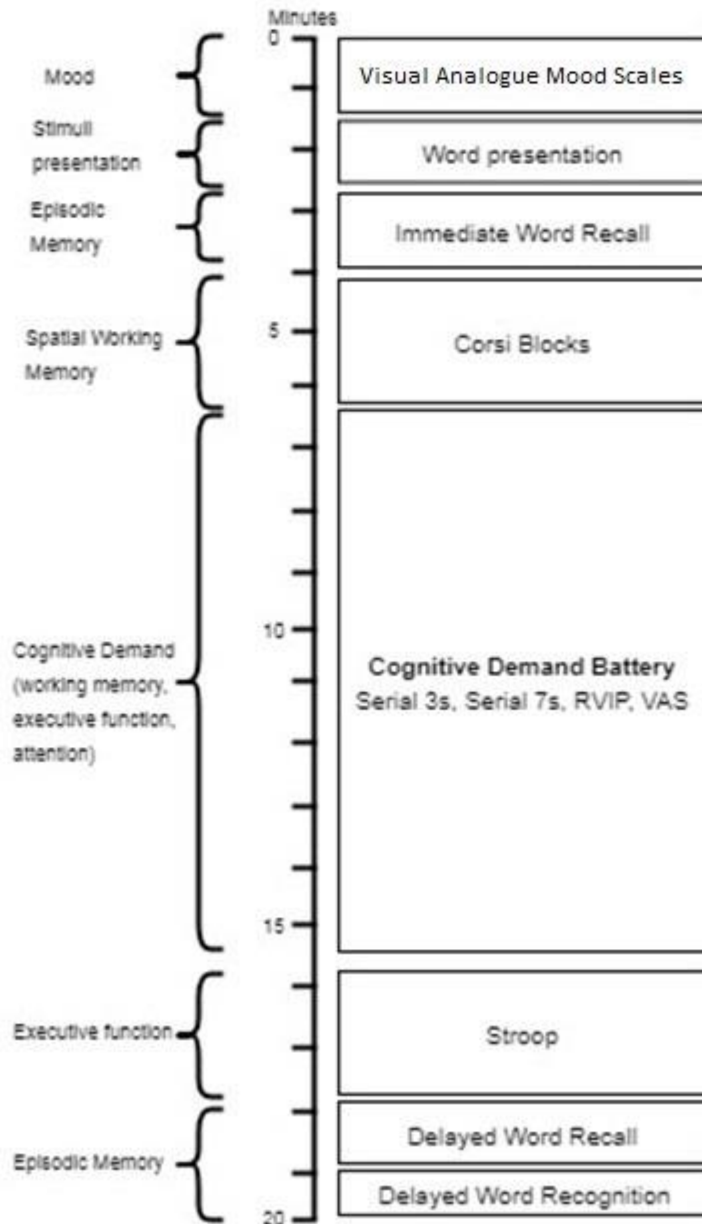

Figure 2.3. COMPASS Cognitive Task Order. The figure depicts the order that cognitive tasks were presented, with approximate completion times. To the left, the Cognitive Domain that the task measures is listed.

2.2.4.1.1.1. Word presentation and Immediate word recall

Fifteen words were presented in the centre of the screen, one at a time, at the rate of 1 per second (with the inter-stimulus duration also lasting 1 second), these words were selected at random from a large bank of words derived from the MRC Psycholinguistic Database (Fearnley, 1997). Words were matched for their frequency, familiarity, word length and concreteness.

Following presentation of the words, the participant was given 60 seconds to write down as many words as they could recall, using the pen and paper provided, the task was scored manually for the number of correct and incorrect responses. Correct words were awarded 1 point each, with half points awarded in the following situations: one letter different; two letters in a word reversed; words with the same stem; for the wrong tense. An error score (1 point) was awarded where a word was not correct and did not fall under any of the half point rules, including any non-sense words. Where the same word was written twice, the second recall was ignored.

Task outcome measures: Number of correct words recalled, number of errors (incorrect words recalled). With higher numbers of correct words recalled and lower numbers of errors, indicating better performance on the task.

2.2.4.1.1.2. Corsi blocks span

In this task, nine identical blue squares appeared on a black screen in non-overlapping positions. A set number of blocks changed colour from blue to red in a randomly generated sequence, during this time the cursor was locked in position. Participants were required to remember this sequence. After the sequence had been presented, participants were instructed to repeat the sequence by clicking on the blocks using the mouse and cursor. This task started at a sequence span of 4, with the task repeated five times at each level of difficulty, the task continued until the participant could no longer correctly recall the sequence. The task continued up to fifteen levels (up to 15 squares in each sequence), as long as participants are making enough correct responses. The task ended when the participant made less than 3 correct responses (out of the five in one level).

Task outcome measures: Span score. This score is calculated by averaging the level of the last 3 correctly completed trials. As an example, here, if the participant correctly responded to all five Level 5 trials, then just one Level 6 trial, their score was calculated as the following:

$$\frac{(5+5+6)}{3} = 5.33$$

With a higher score indicative of better performance on the task.

2.2.4.1.1.3. Serial 3 subtractions

This task lasted 2 minutes in total, to begin participants are presented with a standard instruction screen which informs the participant that they must count backwards in threes as quickly and as accurately as possible. To begin, a random number between 800 and 999 was presented on the screen, participants must use the keyboards linear number keys to enter

their response and then press enter. Once the first response was entered the starting number was cleared from the screen; each of the participants three-digit responses was represented on the screen by three asterisks, which also disappeared once the participant pressed enter – to signal the completion of their response.

On the instruction screen at the start of each task participants are instructed that if they make a mistake they should carry on subtracting from the new incorrect number, with subsequent responses scored as correct in relation to the new number.

Task outcome measures: Number of Total responses, number of Correct responses, number of Errors. With a higher number of correct responses and lower number of errors, indicative of better performance on the task.

2.2.4.1.1.4. Serial 7 subtractions

This task was identical to the Serial 3 subtraction task detailed above, with the exception that participants are required to subtract 7. This task was scored and has the same outcome measures in the same way as Serial 3 subtraction task.

2.2.4.1.1.5. Rapid Visual Information Processing

Participants were required to monitor a continuous series of single digits on the screen, for a period of 5 minutes, to identify strings of three consecutive odd or even numbers. This task lasted a period of 5 minutes in total, during which the numbers 1 to 9 are presented on the screen at the rate of 100 per minute; with eight correct target strings in each minute, presented in a pseudo-random order. The participants were required to respond to the detection of a correct string of numbers, by pressing the centre button (of the four button response pad) as quickly as possible. This task is scored for percentage of target strings correctly detected and the average reaction time (ms) for correct detections.

Task outcome measures: % Overall Accuracy; Reaction time for correct responses (Msecs); Number of False Alarms (Error, here the amount of times the middle button was clicked when there wasn't a correct target string). Here, a higher accuracy on the task; lower (quicker) reaction time and lower number of false alarms (errors) is indicative of better performance on the task.

2.2.4.1.1.6. Visual analogue scales (VAS)

After completion of the previous tasks, participants were required to rate their current subjective "mental fatigue" by using the cursor to position a cross on a visual analogue scale anchored "not at all" (left side) and "extremely" (right side) at the ends. Participants are advised that the end points represent the extremes of the adjective. They must respond how they are feeling at that point of time.

Task outcome measures: A single "Mental Fatigue" score calculated as a percentage along the line from left to right. With a higher score indicating higher levels of Mental Fatigue.

2.2.4.1.1.7. Stroop

A series of colour names (Red, Yellow, Green, Blue) were displayed on the screen, these were written in a coloured font. The word was either presented in the same-coloured font (e.g. the word "Red" presented in red coloured font) named "Congruent" stimuli or the word was presented in a different coloured font (e.g. the word "Red" presented in a blue coloured font) named "Incongruent" stimuli. Participants were required to make a response based on the colour font the word is written in not the colour the word depicts. Participants were shown 60 stimuli in total, which included 30 congruent and 30 incongruent stimuli. Participants were required to use the 4-button coloured response pad to respond to the task, as quickly as possible. The task was scored for % accuracy (Overall, congruent stimuli and incongruent stimuli) and reaction time (msec) (overall, overall correct responses, overall congruent responses, overall incongruent responses, congruent correct responses, incongruent correct responses).

Task outcome measures: % Overall Accuracy; % Accuracy Congruent stimuli; % Accuracy Incongruent stimuli. Reaction time (msecs) for: Overall correct responses, Overall congruent responses, Overall incongruent responses, Congruent correct responses, Incongruent correct responses. Here, a higher accuracy on the task and lower (quicker) reaction time is indicative of better performance on the task.

2.2.4.1.1.8. Delayed word recall

Following completion of above tasks, participants were given 60 seconds to write down as many of the 15 words presented during word presentation (as detailed in section 2.2.4.1.1.1). The task was scored the same as for Immediate Word Recall (as detailed in section 2.2.4.1.1.1).

Task outcome measures: Number of correct words recalled, number of errors (incorrect words recalled). With higher numbers of correct words recalled and lower numbers of errors, indicating better performance on the task.

2.2.4.1.1.9. Word Recognition

A total of thirty words were presented to the participant, comprising of the 15 words presented during stimuli presentation (as detailed in section 2.2.4.1.1.1) plus 15 distractor words. Participants were required to use the response box to make a Yes/No response indicating if the word was within the original set, or if it was a decoy word. Outcomes for this task are accuracy (% correct) and reaction time for correct responses (msec).

Task outcome measures: % Overall Accuracy; % Accuracy Target stimuli; % Accuracy Novel (Decoy) stimuli. Reaction time (msecs) for: overall responses, correct responses, target stimuli and novel (decoy stimuli). Here, a higher accuracy on the task and lower (quicker) reaction time is indicative of better performance on the task.

2.2.4.1.2. Interim cognitive assessments

Participants were required to complete cognitive assessments during the interim supplementation period. These were completed away from the research centre, using participants' own mobile phones via the Cognim^{app} program (BPNRC, Newcastle upon Tyne, UK). The Cognim^{app} program was developed in 2018 and, similar to COMPASS, also allows the user to create a randomised, customised cognitive paradigm, which participants can complete away from the research centre, using their own mobile phone. This program has been used recently on several nutritional intervention trials at Northumbria University, although no published findings are yet available.

Participants completed these assessments on five occasions, firstly the day prior to their first testing visit (Day -1) and at intervals of 7 days during the supplementation period (Days 7, 14, 21 and 28). Participants were prompted to complete the assessment via an email, which was sent at 8am on each of the assessment days, participants then had 24 hours to complete the assessment at a time that suited them. Each of these assessments lasted 10 minutes in total and comprised the cognitive tasks detailed below, Figure 2.4 illustrates the task order and the cognitive domain that each task loads onto. This battery of task was designed to target the same cognitive domains and also be shorter than the study visits assessments to encourage engagement in assessments outside of a lab-based situation. Moreover, whilst ideally

participants would have completed the same tasks as study visits, not all COMPASS tasks are easily transferred to mobile phone administration, where these tasks could be completed with ease.

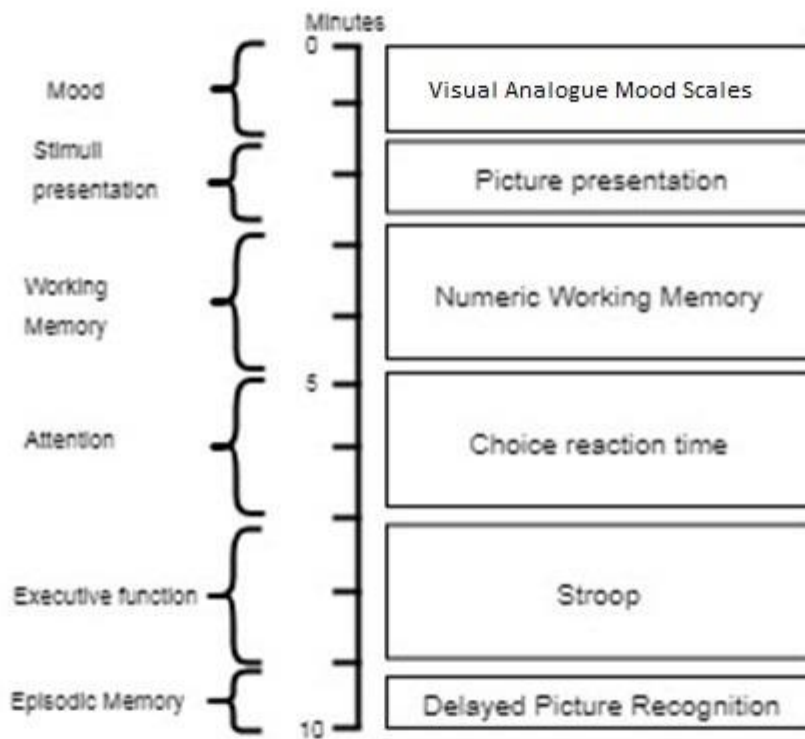

Figure 2.4. The running order of the individual Cognim^{app} cognitive assessments. Tasks are shown in order of completion with approximate timings. On the left the 'cognitive domain' assessed by the task is shown.

2.2.4.1.2.1. Picture presentation

Fifteen coloured photographic images of objects were presented sequentially on screen for the participant to remember at the rate of one every three seconds, each image was presented for one second. These pictures were randomly selected from a large bank of images and different images were presented for each assessment the participant completed.

2.2.4.1.2.2. Numeric working memory (NWM)

Five digits between the numbers 1 to 9 were presented sequentially at random for the participant to hold in their memory. Once the series was complete, this was followed by a series of 30 probe digits (15 targets and 15 distractors), presented one at a time. For each of these digits, the participant was instructed to indicate whether or not the digit had been in the original series, they responded by selecting "Yes" or "No" on the onscreen buttons. The task

consisted of 3 separate (5 digit) trials, presented consecutively. Accuracy (% correct) and mean reaction time (ms) were recorded.

Task outcome measures: % Overall Accuracy; % Accuracy Target stimuli; % Accuracy Novel (Decoy) stimuli. Reaction time (msecs) for: overall responses, correct responses, target stimuli and novel (decoy stimuli). Here, a higher accuracy on the task and lower (quicker) reaction time is indicative of better performance on the task.

2.2.4.1.2.3. Choice reaction time (CRT)

A total of fifty stimuli (arrows) were presented on the screen, with each arrow pointing either left or right and appearing on the screen at irregular intervals. Participants were required to indicate the direction that the arrowhead was presented on their mobile phone screen; they did this by pressing the “Left” or “Right” buttons on their screen. The task was scored for percentage of correct responses and reaction time (msec), the task took ~2 minutes to complete, dependent on the participants speed of reaction.

Task outcome measures: % Overall Accuracy. Reaction time (msecs) for: overall responses and correct responses. Here, a higher accuracy on the task and lower (quicker) reaction time is indicative of better performance on the task.

2.2.4.1.2.4. Stroop

This task was identical as when completed during the inhouse cognitive assessments, with the exception that the coloured buttons were presented on the participants mobile phone screen, below the coloured word, rather than on a separate response pad. A full description of the task can be found in section 2.2.4.1.1.7.

2.2.4.1.2.5. Delayed picture recognition

A series of 15 target pictures, that were presented at the start of the testing assessment, and 15 randomly interspersed decoy pictures are presented on the screen, one at a time. Participants were required to indicate if they have seen the picture before; here to differentiate between the target and decoy words, participants used the “Yes” and “No” buttons on the screen. The task was scored for percentage of correctly recognised pictures and reaction time (ms).

Task outcome measures: % Overall Accuracy, % Accuracy Target stimuli, % Accuracy Novel stimuli. Reaction time (msecs) for: overall responses, correct responses, target stimuli, novel

stimuli. Here, a higher accuracy on the task and lower (quicker) reaction time is indicative of better performance on the task.

2.2.4.2. Cognitive domain data

Alongside assessing cognitive performance on each individual task outcome, consistent with previous resveratrol work (Eschle, 2017), individual task scores were collapsed into relevant outcome measures. This method is frequently implemented in nutrition research (as detailed in Pase & Stough, 2014), including trials utilising the Cognitive Drug Research (CDR) Computerised Assessment System (Stough et al., 2008; Wesnes et al., 2000). This is also common practise within the Brain, Performance and Nutrition Research Centre and has previously shown clearer cognitive effects in various nutritional intervention trials (Avery, 2021; Haskell-Ramsay et al., 2017; Patan, 2019; Wightman et al., 2020; Wightman et al., 2018; Wightman et al., 2021). Data from the current study lends itself to analysis of the following global cognitive domains: Episodic memory, overall accuracy and overall speed.

Cognitive domains were calculated by changing individual task scores into standardised Z scores and clustering these scores into their relevant cognitive domain. The specific calculations for each cognitive domain are outlined below.

2.2.4.2.1. Episodic Memory

The episodic memory data was calculated for COMPASS data only, from standardised values using the following calculation:

> **Episodic Memory** = (Zword recognition accuracy + Zimmediate word recall accuracy + Zdelayed work recall accuracy)/3

2.2.4.2.2. Overall Accuracy

2.2.4.2.2.1. COMPASS

The overall accuracy data was calculated for the COMPASS data from standardised values using the following calculation:

> **Overall accuracy** = (Zserial subtraction 3s accuracy + Zserial subtraction 7s accuracy + Zrvip accuracy + ZStroop accuracy + Zword recognition accuracy + Zimmediate word recall + Zdelayed word recall)/7

2.2.4.2.2.2. Cognim^{app}

The overall accuracy data was calculated for the Cognim^{app} data from standardised values using the following calculation:

Overall accuracy =(ZNWM accuracy + ZCRT accuracy + ZStroop accuracy + Zpicture recognition accuracy)/4

2.2.4.2.3. Overall Speed

2.2.4.2.3.1. COMPASS

The overall speed data was calculated for the COMPASS data from standardised values using the following calculation:

Overall speed = (Zrvip RT + ZStroop RT + Zword recognition RT)/3

2.2.4.2.3.2. Cognim^{app}

The overall speed data was calculated for the Cognim^{app} data from standardised values using the following calculation:

Overall Speed = (ZNWM RT + ZCRT RT + ZStroop RT + ZPicture Recognition RT)/4

2.2.4.3. Mood assessment

2.2.4.3.1. Visual Analogue Mood Scales (VAMS)

To assess acute and chronic changes in mood, the current study used a series of 27 Visual Analogue Scales, which participants completed at the start of each cognitive assessment, both on the testing days and on each interim assessment. These scales comprise 100mm lines which are anchored at either end by the following antonyms detailed within Table 2.2.

Table 2.2. Visual Analogue Mood Scales. Details of antonyms for the 27 VAMS presented to the participants, in order of presentation.

Alert	Inattentive
Lethargic	Energetic
Restless	Calm
Clumsy	Co-ordinated
Weak	Strong
Lively	Sluggish
Happy	Unhappy
Quick-witted	Slow-witted
Satisfied	Unsatisfied
Tranquil	Agitated
Indifferent	Excited
Tense	Relaxed
Anxious	Carefree
Fearful	Fearless
Sharp	Dull
Confused	Clear-headed
Contented	Discontented
Exhausted	Refreshed
Bored	Engaged
Sociable	Unsociable
Friendly	Hostile
Focussed	Unfocussed
Stressed	Carefree
Competent	Incompetent
Peaceful	Troubled
Drowsy	Awake
Motivated	Unmotivated

These scales have been validated in house (unpublished data, in preparation), where 18 of the 27 items can be collapsed into three outcome measures: Alertness, Stress and Tranquillity. Table 2.3. details which scale loads onto the outcome measures.

Table 2.3. Outcomes of Visual Analogue Mood Scales. A previous factor analysis indicated that of the 27 VAMS presented, 18 items load onto 3 outcome measures: 'Alertness' (x11 items), 'Stress' (x4 items) and 'Tranquillity' (x3 items). This table details which scales load onto each factor.

Alertness	Alert	Inattentive
	Lethargic	Energetic
	Clumsy	Co-ordinated
	Lively	Sluggish
	Quick-witted	Slow-witted
	Sharp	Dull
	Exhausted	Refreshed
	Bored	Engaged
	Focussed	Unfocussed
	Drowsy	Awake
	Motivated	Unmotivated
Stress	Tense	Relaxed
	Fearful	Fearless
	Stressed	Carefree
	Peaceful	Troubled
Tranquil	Tranquil	Agitated
	Contented	Discontented
	Friendly	Hostile

2.2.4.3.2. Profile of Mood States (POMs)

The Profile of Mood States (POMs) (McNair, 1992) was used to assess chronic changes in mood, participants completed this at the beginning of each testing visit. The POMs comprises of 65 words and statements that describe feelings that people have, the participants are required to self-report on each of these areas, based on how they are feeling at that exact moment in time. Participants respond using a 5 point Likert scale from 0 – 4, which correspond to the statements: "Not at all", "A little", "Moderately", "Quite a bit" and "Extremely". The POMs provides six scale scores: Anger-Hostility, Confusion-Bewilderment, Depression-Dejection, Fatigue-Inertia, Tension-Anxiety and Vigor-Activity. Total Mood Disturbance (TMD) is also calculated using these scores. For TMD, higher scores are indicative of greater mood disturbance.

2.2.4.4. Blood pressure assessment

Sitting blood pressure and heart rate recordings were collected using a Boso Medicus Prestige (BOSCH + SOHN GmbH u. Co. KG, Jungingen, Germany) blood pressure monitor, using the non-dominant subjects arm, which was supported at the level of the heart and with their feet flat on the floor. Readings were taken upon completion of each of the cognitive assessments. Following both cognitive assessments participants blood pressure was collected as a single measurement, as opposed to the three resting samples obtained as part of eligibility checks during the training visit. Where at the training visit, there were no restrictions placed on participants caffeine intake or exercise prior to measurements (notably here the research centre is located on the fourth floor); both of which may contribute to elevated blood pressure readings. In this situation, allowing participants to sit for a minimum of five minutes before the reading and repeating the reading at one-minute intervals, was deemed necessary to establish an accurate reading. In contrast, within the testing sessions, participants had refrained from caffeine overnight and were sat for a minimum of 30-minutes prior to each reading, whilst completing the cognitive tasks; by controlling for these factors, we can be more confident of an accurate single reading. A secondary reason for this decision is in the feasibility of a single researcher collecting multiple readings from several participants at the same time, whilst this can be completed with ease on a one-to-one screening session, the ability to execute this with multiple participants concurrently, whilst recording the readings accurately, becomes increasingly difficult.

2.2.4.5 Body Mass Index (BMI)

Participants weight (kg) and height (cm) was measured initially at their screening/training visit and BMI was calculated to ascertain eligibility. At the final visit participants weight was measured again, to determine any changes in BMI during supplementation period.

2.2.5. Biological measures

2.2.5.1. Blood sampling

Fasted venous blood samples were collected using 10ml serum and 6ml lithium heparin (LH) vacutainers to assess the following biomarker outcomes: total Cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, C-reactive protein (CRP), interleukin-6 (IL-6), ferric reducing antioxidant power (FRAP), glucose, resveratrol, resveratrol-3-O-D-glucoside, resveratrol-3-0-sulfate, resveratrol-4-O-D-glucoronide. Venous samples were collected on both testing visits before the administration of the day’s treatment and then 1 hour post dose. Samples were inverted 6 times, refrigerated at 5°C and allowed to clot for

at least 1 hour. Samples were processed within 2 hours of collection. 1ml of whole blood was pipetted from the LH tube and immediately stored at -80°C until analysis. The remainder of the samples were centrifuged at 2500 RPM for 10 minutes at room temperature and all resulting plasma and serum was removed and stored at -80°C until analysis.

The following analysis was completed by Samantha Bowerbank, a senior technician within the Applied Sciences department at Northumbria University. Samples were thawed and then vortexed and sonicated for 5 minutes. In a microcentrifuge tube, 200 µL of sample was mixed with 900 µL of 0.1 % formic acid in ethanol and 100 µL of naringenin. Samples were vortexed and sonicated prior to being centrifuged for 10 minutes at 17000 g. The supernatant was removed and transferred into a fresh microcentrifuge tube. The remaining pellet was extracted with 1.2 mL of 83 % aqueous ethanol using the procedure above then both extracts were evaporated to dryness using a sample concentrator. The second extract was reconstituted in 70 µL of ethanol of which 50 µL was transferred into the first extract and 20 µL of taxifolin was added. The solution was then centrifuged and the supernatant transferred to an autosampler vial and 10 µL was analysed via LC-MS/MS.

LC-MS analysis was performed using a Thermo Scientific® surveyor HPLC consisting of an MS pump, autosampler and column oven coupled to a Thermo Scientific® LTQ XL linear ion trap mass spectrometer (Thermo Scientific, Hemel Hempsted). Chromatographic separation was achieved using an Eclipse Plus™ C18 (100 x 4.6 mm, 3.5 µm) column (Agilent, Cheadle) using a gradient mobile phase consisting of A: Water + 0.1 % formic acid and B: Methanol + 0.1 % formic acid.

The mass spectrometer was optimized by auto tuning the MS parameters for resveratrol and was operated in negative selected reaction monitoring mode utilising scan event.

2.2.6. Procedure

Participants were required to attend three sessions at the research centre based within Northumbria University, UK. Initially participants attended a screening/training visit, during which participants were briefed on the requirements of the study, provided informed consent and demographic information. Participant's eligibility was assessed based on the inclusion and exclusion criteria, and those that were eligible were then trained on all of the cognitive and mood measures presented on both COMPASS and Cognimapp.

The day prior (Day -1) to their first testing visit, participants were required to complete the first Cognimapp assessment, this was completed remotely from the laboratory using their own

mobile phones. Participants were instructed to complete this via email, which was sent at 8:00 am, they were told to complete this during the following 24 hours, in a quiet place, free from distractions. This remote assessment of cognitive function and mood took approximately 10 minutes to complete in total.

Participants were required to attend the research centre for two testing visits (Day 1 and Day 29), testing sessions started at 8.00 am and 10.00 am (participants attended consistent time slots for both visits) and lasted 2 hours in total. Participants were required to have fasted for 12 hours, refrained from caffeine for 18 hours and alcohol for 24 hours before arrival to the research centre for each of the visits. These visits took place in a suite of testing facilities within the Brain, Performance and Nutrition Research Centre at Northumbria University, during cognitive assessments participants were visually isolated from each other.

The procedure for each of the testing visits was identical, on arrival participants were checked for continued eligibility to the study requirements and then provided a blood sample. Immediately following this, participants began their baseline cognitive assessment. Firstly, they completed the paper and pencil version of POMs, this was followed by completion of the computerised cognitive assessment and measurements of heart rate and blood pressure. At this point participants consumed their treatment for the day. Participants then completed a second cognitive assessment at 40 minutes post dose, followed by measurements of blood pressure and heart rate. At the end of the testing visit a second blood sample was collected.

During the interim supplementation period the participants were required to complete the mobile cognitive assessments four times on Days 7, 14, 21 and 28, each of these assessments were identical to the initial assessment of Day -1, lasting ~10 minutes each time.

At the end of the first testing visit participants took away their four-week supply of the intervention and the treatment diary to record the consumption of the treatment. This diary and remaining capsules were returned on Day 29 to assess compliance rate. The timelines and assessments of Day 1 and Day 29 are shown in Figure 2.5.

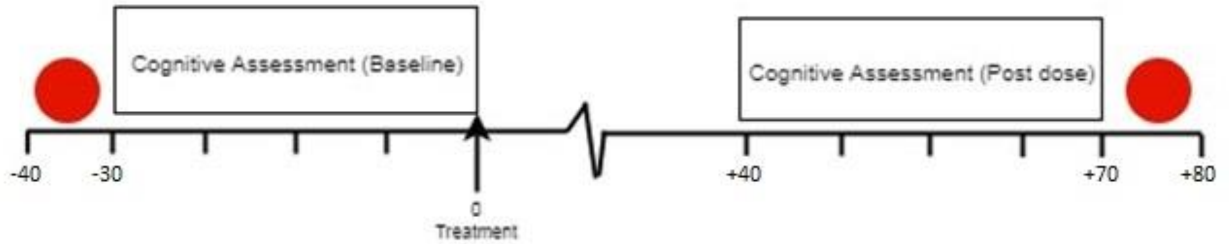

Figure 2.5. Day 1 and Day 29 testing visit timetable. Participants start testing visit with a blood sample, before completing a 30-minute cognitive assessment. Following treatment administration and a 40-minute absorption period, participants completed a second identical cognitive assessment. Followed by a second blood sample, with testing visits lasting approximately 2 hours.

2.2.7. Statistical Methods

2.2.7.1. Data cleaning

One hundred participants were randomised into this study, of which 94 completed both study visits. Each of the six participants who did not complete the study were lost to follow up following visit 1 and did not return for their second visit. Before conducting analyses, deviations from procedure were checked to identify the per protocol population, this resulted in exclusion of four additional participants from the Day 28 analysis. Three due to treatment compliance below 80% and one for antiviral medication use for a diagnosis of shingles during the supplementation period.

Following this, the data was investigated for outlier and anomalous data, initially raw data was visually inspected to identify any 0 values for cognitive assessments, which would indicate failure to respond to task stimuli. All analyses were conducted using SPSS (version 26), where box plots were produced for individual task outcomes to identify potential outliers. These boxplots visually display the spread and skewness of the numerical data; where the box represents the middle 50% of data and the whiskers illustrate the lower and upper 25% of values. Outliers beyond the whiskers are represented with either a circle (if more than one and a half box lengths from the box edge) or an asterisk (those beyond three box lengths from either side); with the latter indicating an extreme outlier. Said extreme outliers were removed, for that task only. Following this, residual values were calculated and histograms produced to visually view the distribution and spread of the data. Any additional values deviating from the normal distribution were then removed, resulting in slightly varying sample sizes for each cognitive task including in the analysis. These data cleaning processes resulted in the following removal of datasets from the COMPASS analysis, as shown in Table 2.4.

Table 2.4. Statistical outliers removed during data cleaning process. Following data cleaning processes, the following datasets were removed. Data is presented for each cognitive task and split by which analysis was impacted by the removal of that dataset.

COMPASS Task	No. participants removed from each analysis		
	Day 1 Acute Analysis	Day 28 Acute Analysis	Pure Chronic Analysis
Immediate and Delayed word recall	1	5	5
Corsi blocks	3	5	5
Serial subtractions of threes	3	9	10
Serial subtractions of sevens	2	6	6
Rapid visual information processing	8	9	11
Stroop	7	9	10
Word recognition	5	11	11

Following this cleaning process, data analysis was conducted.

2.2.7.2. Statistical methods

All data was analysed using analysis of covariance in SPSS (Version 26). All data was analysed for baseline differences via univariate ANOVAs with 'treatment' as a fixed factor; these are reported where they arise and pertain to subsequent effects on the main analyses.

The analysis of all COMPASS cognitive outcomes, blood biomarkers and blood pressure was conducted in three ways: acute effects within Day 1; acute effects within Day 28; and pure chronic effects within Day 28. To analyse this three ANCOVAs were conducted:

1. *Acute effects within Day 1 and Day 28*

To ascertain any acute treatment effects of resveratrol within Day 1, Post-dose data was analysed via ANCOVA with 'treatment' as a fixed factor and their baseline data from that day used as a covariate.

2. *Pure chronic effects on Day 28*

To ascertain if any pure chronic effects of resveratrol supplementation had taken place on Day 28, here, pre- and post-dose data from Day 28 were analysed with 'treatment' as a fixed factor and Day 1 baseline performance as a covariate.

The analysis of the data from the Cognimapp mobile phone interim assessments was conducted as four separate ANCOVAs: Day 7, Day 14, Day 21 and Day 28, each with

‘treatment’ as a fixed factor and their Day 0 baseline data used as a covariate. As the POMS and BMI data were both measured just once during each testing visit; these outcomes were measured via ANCOVA with ‘treatment’ as a fixed factor and Day 1 baseline data as a covariate.

2.3. Results

2.3.1. Compliance and treatment guess

For participants who completed the study, mean compliance was observed to be very good for both treatment groups (97.47% Placebo, 98.06% resveratrol) with a one-way ANOVA identifying no significant differences for compliance between treatment group [$F\,(1, 93)=.12$, $p = .728$]. However, compliance ranged from 57-110%; with three participants removed from analysis due to compliance falling outside the range of >80/<120%. One participant from the placebo group (69%) and two from resveratrol group (73% and 57%).

Participants completed a treatment guess questionnaire (Appendix I) at the end of the final visit and a Chi-Square test of these responses showed no significant differences in participants’ ability to correctly identify whether they had been administered placebo or resveratrol for the duration of the study [$\chi^2\,(1) = .89$, $p = .344$].

2.3.2. Adverse Events

Participants were required to report any adverse events throughout the duration of treatment administration, within their treatment diary. A chi-square test conducted on this data revealed no significant association between treatment and adverse event reporting [$\chi^2\,(1) = 6.00$, $p = .199$].

Table 2.5. Frequency of adverse events. Reported via treatment diary over the 4-week intervention period, reported by treatment group.

Adverse event	Treatment	
	Placebo	Resveratrol
Headache	7	10
Acid reflux	2	1
Muscle ache	1	0
Nose bleeds	0	1
Cold/flu	4	2
Stomach pain	0	1
Migraine	1	0
Shingles	1	0
Unusual mouth taste	1	0
Total	17	15

2.3.3. Physiological Results

2.3.3.1. Study Visit Cognitive Assessments

2.3.3.1.1. Immediate Word Recall

The analysis identified no significant effects of treatment for immediate word recall outcome measures at any timepoint. See Table 2.6.

Table 2.6. Immediate word recall task outcomes for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
		Day 1 Acute						
IWR Correct	Placebo	49	6.64	2.02	6.29	2.18	.31	.58
	Resveratrol	50	6.66	2.35	6.10	1.94		
IWR Incorrect	Placebo	49	.61	.95	.51	.93	.00	.94
	Resveratrol	50	.66	.84	.54	.99		
		Day 28 Acute						
IWR Correct	Placebo	43	6.75	1.86	5.87	2.00	2.83	.09
	Resveratrol	46	6.91	2.29	6.54	1.94		
IWR Incorrect	Placebo	43	.56	.88	.67	.96	.61	.43
	Resveratrol	46	.65	.92	.57	.91		
		Pure Chronic						
		Day 1 A1			Day 28 A1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
IWR Correct	Placebo	43	6.64	2.02	6.75	1.86	.28	.59
	Resveratrol	46	6.66	2.35	6.91	2.29		
IWR Incorrect	Placebo	43	.61	.95	.56	.88	.12	.72
	Resveratrol	46	.66	.84	.65	.92		

2.3.3.1.2. Corsi blocks

The analysis identified no significant effects of treatment for Corsi block span score at any timepoint. See Table 2.7.

Table 2.7. Corsi block task outcome for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main effects	
		n	Mean	SD	Mean	SD	F	p
		Day 1						
Corsi block	Placebo	48	6.12	.82	6.16	.85	1.47	.23
span	Resveratrol	49	6.29	.74	6.11	.74		
		Day 28						
Corsi block	Placebo	44	6.10	.74	6.15	.75	.27	.61
span	Resveratrol	45	6.02	.89	6.06	.68		

		Pure Chronic						
		Day 1 A1			Day 28 A1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Corsi block	Placebo	44	6.12	.82	6.10	.74	1.56	.21
span	Resveratrol	46	6.29	.74	6.02	.899		

2.3.3.1.3. Serial 3 subtractions

A significant effect of treatment for total number of subtractions of threes was identified on Day 28 after controlling for Day 28 baseline scores, $F(1,82) = 4.11$, p=.04, d = .45. With placebo treatment group performing more (mean = 44.28) subtractions than the resveratrol group (mean = 41.24). No additional significant effects of treatment were observed for any outcomes of the serial threes task at any timepoint. See Table 2.8.

Table 2.8. Serial subtraction of threes outcome for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects		
		n	Mean	SD	Mean	SD	df	F	p
					Day 1 Acute				
SS3 Total	Placebo	49	39.49	14.94	42.24	16.15	1	1.36	.25
	Resveratrol	48	40.25	14.38	41.77	15.10			
SS3 Correct	Placebo	49	37.65	15.60	39.76	17.18	1	2.15	.14
	Resveratrol	48	38.58	14.87	38.79	15.38			
SS3 Errors	Placebo	49	1.84	2.22	2.49	2.94	1	1.65	.20
	Resveratrol	48	1.67	1.66	2.98	2.59			
					Day 28 Acute				
SS3 Total	Placebo	43	39.45	14.72	**44.28**	**16.25**	**1**	**4.11**	**.04***
	Resveratrol	42	39.21	13.30	**41.24**	**15.69**			
SS3 Correct	Placebo	43	37.55	15.32	41.35	16.70	1	2.40	.12
	Resveratrol	42	37.29	13.70	38.71	15.88			
SS3 Errors	Placebo	43	1.68	1.78	2.93	2.72	1	1.11	.29
	Resveratrol	42	1.93	2.25	2.52	2.38			
					Pure Chronic				
		Day 1 A1			Day 28 A1		Main Effects		
		n	Mean	SD	Mean	SD		F	p
SS3 Total	Placebo	44	39.49	14.94	39.45	14.72	1	.56	.46
	Resveratrol	41	40.25	14.38	39.83	12.84			
SS3 Correct	Placebo	44	37.65	15.60	37.55	15.32	1	.53	.47
	Resveratrol	41	38.58	14.87	37.88	13.23			
SS3 Errors	Placebo	44	1.84	2.22	1.68	1.78	1	.18	.67
	Resveratrol	41	1.67	1.66	1.95	2.28			

2.3.3.1.4. Serial 7 subtractions

The analysis identified no significant effects of treatment for any outcome of serial sevens subtraction task at any timepoint. See Table 2.9.

Table 2.9. Serial subtractions of sevens outcome for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
			Day 1 Acute					
SS7 Total	Placebo	49	25.05	11.50	26.65	12.95	1.94	.17
	Resveratrol	49	24.12	10.18	26.76	11.60		
SS7 Correct	Placebo	49	22.76	11.77	24.16	13.90	2.16	.15
	Resveratrol	49	21.65	10.38	24.37	11.80		
SS7 Errors	Placebo	49	2.29	2.16	2.49	2.21	.16	.69
	Resveratrol	49	2.47	2.40	2.39	1.92		
			Day 28 Acute					
SS7 Total	Placebo	43	25.77	12.07	27.26	12.76	1.17	.28
	Resveratrol	45	25.91	10.59	26.53	10.93		
SS7 Correct	Placebo	43	23.52	12.33	24.56	12.75	.46	.50
	Resveratrol	45	23.69	10.28	24.13	10.92		
SS7 Errors	Placebo	43	2.25	2.26	2.70	2.77	.31	.57
	Resveratrol	45	2.22	2.06	2.40	1.88		
			Pure Chronic					
		Day 1 A1			Day 28 A1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
SS7 Total	Placebo	44	25.05	11.50	25.77	12.07	.44	.50
	Resveratrol	45	24.12	10.18	25.91	10.59		
SS7 Correct	Placebo	44	22.76	11.77	23.52	12.33	.68	.41
	Resveratrol	45	21.65	10.38	23.69	10.28		
SS7 Errors	Placebo	44	2.29	2.16	2.25	2.26	.07	.79
	Resveratrol	45	2.47	2.40	2.22	2.06		

2.3.3.1.5. Rapid Visual Information Processing

The analysis identified a trend towards a significant effect of treatment for correct reaction on Day 28 after controlling for Day 28 baseline scores, $F(1,82) = 3.92$, p=.051, d = .43. With the resveratrol treatment group performing more quickly (mean = 504.75 msec) than the placebo group (mean = 519.36 msec).

Additionally, a trend towards a significant effect of treatment for false alarms was identified on Day 28 after controlling for Day 28 baseline scores, $F(1,82) = 3.49$, p=.065, d = .41. With the resveratrol treatment group performing fewer false alarms (mean = 1.53) than the placebo group (mean = 2.67). No additional significant effects of treatment were observed for any outcome of RVIP task at any timepoint. See Table 2.10.

Table 2.10. RVIP outcomes for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
		Day 1 Acute						
RVIP Accuracy	Placebo	47	62.50	21.87	63.51	22.08	.57	.45
	Resveratrol	45	60.37	18.71	64.50	21.73		
RVIP Correct RT	Placebo	47	509.53	55.18	519.50	56.46	.76	.38
	Resveratrol	45	494.62	55.91	499.50	57.08		
RVIP False alarms	Placebo	47	2.48	2.65	1.87	1.76	.00	.99
	Resveratrol	45	2.81	2.63	1.91	2.11		
		Day 28						
RVIP Accuracy	Placebo	42	60.87	22.54	62.67	23.44	1.51	.22
	Resveratrol	43	63.77	19.09	61.91	20.76		
RVIP Correct RT	Placebo	42	518.26	67.25	**519.36**	**53.63**	**3.92**	**.05[t]**
	Resveratrol	43	508.16	49.84	**504.75**	**48.13**		
RVIP False alarms	Placebo	42	2.84	3.92	**2.67**	**2.86**	**3.49**	**.06[t]**
	Resveratrol	43	1.95	2.40	**1.53**	**1.60**		
		Pure Chronic						
		Day 1 A1			Day 28 A1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
RVIP Accuracy	Placebo	41	62.50	21.87	61.76	21.86	2.61	.11
	Resveratrol	42	60.37	18.71	64.52	18.68		
RVIP Correct RT	Placebo	41	509.53	55.18	514.67	58.75	.064	.80
	Resveratrol	42	494.62	55.91	508.00	50.43		
RVIP False alarms	Placebo	41	2.48	2.65	2.78	4.00	1.36	.24
	Resveratrol	42	2.81	2.63	1.98	2.42		

2.3.3.1.6. Mental fatigue VAS

The analysis identified a trend towards a significant effect of treatment for mental fatigue VAS on Day 28 after controlling for Day 28 baseline scores (pure chronic effect), $F(1,87) = 3.64$, $p=.059$, $d = .40$. With the resveratrol treatment group reporting feeling less mentally fatigued (mean = 52.37) than the placebo group (mean = 58.57). No additional significant effects of treatment were observed at any timepoint. See Table 2.11.

Table 2.11. Mental fatigue VAS outcomes for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
		Day 1 Acute						
Mental Fatigue VAS	Placebo	50	56.60	12.71	60.36	13.23	1.04	.31
	Resveratrol	50	54.18	16.19	56.68	14.69		
		Day 28 Acute						
Mental Fatigue VAS	Placebo	44	58.57	14.20	61.34	14.85	.01	.97
	Resveratrol	46	46	52.37	56.93	16.18		
		Pure Chronic						
		Day 1 A1			Day 28 A1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Mental Fatigue VAS	Placebo	44	56.60	12.71	**58.57**	**14.20**	**3.64**	**.05**[t]
	Resveratrol	46	54.18	16.19	**52.37**	**15.04**		

2.3.3.1.7. Stroop

The analysis identified a significant effect of treatment for overall congruent reaction time on Day 1 after controlling for Day 1 baseline scores, F(1,90) = 5.11, *p*=.026 *d* = .47. With the placebo treatment group performing more quickly (mean = 680.80 msec) than the resveratrol group (mean = 682.83 msec).

Additionally, a significant effect of treatment for correct congruent reaction time was identified on Day 1 after controlling for Day 1 baseline scores, F(1,90) = 5.02, *p*=.027, *d* = .47. With the placebo treatment group performing more quickly (mean = 681.94 msec) than the resveratrol group (mean = 684.05 msec). No additional significant effects of treatment were observed for any outcome of Stroop task at any timepoint. See Table 2.12.

Table 2.12. Stroop outcomes for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
		Day 1 Acute						
Overall Accuracy	Placebo	47	97.74	1.95	97.97	1.90	.22	.64
	Resveratrol	46	97.53	2.69	97.97	2.74		
Overall RT	Placebo	47	717.35	117.03	701.93	105.00	1.71	.19
	Resveratrol	46	754.14	123.65	719.58	101.62		
Correct RT	Placebo	47	718.45	116.05	703.23	105.26	1.74	.19
	Resveratrol	46	755.10	123.35	720.71	102.18		
Congruent Accuracy	Placebo	47	97.63	2.37	98.58	1.80	.87	.35
	Resveratrol	46	97.60	2.95	98.11	2.95		
	Placebo	47	97.84	2.42	97.37	3.10	2.18	.14

Incongruent Accuracy	Resveratrol	46	97.46	2.99	97.82	3.52		
Congruent RT	Placebo	47	684.17	106.89	**680.80**	**109.65**	**5.11**	**.02***
	Resveratrol	46	721.17	116.42	**682.83**	**98.69**		
Incongruent RT	Placebo	47	750.52	134.94	723.03	109.67	.26	.60
	Resveratrol	46	787.11	144.34	756.33	112.47		
Correct Congruent RT	Placebo	47	685.31	106.60	**681.94**	**109.58**	**5.02**	**.02***
	Resveratrol	46	722.02	116.62	**684.05**	**99.11**		
Correct Incongruent RT	Placebo	47	751.63	133.81	724.75	110.32	.16	.68
	Resveratrol	46	788.57	144.40	757.28	112.83		
			Day 28 Acute					
Overall Accuracy	Placebo	43	97.79	2.23	97.28	2.54	1.61	.20
	Resveratrol	42	98.01	2.44	98.01	2.60		
Overall RT	Placebo	43	706.09	97.04	690.41	91.93	.38	.53
	Resveratrol	42	745.06	144.07	725.76	113.46		
Correct RT	Placebo	43	706.19	96.64	691.25	91.23	.38	.53
	Resveratrol	42	745.59	144.24	726.75	113.06		
Congruent Accuracy	Placebo	43	97.98	2.19	97.21	3.63	.46	.49
	Resveratrol	42	97.93	2.94	97.69	3.78		
Incongruent Accuracy	Placebo	43	97.59	3.51	97.36	3.53	1.69	.19
	Resveratrol	42	98.09	2.86	98.33	2.35		
Congruent RT	Placebo	43	680.98	103.14	669.05	90.25	.31	.57
	Resveratrol	42	714.65	125.46	699.28	102.60		
Incongruent RT	Placebo	43	731.20	100.20	711.77	102.45	.49	.48
	Resveratrol	42	775.48	171.40	752.24	130.73		
Correct Congruent RT	Placebo	43	681.42	103.55	670.29	89.63	.29	.58
	Resveratrol	42	714.83	125.74	699.81	101.86		
Correct Incongruent RT	Placebo	43	731.32	99.72	712.34	101.86	.51	.47
	Resveratrol	42	776.37	171.40	753.56	131.24		
			Pure Chronic					
			Day 1 A1		Day 28 A1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Overall Accuracy	Placebo	42	97.74	1.95	97.81	2.25	.60	.43
	Resveratrol	42	97.53	2.69	98.01	2.44		
Overall RT	Placebo	42	717.35	117.03	706.14	98.21	.37	.54
	Resveratrol	42	754.14	123.65	745.06	144.07		
Correct RT	Placebo	42	718.45	116.05	706.28	97.81	.37	.54
	Resveratrol	42	755.10	123.35	745.59	144.24		
Congruent Accuracy	Placebo	42	97.63	2.37	98.01	2.21	.00	.99
	Resveratrol	42	97.60	2.95	97.93	2.94		
Incongruent Accuracy	Placebo	42	97.84	2.42	97.61	3.55	1.05	.30
	Resveratrol	42	97.46	2.99	98.09	2.86		
Congruent RT	Placebo	42	684.17	106.89	680.15	104.24	.13	.71
	Resveratrol	42	721.17	116.42	714.65	125.46		
Incongruent RT	Placebo	42	750.52	134.94	732.14	101.22	.68	.40
	Resveratrol	42	787.11	144.34	775.48	171.40		
Correct Congruent RT	Placebo	42	685.31	106.60	680.64	104.68	.12	.73
	Resveratrol	42	722.02	116.62	714.83	125.74		
Correct Incongruent RT	Placebo	42	751.63	133.81	732.29	100.72	.69	.40
	Resveratrol	42	788.57	144.40	776.37	171.40		

2.3.3.1.8. Delayed word recall

The analysis identified no significant effects of treatment for any outcome of delayed word recall task at any timepoint. See Table 2.13.

Table 2.13. Delayed word recall outcome for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
		Day 1 Acute						
DWR Correct	Placebo	49	4.54	2.29	3.44	2.62	.11	.74
	Resveratrol	50	4.81	2.56	3.44	2.31		
DWR Incorrect	Placebo	49	1.00	1.24	1.14	1.78	.03	.84
	Resveratrol	50	.82	1.11	.96	1.37		
		Day 28 Acute						
DWR Correct	Placebo	43	5.14	2.23	3.55	2.17	.80	.37
	Resveratrol	46	5.20	2.73	3.96	2.68		
DWR Incorrect	Placebo	43	.72	1.26	1.19	1.73	2.71	.10
	Resveratrol	46	.85	1.24	.87	1.50		
		Pure Chronic						
		Day 1 A1			Day 28 A1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
DWR Correct	Placebo	43	4.54	2.29	5.14	2.23	.00	.97
	Resveratrol	46	4.81	2.56	5.20	2.73		
DWR Incorrect	Placebo	43	1.00	1.24	.72	1.26	.62	.43
	Resveratrol	46	.82	1.11	.85	1.24		

2.3.3.1.9. Delayed word recognition

The analysis identified a significant effect of treatment for overall reaction time on Day 1 after controlling for Day 1 baseline scores, $F(1,92) = 4.34$, $p = .040$, $d = .43$. With the placebo treatment group performing more quickly (mean = 889.68 msec) than the resveratrol group (mean = 936.50 msec).

Additionally, a significant effect of treatment for 'No' reaction time was identified on Day 1 after controlling for Day 1 baseline scores, $F(1,92) = 6.14$, $p = .015$, $d = .52$. With the placebo treatment group performing more quickly (mean = 889.19 msec) than the resveratrol group (mean = 974.12 msec). No additional significant effects of treatment were observed for any outcome of the word recognition task at any timepoint. See Table 2.14.

Table 2.14. Delayed word recognition outcome for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
		Day 1 Acute						
Word recognition Accuracy	Placebo	45	79.49	8.01	78.51	12.05	.55	.45
	Resveratrol	50	77.60	9.96	76.06	9.39		
Word recognition Overall RT	Placebo	45	918.72	149.85	**889.68**	**144.25**	**4.34**	**.04***
	Resveratrol	50	920.14	187.68	**936.50**	**191.55**		
Word recognition Correct RT	Placebo	45	887.21	140.29	856.52	126.26	2.18	.14
	Resveratrol	50	898.79	173.08	898.22	183.96		
Word recognition Correct Yes %	Placebo	45	69.71	14.45	71.70	17.00	.10	.74
	Resveratrol	50	65.06	17.27	68.13	16.56		
Word recognition Correct No %	Placebo	45	89.27	11.45	85.33	15.08	.76	.38
	Resveratrol	50	90.13	11.60	83.99	13.99		
Word recognition Yes RT	Placebo	45	913.40	173.80	890.16	164.78	.28	.59
	Resveratrol	50	902.21	207.82	898.94	193.36		
Word recognition No RT	Placebo	45	924.07	165.66	**889.19**	**159.57**	**6.14**	**.01***
	Resveratrol	50	938.08	206.91	**974.12**	**239.69**		
		Day 28 Acute						
Word recognition Accuracy	Placebo	38	82.64	9.52	78.50	10.12	.34	.55
	Resveratrol	45	78.29	10.09	76.74	10.13		
Word recognition Overall RT	Placebo	38	897.07	147.30	908.32	164.88	.75	.38
	Resveratrol	45	920.42	191.67	911.31	172.54		
Word recognition Correct RT	Placebo	38	875.40	154.29	871.64	150.47	.47	.49
	Resveratrol	45	879.96	176.73	893.30	173.89		
Word recognition Correct Yes %	Placebo	38	72.13	16.95	71.57	16.00	.07	.78
	Resveratrol	45	67.11	18.60	67.25	18.73		
Word recognition Correct No %	Placebo	38	93.16	8.98	85.43	14.27	2.08	.15
	Resveratrol	45	89.48	11.84	86.22	14.86		
Word recognition Yes RT	Placebo	38	892.27	167.74	906.25	187.57	.10	.75
	Resveratrol	45	902.67	224.23	904.04	203.88		
Word recognition No RT	Placebo	38	901.86	178.69	910.39	179.24	.22	.63
	Resveratrol	45	938.17	212.25	918.58	197.64		
		Pure Chronic						
		Day 1 A1			Day 28 A1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Word recognition Accuracy	Placebo	39	79.49	8.01	82.64	9.52	2.65	.10
	Resveratrol	45	77.60	9.96	78.29	10.09		
Word recognition Overall RT	Placebo	39	918.72	149.85	897.07	147.30	.46	.49
	Resveratrol	45	920.14	187.68	920.42	191.67		
Word recognition Correct RT	Placebo	39	887.21	140.29	875.40	154.29	.08	.77
	Resveratrol	45	898.79	173.08	879.96	176.76		
Word recognition Correct Yes %	Placebo	39	69.71	14.45	72.13	16.95	.51	.47
	Resveratrol	45	65.06	17.27	67.11	18.60		
Word recognition Correct No %	Placebo	39	89.27	11.45	93.16	8.98	2.53	.11
	Resveratrol	45	90.13	11.60	89.48	11.84		
Word recognition Yes RT	Placebo	39	913.40	173.80	892.27	167.74	.19	.65
	Resveratrol	45	902.21	207.82	902.67	224.23		
Word recognition No RT	Placebo	39	924.07	165.66	901.86	178.69	.31	.57
	Resveratrol	45	938.08	206.91	938.17	212.25		

2.3.3.1.10 COMPASS Cognitive Domains

2.3.3.1.10.1 Episodic Memory

The analysis identified no significant effects of treatment for episodic memory domain at any timepoint. See Table 2.15.

Table 2.15. Episodic memory cognitive domain outcomes for placebo and resveratrol treatment groups. Data presented are Z composite scores, calculated by clustering relevant tasks. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
		Day 1 Acute						
Episodic Memory	Placebo	49	.00	.67	.041	.87	.41	.52
	Resveratrol	50	-.02	.85	-.06	.70		
		Day 28 Acute						
Episodic Memory	Placebo	43	.04	.73	-.01	.71	.74	.39
	Resveratrol	46	-.09	.82	.00	.78		
		Pure Chronic						
		Day 1 A1			Day 28 A1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Episodic Memory	Placebo	44	.00	.67	.04	.73	.45	.50
	Resveratrol	46	-.02	.85	-.09	.82		

2.3.3.1.10.2. Overall Accuracy

The analysis identified no significant effects of treatment for overall accuracy domain at any timepoint. See Table 2.16.

Table 2.16. Overall accuracy cognitive domain outcomes for placebo and resveratrol treatment groups. Data presented are Z composite scores, calculated by clustering relevant tasks. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
		Day 1 Acute						
Overall Accuracy	Placebo	50	.01	.54	.01	.57	.15	.69
	Resveratrol	50	-.02	.49	-.03	.49		
		Day 28 Acute						
Overall Accuracy	Placebo	44	.00	.55	-.004	.53	.01	.92
	Resveratrol	46	-.01	.50	-.008	.47		
		Pure Chronic						
		Day 1 A1			Day 28 A1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Overall Accuracy	Placebo	44	.01	.54	.00	.55	.08	.76
	Resveratrol	46	-.02	.49	-.01	.50		

2.3.3.1.10.3. Overall Speed

The analysis identified no significant effects of treatment for overall speed domain at any timepoint. See Table 2.17.

Table 2.17. Overall speed cognitive domain outcomes for placebo and resveratrol treatment groups. Data presented are Z composite scores, calculated by clustering relevant tasks. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
		Day 1 Acute						
Overall Speed	Placebo	50	-.01	.80	-.03	.74	.39	.56
	Resveratrol	50	.02	.88	.05	.89		
		Day 28 Acute						
Overall Speed	Placebo	44	-.04	.69	-.05	.74	.03	.86
	Resveratrol	46	.08	.91	.06	.87		
		Pure Chronic						
		Day 1 A1			Day 28 A1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Overall Speed	Placebo	44	-.01	.80	-.04	.69	.63	.42
	Resveratrol	46	.02	.88	.08	.91		

2.3.3.2. Interim cognitive assessments via Cognim[app]

2.3.3.2.1. Numeric working memory

The analysis identified no significant effects of treatment for numeric working memory at any timepoint. See Table 2.18.

Table 2.18. Numeric memory task outcomes for placebo and resveratrol treatment groups. Baseline (Day 0) raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects from ANCOVA analysis, split by day of assessment (Days 7, 14, 21 and 28).

		Baseline (Day 0)			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
		Day 7						
Accuracy decoy	Placebo	40	44.01	.84	43.51	1.23	.11	.73
	Resveratrol	40	43.61	1.03	43.45	1.53		
Accuracy overall	Placebo	40	96.69	2.93	96.85	2.97	.13	.71
	Resveratrol	40	97.12	2.56	96.69	3.11		
Accuracy target	Placebo	40	52.68	2.71	53.33	2.34	.25	.61
	Resveratrol	40	53.51	2.36	53.24	1.94		
Correct RT	Placebo	40	830.42	171.78	810.16	187.66	.011	.91
	Resveratrol	40	816.40	177.22	792.18	177.59		
RT Decoy	Placebo	40	903.26	182.35	902.30	240.69	.50	.47
	Resveratrol	40	896.47	209.97	868.19	211.68		
RT Overall	Placebo	40	834.17	172.63	819.52	206.23	.03	.85
	Resveratrol	40	821.05	179.55	764.65	180.16		
RT Target	Placebo	40	778.89	174.82	753.29	189.75	.11	.73
	Resveratrol	40	760.72	167.07	735.82	163.07		
		Day 14						
Accuracy decoy	Placebo	36	44.01	.84	43.89	1.03	.76	.38
	Resveratrol	41	43.61	1.03	43.50	1.16		

Accuracy overall	Placebo	36	96.69	2.93	97.46	3.13	1.72	.19
	Resveratrol	41	97.12	2.56	96.69	2.80		
Accuracy target	Placebo	36	52.68	2.71	53.56	2.43	1.12	.29
	Resveratrol	41	53.51	2.36	53.17	2.19		
Correct RT	Placebo	36	830.42	171.78	806.64	175.61	.15	.69
	Resveratrol	41	816.40	177.22	773.64	175.42		
RT Decoy	Placebo	36	903.26	182.35	880.74	211.37	.04	.82
	Resveratrol	41	896.47	209.97	851.70	222.72		
RT Overall	Placebo	36	834.17	172.63	809.17	177.83	.13	.71
	Resveratrol	41	821.05	179.55	776.16	178.31		
RT Target	Placebo	36	778.89	174.82	751.91	162.32	.36	.55
	Resveratrol	41	760.72	167.07	715.73	151.31		
Day 21								
Accuracy decoy	Placebo	38	44.01	.84	43.95	.97	1.83	.17
	Resveratrol	38	43.61	1.03	43.43	1.42		
Accuracy overall	Placebo	38	96.69	2.93	97.33	2.99	1.51	.22
	Resveratrol	38	97.12	2.56	96.81	3.48		
Accuracy target	Placebo	38	52.68	2.71	53.38	2.47	.43	.51
	Resveratrol	38	53.51	2.36	53.39	2.59		
Correct RT	Placebo	38	830.42	171.78	766.26	151.58	1.82	.18
	Resveratrol	38	816.40	177.22	782.61	167.58		
RT Decoy	Placebo	38	903.26	182.35	838.25	180.39	1.43	.23
	Resveratrol	38	896.47	209.97	862.44	217.96		
RT Overall	Placebo	38	834.17	172.63	766.42	150.59	1.87	.17
	Resveratrol	38	821.05	179.55	784.00	168.64		
RT Target	Placebo	38	778.89	174.82	708.96	138.69	1.78	.18
	Resveratrol	38	760.72	167.07	721.25	141.22		
Day 28								
Accuracy decoy	Placebo	34	44.01	.84	43.75	1.01	.63	.42
	Resveratrol	38	43.61	1.03	43.43	1.39		
Accuracy overall	Placebo	34	96.69	2.93	97.42	2.67	.95	.33
	Resveratrol	38	97.12	2.56	96.75	2.62		
Accuracy target	Placebo	34	52.68	2.71	53.66	1.99	.58	.44
	Resveratrol	38	53.51	2.36	53.31	1.92		
Correct RT	Placebo	34	830.42	171.78	762.71	150.94	2.02	.16
	Resveratrol	38	816.40	177.22	802.47	179.07		
RT Decoy	Placebo	34	903.26	182.35	827.11	181.07	2.15	.14
	Resveratrol	38	896.47	209.97	883.32	214.19		
RT Overall	Placebo	34	834.17	172.63	764.04	152.94	2.01	.16
	Resveratrol	38	821.05	179.55	806.04	183.53		
RT Target	Placebo	34	778.89	174.82	713.59	139.74	1.25	.26
	Resveratrol	38	760.72	167.07	744.22	175.88		

2.3.3.2.2. Choice reaction time

The analysis identified a significant effect of treatment for overall accuracy on Day 21 after controlling for Day 0 baseline scores, $F(1,80) = 6.38$, $p = .013$, $d = .56$. With the resveratrol treatment group performing more accurately (mean = 98.93 %) than the placebo group (mean = 97.86 %).

No additional significant effects of treatment were observed for any outcome of the choice reaction time task at any timepoint. See Table 2.19.

Table 2.19. Choice reaction time outcome for placebo and resveratrol treatment groups. Baseline (Day 0) raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects from ANCOVA analysis, split by day of assessment (Days 7, 14, 21 and 28).

		Baseline (Day 0)			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
		Day 7						
Accuracy overall	Placebo	45	98.30	2.16	98.31	2.55	.09	.76
	Resveratrol	43	98.39	1.81	98.23	2.59		
Reaction time	Placebo	45	526.95	70.56	525.56	71.20	.02	.87
	Resveratrol	43	98.39	1.81	98.23	2.59		
		Day 14						
Accuracy overall	Placebo	41	98.30	2.16	98.00	2.53	.00	.97
	Resveratrol	44	98.39	1.81	98.09	2.86		
Reaction time	Placebo	41	526.95	70.56	519.06	69.41	.13	.71
	Resveratrol	44	548.39	97.34	530.64	84.21		
		Day 21						
Accuracy overall	Placebo	42	98.30	2.16	**97.86**	**2.31**	**6.38**	**.01***
	Resveratrol	41	98.39	1.81	**98.93**	**1.49**		
Reaction time	Placebo	42	526.95	70.56	512.10	76.13	.28	.59
	Resveratrol	41	548.39	97.34	535.32	79.45		
		Day 28						
Accuracy overall	Placebo	37	98.30	2.16	98.97	1.46	.21	.64
	Resveratrol	44	98.39	1.81	99.18	1.57		
Reaction time	Placebo	37	526.95	70.56	527.53	74.18	.06	.79
	Resveratrol	44	548.39	97.34	536.96	97.46		

2.3.3.2.3. Stroop

The analysis identified no significant effects of treatment for Stroop at any timepoint. See Table 2.20.

Table 2.20. Stroop task outcomes for placebo and resveratrol treatment groups. Baseline (Day 0) raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects from ANCOVA analysis, split by day of assessment (Days 7, 14, 21 and 28).

		Baseline (Day 0)			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
		Day 7						
Accuracy congruous	Placebo	46	49.71	.72	49.56	.74	.00	.97
	Resveratrol	42	49.43	.99	49.52	.92		
Accuracy incongruous	Placebo	46	49.57	1.01	49.49	.98	.02	.88
	Resveratrol	42	49.57	.88	49.52	1.12		
Accuracy overall	Placebo	46	99.29	1.46	99.05	1.34	.03	.84
	Resveratrol	42	99.00	1.46	99.04	1.61		
RT Congruous	Placebo	46	681.08	103.25	675.10	108.66	1.58	.21
	Resveratrol	42	690.20	114.37	654.45	85.13		
RT incongruous	Placebo	46	733.56	130.92	716.19	112.21	1.81	.18
	Resveratrol	42	761.06	165.36	698.95	123.88		
RT Overall	Placebo	46	706.90	115.06	695.26	109.22	1.93	.16
	Resveratrol	42	724.83	135.37	676.80	101.53		
		Day 14						
Accuracy congruous	Placebo	40	49.71	.72	49.66	.67	.01	.89
	Resveratrol	40	49.43	.99	49.54	.84		
Accuracy incongruous	Placebo	40	49.57	1.01	49.33	1.63	.10	.74
	Resveratrol	40	49.57	.88	49.41	.96		

Accuracy overall	Placebo	40	99.29	1.46	98.99	1.99	.42	.51
	Resveratrol	40	99.00	1.46	98.95	1.54		
RT Congruous	Placebo	40	681.08	103.25	651.39	101.28	.21	.64
	Resveratrol	40	690.20	114.37	655.83	109.88		
RT incongruous	Placebo	40	733.56	130.92	689.46	101.25	.30	.58
	Resveratrol	40	761.06	165.36	700.15	123.22		
RT Overall	Placebo	40	706.90	115.06	670.02	99.09	.44	.50
	Resveratrol	40	724.83	135.37	678.11	114.70		
Day 21								
Accuracy congruous	Placebo	43	49.71	.72	49.34	.97	.09	.76
	Resveratrol	41	49.43	.99	49.34	1.22		
Accuracy incongruous	Placebo	43	49.57	1.01	49.22	1.17	.55	.45
	Resveratrol	41	49.57	.88	49.38	1.03		
Accuracy overall	Placebo	43	99.29	1.46	98.56	1.52	.52	.47
	Resveratrol	41	99.00	1.46	98.73	1.77		
RT Congruous	Placebo	43	681.08	103.25	645.96	97.02	1.44	.23
	Resveratrol	41	690.20	114.37	661.40	121.78		
RT incongruous	Placebo	43	733.56	130.92	684.91	111.18	.012	.91
	Resveratrol	41	761.06	165.36	690.91	124.18		
RT Overall	Placebo	43	706.90	115.06	663.85	102.76	.56	.45
	Resveratrol	41	724.83	135.37	675.43	120.79		
Day 28								
Accuracy congruous	Placebo	34	49.71	.72	49.60	.82	.01	.91
	Resveratrol	43	49.43	.99	49.49	.93		
Accuracy incongruous	Placebo	34	49.57	1.01	49.46	1.06	1.44	.23
	Resveratrol	43	49.57	.88	49.10	1.37		
Accuracy overall	Placebo	34	99.29	1.46	99.06	1.30	.97	.32
	Resveratrol	43	99.00	1.46	98.60	1.74		
RT Congruous	Placebo	34	681.08	103.25	654.67	105.72	.48	.49
	Resveratrol	43	690.20	114.37	656.48	105.84		
RT incongruous	Placebo	34	733.56	130.92	702.89	135.62	.04	.82
	Resveratrol	43	761.06	165.36	689.55	119.89		
RT Overall	Placebo	34	706.90	115.06	678.48	119.07	.05	.81
	Resveratrol	43	724.83	135.37	672.13	110.00		

2.3.3.2.4. Delayed picture recognition

The analysis identified a trend towards a significant effect of treatment for correct reaction time on Day 7 after controlling for Day 0 baseline scores, $F(1,73) = 3.24$, $p = .076$, $d = .42$. With the resveratrol treatment group performing more quickly (mean = 865.96 msec) than the placebo group (mean = 903.73 msec).

Additionally, a trend towards a significant effect of treatment for target reaction time was identified on Day 7 after controlling for Day 0 baseline scores, $F(1,7) = 3.13$, $p = .081$, $d = .41$. With the resveratrol treatment group performing more quickly (mean = 847.38 msec) than the placebo group (mean = 893.64 msec).

A significant effect of treatment for correct reaction time was identified on Day 28 after controlling for Day 0 baseline scores, $F(1,71) = 4.42$, $p = .039$, $d = .50$. With the placebo

treatment group performing more quickly (mean = 889.42 msec) than the resveratrol group (mean = 940.73 msec).

A trend towards a significant effect of treatment for overall reaction time was identified on Day 28 after controlling for Day 0 baseline scores, $F(1,71) = 3.20$, $p = .078$, $d = .42$. With the placebo treatment group performing more quickly (mean = 905.93 msec) than the resveratrol group (mean = 946.28 msec).

No additional significant effects of treatment were observed for any outcome of picture recognition task at any timepoint. See Table 2.21.

Table 2.21. Picture recognition outcomes for placebo and resveratrol treatment groups. Baseline (Day 0) raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects from ANCOVA analysis, split by day of assessment (Days 7, 14, 21 and 28).

		Baseline (Day 0)			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
				Day 7				
Accuracy decoy	Placebo	41	48.58	1.93	47.64	3.43	2.63	.10
	Resveratrol	35	47.52	2.63	48.47	2.19		
Accuracy overall	Placebo	41	95.24	4.38	93.17	6.23	1.35	.24
	Resveratrol	35	92.17	6.85	93.52	6.581		
Accuracy target	Placebo	41	46.66	3.80	45.52	5.08	.26	.60
	Resveratrol	35	44.65	5.73	45.04	5.68		
RT Correct	Placebo	41	894.71	153.14	**903.73**	**150.37**	**3.24**	**.07[t]**
	Resveratrol	35	897.21	134.33	**865.96**	**115.21**		
RT Decoy	Placebo	41	948.50	206.37	943.54	183.76	1.20	.27
	Resveratrol	35	944.93	157.68	912.40	142.62		
RT Overall	Placebo	41	903.24	159.08	918.59	166.24	2.79	.09
	Resveratrol	35	908.07	136.96	879.89	130.73		
RT Target	Placebo	41	857.99	139.14	**893.64**	**165.75**	**3.13**	**.08[t]**
	Resveratrol	35	871.20	132.06	**847.38**	**143.36**		
				Day 14				
Accuracy decoy	Placebo	41	48.58	1.93	48.37	2.59	.35	.55
	Resveratrol	41	47.52	2.63	48.61	2.78		
Accuracy overall	Placebo	41	95.24	4.38	93.73	6.67	.03	.85
	Resveratrol	41	92.17	6.85	91.87	7.52		
Accuracy target	Placebo	41	46.66	3.80	45.36	6.14	.63	.42
	Resveratrol	41	44.65	5.73	43.25	7.12		
RT Correct	Placebo	41	894.71	153.14	887.64	122.35	.00	.95
	Resveratrol	41	897.21	134.33	884.70	136.70		
RT Decoy	Placebo	41	948.50	206.37	938.83	201.22	1.01	.31
	Resveratrol	41	944.93	157.68	301.71	151.69		
RT Overall	Placebo	41	903.24	159.08	901.03	142.36	.02	.87
	Resveratrol	41	908.07	136.96	893.06	142.92		
RT Target	Placebo	41	857.99	139.14	863.23	119.33	.66	.41
	Resveratrol	41	871.20	132.06	884.40	150.89		
				Day 21				
Accuracy decoy	Placebo	38	48.58	1.93	48.42	2.65	.81	.37
	Resveratrol	39	47.52	2.63	48.80	1.94		
Accuracy overall	Placebo	38	95.24	4.38	93.86	4.74	.26	.61
	Resveratrol	39	92.17	6.85	91.53	8.01		
Accuracy target	Placebo	38	46.66	3.80	45.43	3.90	1.16	.28
	Resveratrol	39	44.65	5.73	42.73	7.56		

RT Correct	Placebo	38	894.71	153.14	878.84	159.93	.90	.34
	Resveratrol	39	897.21	134.33	898.89	150.30		
RT Decoy	Placebo	38	948.50	206.37	905.04	178.55	1.60	.20
	Resveratrol	39	944.93	157.68	931.03	178.64		
RT Overall	Placebo	38	903.24	159.08	891.95	172.68	.65	.42
	Resveratrol	39	908.07	136.96	910.46	151.98		
RT Target	Placebo	38	857.99	139.14	878.86	207.99	.04	.84
	Resveratrol	39	871.20	132.06	889.89	160.02		
			Day 28					
Accuracy decoy	Placebo	33	48.58	1.93	48.28	2.64	.06	.79
	Resveratrol	41	47.52	2.63	47.56	4.34		
Accuracy overall	Placebo	33	95.24	4.38	93.63	6.25	.12	.72
	Resveratrol	41	92.17	6.85	90.73	9.32		
Accuracy target	Placebo	33	46.66	3.80	45.35	5.46	.42	.51
	Resveratrol	41	44.65	5.73	43.17	7.26		
RT Correct	Placebo	33	894.71	153.14	**889.42**	**137.40**	**4.42**	**.03***
	Resveratrol	41	897.21	134.33	**940.73**	**175.94**		
RT Decoy	Placebo	33	948.50	206.37	935.83	183.77	2.11	.15
	Resveratrol	41	944.93	157.68	973.18	189.75		
RT Overall	Placebo	33	903.24	159.08	**905.93**	**144.67**	**3.20**	**.07**^{t}
	Resveratrol	41	908.07	136.96	**946.28**	**162.27**		
RT Target	Placebo	33	857.99	139.14	876.03	146.19	1.76	.18
	Resveratrol	41	871.20	132.06	919.38	166.41		

2.3.3.2.5. Cognim^{app} Cognitive Domains

2.3.3.2.5.1. Overall Accuracy

The analysis identified no significant effects of treatment for overall accuracy domain at any timepoint. See Table 2.22.

Table 2.22. Overall accuracy cognitive domain outcomes for placebo and resveratrol treatment groups. Data included here are standardised Z scores, calculated by clustering relevant tasks. Baseline (Day 0) raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects from ANCOVA analysis, split by day of assessment (Days 7, 14, 21 and 28).

		Baseline (Day 0)			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
			Day 7					
Overall Accuracy	Placebo	34	.08	.48	.10	.57	.00	.96
	Resveratrol	40	-.11	.67	-.00	.63		
			Day 14					
Overall Accuracy	Placebo	34	.08	.48	.15	.59	.77	.38
	Resveratrol	40	-.11	.67	-.06	.61		
			Day 21					
Overall Accuracy	Placebo	34	.08	.48	.07	.58	.34	.55
	Resveratrol	40	-.11	.67	-.00	.63		
			Day 28					
Overall Accuracy	Placebo	34	.08	.48	.04	.79	.18	.66
	Resveratrol	40	-.11	.67	-.13	.06		

2.3.3.2.5.2. Overall Speed

The analysis identified no significant effects of treatment for overall speed domain at any timepoint. See Table 2.23.

Table 2.23. Overall speed cognitive domain outcomes for placebo and resveratrol treatment groups. Data included here are standardised Z scores, calculated by clustering relevant tasks. Baseline (Day 0) raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects from ANCOVA analysis, split by day of assessment (Days 7, 14, 21 and 28).

		Baseline (Day 0)			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
				Day 7				
Overall Speed	Placebo	34	-.03	.82	.14	.90	.67	.41
	Resveratrol	40	.06	.84	.01	.85		
				Day 14				
Overall Speed	Placebo	34	-.03	.82	-.00	.74	.23	.63
	Resveratrol	40	.06	.84	-.00	.86		
				Day 21				
Overall Speed	Placebo	34	-.03	.82	.00	.83	.94	.33
	Resveratrol	40	.06	.84	.05	.87		
				Day 28				
Overall Speed	Placebo	34	-.03	.82	-.06	.82	1.56	.21
	Resveratrol	40	.06	.84	.02	.80		

2.3.3.3. Mood

2.3.3.3.1. Visual Analogue Mood Scales

2.3.3.3.1.1. Study visits

The analysis identified a significant effect of treatment for Tranquillity on Day 1 after controlling for Day 1 baseline scores, $F(1,97) = 5.78$, $p = .018$, $d = .49$. With the resveratrol treatment group reporting feeling more tranquil (mean = 68.88) than the placebo group (mean = 63.86).

No additional significant effects of treatment were observed for any VAMS outcome at any timepoint. See Table 2.24.

Table 2.24. Visual analogue mood scales outcomes for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

			Baseline		Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
			Day 1 Acute					
Alertness	Placebo	50	59.90	15.45	58.85	14.46	.03	.84
	Resveratrol	50	62.78	14.68	60.82	13.01		
Stress	Placebo	50	41.62	13.22	40.72	12.97	.19	.66
	Resveratrol	50	37.74	12.90	38.29	11.60		
Tranquillity	Placebo	50	65.86	15.47	**63.86**	**15.32**	**5.78**	**.01***
	Resveratrol	50	69.00	12.39	**68.88**	**10.56**		
			Day 28 Acute					
Alertness	Placebo	44	62.46	14.99	61.10	14.05	.11	.73
	Resveratrol	46	63.31	13.90	62.25	14.09		
Stress	Placebo	44	39.31	13.49	39.28	12.55	.72	.39
	Resveratrol	46	38.16	13.89	39.76	13.48		
Tranquillity	Placebo	44	66.98	14.22	66.53	13.27	1.23	.26
	Resveratrol	46	68.87	12.28	66.82	12.25		
			Pure Chronic					
			Day 1 A1		Day 28 A1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Alertness	Placebo	44	59.90	15.45	62.46	14.99	.22	.64
	Resveratrol	46	62.78	14.68	63.31	13.90		
Stress	Placebo	44	41.62	13.22	39.31	13.49	.47	.49
	Resveratrol	46	37.74	12.90	38.16	13.89		
Tranquillity	Placebo	44	65.86	15.47	66.98	14.22	.01	.89
	Resveratrol	46	69.00	12.39	68.87	12.28		

2.2.3.3.1.2. Interim mood assessments via Cognim^{app}

The analysis identified no significant effects of treatment for any VAMS output at any timepoint. See Table 2.25.

Table 2.25. Visual analogue mood scales outcomes for placebo and resveratrol treatment groups. Baseline (Day 0) raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects from ANCOVA analysis, split by day of assessment (Days 7, 14, 21 and 28).

		Baseline (Day 0)			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Day 7								
Alertness	Placebo	35	65.53	12.27	63.88	15.52	.23	.62
	Resveratrol	41	62.14	16.24	64.01	15.13		
Stress	Placebo	35	37.20	13.58	40.43	15.81	2.49	.11
	Resveratrol	41	36.33	13.56	36.32	13.33		
Tranquillity	Placebo	35	68.02	13.60	65.63	16.94	.32	.57
	Resveratrol	41	67.97	13.85	66.92	14.59		
Day 14								
Alertness	Placebo	35	65.53	12.27	65.07	15.49	.03	.84
	Resveratrol	41	62.14	16.24	64.27	13.13		
Stress	Placebo	35	37.20	13.58	36.21	14.24	.64	.42
	Resveratrol	41	36.33	13.56	38.15	13.15		
Tranquillity	Placebo	35	68.02	13.60	67.29	13.75	.27	.60
	Resveratrol	41	67.97	13.85	68.29	12.79		
Day 21								
Alertness	Placebo	35	65.53	12.27	63.64	15.84	1.19	.27
	Resveratrol	41	62.14	16.24	65.25	14.55		
Stress	Placebo	35	37.20	13.58	38.20	13.99	.37	.54
	Resveratrol	41	36.33	13.56	36.45	15.26		
Tranquillity	Placebo	35	68.02	13.60	65.83	16.16	.00	.94
	Resveratrol	41	67.97	13.85	65.82	13.50		
Day 28								
Alertness	Placebo	35	65.53	12.27	61.46	18.46	.37	.54
	Resveratrol	41	62.14	16.24	62.00	16.13		
Stress	Placebo	35	37.20	13.58	39.39	15.87	.05	.82
	Resveratrol	41	36.33	13.56	39.69	15.16		
Tranquillity	Placebo	35	68.02	13.60	65.16	16.04	.12	.72
	Resveratrol	41	67.97	13.85	64.11	16.29		

2.3.3.3.2. Profile of Mood Scales

The analysis identified a trend towards a significant effect of treatment for Anger-Hostility on Day 28 after controlling for Day 1 scores, $F(1,77) = 3.19$, $p = .078$, $d = .41$. With the resveratrol treatment group reporting feeling less angry (mean = .97) than the placebo group (mean = 1.33).

No additional significant effects of treatment were observed for any POMS outcome. See Table 2.26.

Table 2.26. Profile of Mood scales outcomes for placebo and resveratrol treatment groups. Baseline (Day 1) raw scores and post-dose (Day 28) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Tension-Anxiety	Placebo	45	5.60	4.57	4.98	4.48	.78	.37
	Resveratrol	46	5.64	4.65	4.65	4.20		
Depression-Dejection	Placebo	39	1.49	2.35	1.44	2.73	.13	.71
	Resveratrol	38	1.24	2.01	.84	1.55		
Anger-Hostility	Placebo	42	1.38	2.24	**1.33**	**2.24**	**3.19**	**.07**[t]
	Resveratrol	38	1.98	2.73	**.97**	**1.68**		
Vigour-Activity	Placebo	45	16.82	6.72	17.93	6.92	.00	.94
	Resveratrol	46	18.20	7.47	18.24	6.61		
Fatigue-Inertia	Placebo	45	5.42	4.07	5.13	4.43	.49	.48
	Resveratrol	45	4.31	3.72	4.29	4.54		
Confusion-Bewilderment	Placebo	45	7.98	4.74	6.36	3.89	.84	.36
	Resveratrol	46	7.06	4.76	6.87	5.34		
Friendliness	Placebo	45	15.80	4.38	16.07	3.57	.11	.74
	Resveratrol	38	16.14	4.19	15.95	3.48		
Total Mood Disturbance	Placebo	45	4.84	18.38	2.36	20.97	.54	.46
	Resveratrol	46	2.22	19.34	-.07	18.59		

2.3.3.4. Blood pressure

The analysis identified no significant effects of treatment for any blood pressure output at any timepoint. See Table 2.27.

Table 2.27. Blood pressure outcomes for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

			Baseline		Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Day 1 Acute								
Systolic	Placebo	50	112.40	13.98	112.40	13.98	.03	.85
	Resveratrol	50	113.54	13.60	113.54	13.60		
Diastolic	Placebo	50	73.34	9.98	73.34	9.98	2.46	.12
	Resveratrol	50	76.68	10.43	76.68	10.43		
Heart Rate	Placebo	50	65.98	11.35	65.98	11.35	1.62	.20
	Resveratrol	50	64.70	10.56	64.70	10.56		
Day 28 Acute								
Systolic	Placebo	47	114.49	12.56	116.85	13.24	.17	.68
	Resveratrol	47	114.49	12.98	116.11	12.64		
Diastolic	Placebo	47	72.87	10.70	76.55	10.55	.25	.61
	Resveratrol	47	76.57	9.82	78.70	10.00		
Heart Rate	Placebo	47	65.47	9.78	64.45	12.04	.84	.36
	Resveratrol	47	64.98	10.53	62.87	9.42		
Pure Chronic								
			Day 1 A1		Day 28 A1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Systolic	Placebo	47	112.40	13.98	114.49	12.56	.33	.56
	Resveratrol	47	113.54	13.60	114.49	12.98		
Diastolic	Placebo	47	73.34	9.98	72.87	10.70	.55	.45
	Resveratrol	47	76.68	10.43	76.57	9.82		
Heart Rate	Placebo	47	65.98	11.35	65.47	9.78	.31	.57
	Resveratrol	47	64.70	10.56	64.98	10.53		

2.3.3.5. BMI

The analysis identified no significant effects of treatment for BMI or weight change. See Table 2.28.

Table 2.28. BMI and weight for placebo and resveratrol treatment groups. Baseline (weight and BMI at training visit) raw scores and post-dose (Day 28) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects from ANCOVA analysis.

			Baseline		Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Weight	Placebo	47	72.51	16.60	72.43	16.31	.70	.40
	Resveratrol	47	70.60	10.79	70.87	11.07		
BMI	Placebo	47	25.71	5.08	25.83	5.17	.53	.46
	Resveratrol	47	24.98	3.29	25.12	3.37		

2.3.4. Biological Results

2.3.4.1. Blood samples

The analysis identified a significant effect of treatment on resveratrol-3-0-sulfate concentration on Day 1 after controlling for Day 1 baseline scores, $F(1,27) = 12.20$, $p = .002$, $d = 1.34$. With the resveratrol treatment group having a higher concentration (mean = 876.75 ng/mL) than the placebo group (mean = 507.87 ng/mL). The same significant effect was observed on Day 28 after controlling for Day 28 baseline scores, $F(1,20) = 7.24$, $p = .014$, $d = 1.20$. With the resveratrol treatment group having a higher concentration (mean = 1114.98 ng/mL) than the placebo group (mean = 548.41 ng/mL).

Additionally a significant effect of treatment on resveratrol-4-O-D-glucuronide concentration was observed on Day 1 after controlling for Day 1 baseline scores, $F(1,27) = 4.86$, $p = .036$, $d = .85$. With the resveratrol treatment group having a higher concentration (mean = 380.09 ng/mL) than the placebo group (mean = 242.74 ng/mL). The same significant effect was observed on Day 28 after controlling for Day 28 baseline scores, $F(1,23) = 5.65$, $p = .026$, $d = .99$. With the resveratrol treatment group having a higher concentration (mean = 424.92 ng/mL) than the placebo group (mean = 340.42 ng/mL).

A trend towards a significant effect of treatment was observed on triglyceride concentration on Day 1 after controlling for Day 1 baseline scores, $F(1,26) = 3.95$, $p = .057$, $d = .77$. With the resveratrol treatment group having a lower concentration (mean = 63.04 ng/mL) than the placebo group (mean = 80.95 ng/mL). This effect was identified as significant on Day 28 after controlling for Day 28 baseline scores, $F(1,23) = 5.25$, $p = .031$, $d = .96$. With the resveratrol treatment group having a lower concentration (mean = 79.48 ng/mL) than the placebo group (mean = 87.60 ng/mL).

Additionally, a trend towards a significant effect of treatment on cholesterol concentration was observed on Day 28 after controlling for Day 1 baseline scores (pure chronic effect), $F(1,22) = 4.31$, $p=.050$, $d = .89$. With the placebo treatment group having a lower concentration (mean = 100.99 ng/mL) than the resveratrol group (mean = 118.53 ng/mL).

A trend towards a significant effect of treatment on HDL concentration was observed on Day 28 after controlling for Day 1 baseline scores (pure chronic effect), $F(1,22) = 4.24$, $p = .051$. $d = .87$. With the placebo treatment group having a higher concentration (mean = 125.56 ng/mL) than the resveratrol group (mean = 85.01 ng/mL).

No additional significant effects of treatment were observed for any blood biomarker outcomes at any timepoint. See Table 2.29.

Table 2.29. Blood biomarker outcomes for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 28, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 28 baseline (assessment 1) estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Day 1 Acute								
Cholesterol	Placebo	15	95.10	33.21	93.53	34.65	.00	.95
	Resveratrol	14	96.84	28.78	95.20	37.82		
CRP	Placebo	14	43.61	16.99	42.96	9.26	.27	.60
	Resveratrol	14	39.83	10.12	41.15	9.62		
FRAP	Placebo	15	57.26	17.97	57.74	20.44	.08	.77
	Resveratrol	14	56.40	16.37	61.39	10.21		
Glucose	Placebo	14	61.24	13.78	64.09	20.73	1.47	.23
	Resveratrol	14	59.60	10.21	60.86	10.55		
HDL	Placebo	15	102.78	43.49	115.91	46.27	2.11	.15
	Resveratrol	13	94.62	32.17	93.49	28.42		
IL-6	Placebo	15	1.34	6.09	.00	.00	/	/
	Resveratrol	14	3.20	15.86	.00	.00		
LDL	Placebo	15	111.43	24.95	106.65	25.69	.05	.82
	Resveratrol	14	99.87	22.12	100.99	28.21		
Resveratrol-3-O-D-glucoside	Placebo	15	1730.05	792.04	1720.64	869.99	.00	.93
	Resveratrol	15	1519.43	758.30	1475.82	794.22		
Resveratrol	Placebo	16	320.44	137.45	314.73	141.75	1.24	.27
	Resveratrol	13	353.41	112.22	392.38	183.06		
Resveratrol-3-0-sulfate	Placebo	16	374.31	228.83	**412.71**	**231.63**	**12.20**	**.002***
	Resveratrol	14	425.79	306.68	**876.75**	**507.87**		
Resveratrol-4-O-D-glucoronide	Placebo	16	259.05	127.44	**242.74**	**115.87**	**4.86**	**.03***
	Resveratrol	14	288.31	169.29	**380.09**	**208.95**		
Triglycerides	Placebo	15	79.21	55.28	**80.95**	**40.57**	**3.95**	**.05[t]**
	Resveratrol	14	80.87	61.94	**63.04**	**25.45**		
Day 28 Acute								
Cholesterol	Placebo	14	107.36	33.70	104.21	34.94	1.41	.24
	Resveratrol	12	102.40	36.18	107.83	28.89		
CRP	Placebo	10	46.65	17.32	40.13	7.97	.47	.50
	Resveratrol	11	42.16	11.99	42.87	11.35		
FRAP	Placebo	14	59.02	20.14	58.41	30.51	.00	.94
	Resveratrol	12	60.94	23.67	63.24	11.72		
Glucose	Placebo	14	63.79	18.39	70.53	23.27	.81	.37
	Resveratrol	12	68.94	22.34	68.47	20.02		
HDL	Placebo	14	109.50	51.90	89.98	45.21	.92	.34
	Resveratrol	12	91.67	27.39	104.97	40.88		
IL-6	Placebo	14	3.05	16.82	.00	.00	1.17	.28
	Resveratrol	12	.23	1.13	.76	.26		
LDL	Placebo	14	111.62	25.81	108.11	30.18	.42	.52
	Resveratrol	12	100.00	32.45	109.13	28.34		
Resveratrol-3-O-D-glucoside	Placebo	33	Technical issues with analysis				/	/
	Resveratrol	25						

Resveratrol	Placebo	14	410.35	190.47	398.57	105.52	1.73	.20
	Resveratrol	12	371.34	188.94	425.46	186.71		
Resveratrol-3-0-sulfate	Placebo	12	535.06	223.41	**548.41**	**167.37**	**7.24**	**.01***
	Resveratrol	11	626.26	298.84	**1114.98**	**630.81**		
Resveratrol-4-O-D-glucoronide	Placebo	14	259.05	127.44	**340.42**	**124.96**	**5.65**	**.02***
	Resveratrol	12	288.31	169.29	**424.92**	**255.70**		
Triglycerides	Placebo	14	75.54	46.75	**87.60**	**48.05**	**5.25**	**.03***
	Resveratrol	12	90.80	56.01	**79.48**	**53.85**		
		Pure Chronic						
		Day 1 S1			Day 28 S1		Main Effects	
		n	Mean	SD	Mean	SD	F	p
Cholesterol	Placebo	13	95.10	33.21	**100.99**	**27.74**	**4.31**	**.05^{t}**
	Resveratrol	12	96.84	28.78	**118.53**	**21.93**		
CRP	Placebo	13	43.61	16.99	51.11	23.00	1.37	.25
	Resveratrol	12	39.83	10.12	41.70	11.05		
FRAP	Placebo	13	57.26	17.97	52.81	20.30	.36	.55
	Resveratrol	12	56.40	16.37	61.63	24.52		
Glucose	Placebo	13	61.24	13.78	70.14	16.92	.18	.66
	Resveratrol	12	59.60	10.21	76.58	26.18		
HDL	Placebo	13	102.78	43.49	**125.56**	**61.55**	**4.24**	**.05^{t}**
	Resveratrol	12	94.62	32.17	**85.01**	**29.89**		
IL-6	Placebo	13	1.34	6.09	.00	.00	/	/
	Resveratrol	12	3.20	15.86	.00	.00		
LDL	Placebo	13	111.43	24.95	121.13	26.32	1.15	.29
	Resveratrol	12	99.87	22.12	98.59	42.40		
Resveratrol-3-O-D-glucoside	Placebo	22	1730.05	792.04	1969.81	713.70	.46	.49
	Resveratrol	18	1519.43	758.30	1891.46	862.50		
Resveratrol	Placebo	13	320.44	137.45	422.79	125.26	.47	.49
	Resveratrol	12	353.41	112.22	359.38	239.63		
Resveratrol-3-0-sulfate	Placebo	11	374.31	228.83	547.79	151.18	.39	.53
	Resveratrol	11	425.79	306.68	661.46	408.49		
Resveratrol-4-O-D-glucoronide	Placebo	13	259.05	127.44	339.53	116.03	.85	.36
	Resveratrol	12	288.31	169.29	283.57	153.99		
Triglycerides	Placebo	13	79.21	55.28	87.49	40.91	.084	.77
	Resveratrol	12	80.87	61.94	93.02	57.17		

2.4 Discussion

The aim of the current study was to investigate the potential for resveratrol to act as a cognitive enhancer in a more diverse demographic than previous investigations. Where previous work has suggested that acute supplementation in young, healthy adults is ineffective; it was hypothesised that cognitive enhancing effects may be clearer within a more diverse demographic group (both in terms of age and weight status). Within this group it was proposed that a range of inflammatory statuses would be observed; where resveratrol supplementation would have the potential to reduce inflammation and in turn, enhance cognitive performance.

Overall, as summarised in Table 2.30 the findings from the current study showed significant effects of resveratrol supplementation on choice reaction time accuracy (Day 21 cognimapp assessment); subjective ratings of tranquillity (Day 1) and concentrations of resveratrol-3-O-

sulfate (Day 1 and Day 28), resveratrol-4-O-D-glucuronide (Day 1 and Day 28) and triglycerides (Day 28). Additionally, trends towards significant effects were observed for reaction time and false alarms for RVIP (Day 28); ratings of mental fatigue (pure chronic effect); correct and target reaction time during picture recognition (Day 7 cognimapp assessment); anger/hostility POMS outcome; and triglycerides (Day 1). However, significant effects of placebo supplementation were observed on total number of subtractions of threes (Day 28); overall congruent and correct congruent reaction time during stroop (Day 1); overall reaction time and 'No' reaction time during word recognition (Day 1); correct and overall reaction time for picture recognition (Day 28 cognimapp assessment); and concentrations of cholesterol and HDL (pure chronic effects). No significant effects of treatment were identified during the completion of immediate word recall, corsi blocks, subtractions of sevens, delayed word recall tasks or episodic memory, overall accuracy and overall speed domains on study visit assessments. Nor were any significant treatment effects observed during completion of numeric working memory, stroop, overall accuracy and overall speed cognitive domains and VAMS during interim mobile phone assessments. Additionally, no treatment effects were observed for any blood pressure outcomes, BMI or concentrations of CRP, FRAP, glucose, LDL, resveratrol or resveratrol-3-O-D-glucoside nor on inflammation (IL-6).

Table 2.30. Summary of study findings. Summarising all significant and trending towards significant findings for all outcome measures from the study. Split by results in favour of resveratrol and placebo treatment groups. ↑ = increased score. ↓ = reduced score. * = significant (p<.005). [t] = trend towards significant

Outcome measure	500 mg Resveratrol	Placebo
	COMPASS Assessments	
Serial 3 subtractions	/	↑ Total Subs Day 28 (Acute)*
Rapid Visual Information Processing	↓ Correct RT Day 28 (Acute)[t] ↓ False Alarms Day 28 (Acute)[t]	/
Mental Fatigue VAS	↓ Mentally fatigued Day 28* (Pure Chronic)[t]	/
Stroop	/	↓ Overall Congruent RT Day 1 (Acute)* ↓ Correct Congruent RT Day 1 (Acute)*
Delayed Word Recall	/	↓ Overall RT Day 1 (Acute)* ↓ 'No' RT Day 1 (Acute)*
	Interim Cognitive Assessments (Cognim^{app})	
Choice Reaction Time	↑ Overall Accuracy Day 21*	/
Delayed Picture Recognition	↓ Correct RT Day 7[t] ↓ Target RT Day 7[t]	↓ Correct RT Day 28* ↓ Overall RT Day 28[t]
	Mood	
Visual Analogue Scales	↑ Tranquillity Day 1 (Acute)*	/
Profile of Mood States	↓ Anger-Hostility Day 1 (Acute)[t]	/
	Blood Biomarkers	
Cholesterol	/	↓ Day 28 (Pure chronic)[t]
HDL	/	↑ Day 28 (Pure chronic)[t]
Resveratrol-3-0-sulfate	↑ Day 1 (Acute)* ↑ Day 28 (Acute)*	/
Resveratrol-4-O-D-glucoronide	↑ Day 1 (Acute)* ↑ Day 28 (Acute)*	/
Triglycerides	↓ Day 1 (Acute)[t] ↓ Day 28 (Acute)*	/

As with previous work, the current study provides limited support for a cognitive enhancing role of resveratrol. Despite some treatment related effects in favour of resveratrol, much of these are limited to trends towards significance and there are no effects observed when considering global cognitive domains; interpretation of which can often cut through the 'noise' of individual task performance. Additionally, much of the significant treatment effects observed show reduced performance following resveratrol supplementation. Despite this, there was some limited evidence of modulation of subjective mood with an increase in rates of 'tranquillity' and trends towards reductions of 'mental fatigue' and 'anger/hostility' following resveratrol supplementation. However, in absence of clear cognitive enhancing effects, this study provides little support of resveratrol ability to modulate cognition within this demographic.

The cognitive tasks utilised within this study include those used previously (Eschle et al., 2020; Kennedy et al., 2010; Wightman et al., 2019; Wightman, Haskell-Ramsay, Reay, et al., 2015), with the inclusion of the novel interim task administration over Cognimapp. As previous research has failed to observe clear cognitive enhancing effects, the rationale behind incorporating this range of tasks was to provide a broad assessment over a range of cognitive domains via the newly available Cognimapp platform. Despite this, the current study supports the lack of cognitive findings that have been observed previously in younger cohorts. As with young, healthy participants, a potential explanation for the null findings in this demographic is that they might not be sufficiently cognitively compromised by the tasks alone, as they are likely to be near the peak of their cognitive abilities. Previously, studies have aimed to overcome this by increasing the cognitive demand, either by increasing the length of cognitive assessments, or by repeating assessments over a longer period. This study employed a number of tasks; however, each assessment was over a relatively short time frame (30 minutes) and repeated just once on each testing visit. This design was for two reasons: firstly, it was hypothesised that the older and more diverse demographic were more likely to be cognitively compromised than those previously investigated and secondly, for practical purposes; as this design allowed for two cohorts of participants each morning. However, based on the lack of findings it seems probable that a more cognitively demanding paradigm is necessary to observe cognitive enhancing effects in a healthy cohort.

Considering specifically here, the interim cognitive assessments administered via Cognim^{app} during the supplementation period. The novel addition of interim cognitive assessments to this field of research has numerous advantages, when used as a complementary addition, alongside more traditional assessment methods in this field of research. These include: (1) reducing the requirement for participants to attend lengthy in person assessment visits, (2) the capability to monitor treatment response more often, (3) reducing retrospective recall bias,

specifically here, in measurement of mood outcomes which often rely on participants responding to questions relating to the previous month, (4) the ability to assess treatment-related changes in mood and cognitive performance in real-world settings. However, as an emerging direction of research, it is necessary to also acknowledge limitations to this methodology and considerations that should be taken it the future when adopting this approach.

Here it is important to consider that traditionally, cognitive assessments are delivered in highly controlled, artificial environments which do not reflect “typical” day-to-day cognitive functioning, which is a key advantage to remote cognitive assessment. Nevertheless, the traditional highly-controlled approach, allows more certainty that treatment-related effects can be attributed to the intervention only, by controlling for extraneous factors including, but not limited to, (1) caffeine intake, (2) consumption of other food products or supplements, (3) noise and distraction from tasks, (4) time of day assessments completed, (5) consistency between all participants in the trial. It must be noted that whilst the flexibility in remote delivery is advantageous to research participants; the completion of cognitive tasks in an unsupervised environment has its disadvantages. Explicitly here, it does not allow for supervision of the level of focus and engagement to cognitive tasks, likewise, there is a greater likelihood of task distraction; resulting in reduced performance on tasks (as reviewed further in Moore, Swendson, & Depp, 2017).

A key limitation of the present trial is that in the interest of ensuring engagement in the remote aspect of the trial, participants were allowed to complete the assessments at any time of day that suited them. Whilst this decision was made to encourage completion of the remote assessments, this did not account for vast differences in assessment completion time within and between participants. As detailed further within Weizenbaum, Torous and Fulford (2020), the time of day, has been previously shown to impact cognitive performance due to internal circadian rhythms. With a key review paper, suggesting time of day had a significant impact on performance of numerous cognitive tasks, relating to attention, executive functioning and memory (Schmidt, Collette, Cajochen & Peigneux, 2007). As such, future trials incorporating mobile assessments, should ensure they employ tighter restriction on assessment completion time, overcoming the oversight in design within the current trial.

Moreover, whilst participants were instructed to complete the short assessments in a quiet location, free of distractions; the unsupervised nature of remote assessments makes it difficult to assess the engagement of participants. Surrounding noise also raises its own issue, with well documented links between auditory distraction and impaired cognitive performance

(detailed within Weizenbaum et al., 2020); the current remote assessment technology relies on participants following instruction to complete assessments alone in quiet locations, rather than somewhere exposed to irregular, distracting noise for example on public transport. Furthermore, completion on a participants own mobile phone raises its own issues, where not only can participants be distracted by their environment, but also by notifications on their phone, during completion of tasks. Recent literature has suggested objective measures of effort could be utilise eye-tracking sensors during assessment completion; measurement of ambient noise during mobile assessment could also be conducted via the phone's microphone, to determine audio distractions during assessment completion; these however come with the obvious cost of technological development and potentially concerns of privacy and security from participants (Moore et al., 2017; Weizenbaum et al., 2020).

A further relevant point to consider is that validation work within the Brain, Performance and Nutrition Research Centre (unpublished data), employed Cognim^{app} assessments completed alongside COMPASS assessments, with both completed within the same laboratory-based setting. Therefore, whilst this data indicated that the mode of cognitive task delivery had no impact on cognitive performance in the research centre; this work did not account for the above external factors, stressing the requirement of additional validation when utilising the software away from the research centre. Therefore, whilst the incorporation of remote cognitive assessments is an exciting step-forwards in the field; the above highlights that we are still in the early stages of development and whilst there is certain promise of the possibilities for mobile cognitive assessments to change the way research is conducted, there are numerous important factors that must be considered when incorporating this into future paradigms.

Whilst the anti-inflammatory potential of resveratrol has been documented previously, specifically the ability to inhibit pro-inflammatory signalling cascades (Spencer et al., 2012); this study failed to observe any treatment effects on markers of inflammation (IL-6 and CRP). Although previous studies have observed reductions in these markers (or similar) following resveratrol supplementation (BaGen et al., 2018; Militaru et al., 2013; Shi et al., 2017); supplementary studies have not observed an anti-inflammatory effect despite prolonged resveratrol supplementation (Kjær et al., 2017; Yoshino et al., 2012). Indeed, recent meta-analyses have indicated that to observe anti-inflammatory effects, study designs should incorporate a dosage of ≥500 mg per day over a period of ≥10 weeks (Gorabi et al., 2021; Omraninava et al., 2021), therefore a longer supplementation period may be necessary to exert an anti-inflammatory effect.

It is important to note a few points here. The first is that due to physiological differences in the vasculature of older (in this case, older than the 18–35-year-old cohorts historically utilized in this area) participants, the inability to collect intravenous blood samples resulted in a lower sample size for this aspect of the study than anticipated and the relatively small, heterogeneous sample achieved could have been insufficient to observe changes. Secondly, due to the Covid-19 pandemic, analysis of biological samples within this thesis were significantly delayed. As such, serum samples collected within this trial were stored at -80°C for approximately 2 years. Whilst, to the best of our knowledge there is no literature to suggest storage of this length will reduce stability of the samples, it is nevertheless a potentially important consideration, which could account for this observed lack of findings. Lastly, IL6 may have been an insensitive marker to inflammatory changes in this cohort. Here, analysis was unable to detect low concentrations in samples and this leads to two possibilities. The first is that some methodological difference in the analytical process rendered the technique as less sensitive to IL6 than in the previously mentioned resveratrol trials. The second is that IL6 is simply not an appropriate barometer of inflammatory changes in this paradigm (either due to the intervention, the procedure of the study (e.g. too short an intervention period) and/or the demographic used (e.g. inflammation was not sufficiently compromised for IL6 to be actioned)).

Similarly, whilst this study assumed that a more diverse demographic would result in a broad range of inflammatory statuses, a larger understanding of individuals inflammatory profile would have been beneficial on enrolment to the study. Here, a greater understanding of inflammatory status of participants could be used in the future for the stratification of participants and to investigate the effects of resveratrol supplementation in individual inflammatory groups. This study also did not account for individual differences that may impact upon inflammatory status, irrespective of body mass index. Specifically, considering individual dietary profiles, particularly consumption of dietary elements such as anti-inflammatory polyphenols, which may provide a protective effect on inflammation, regardless of BMI status. The relatively high consumption of fruit and vegetables and baseline plasma resveratrol metabolite levels in both treatment groups, certainly suggests that participants enrolled in the study consume a fairly healthy diet and therefore may not be the best candidates to benefit from resveratrol supplementation. Supplementary work should collect information on participants dietary habits, to investigate this further.

This study also indicated mixed findings in terms of the impact of resveratrol supplementation on biomarkers of cholesterol; with significant reductions in triglycerides observed but increases in total cholesterol and reductions in HDL. Here, a typical beneficial response would

be reductions in total cholesterol, LDL and triglycerides, paired with an increase in HDL (Arsenault et al., 2009). Reductions in triglycerides is consistent with previous work, specifically in animal models (Andrade et al., 2014; Cho et al., 2012) and in humans when presented as a nutraceutical formula (with epigallocatechin-3-gallate, quercetin and grape extracts) (Most et al., 2016; Qureshi et al., 2012; Tomé-Carneiro et al., 2012) and in isolation in obese individuals (Timmers et al., 2011) and those with dyslipidaemia (Simental-Mendía & Guerrero-Romero, 2019). In contrast to the current study findings, resveratrol supplementation has been previously shown to decrease total cholesterol (Bhatt et al., 2012; Simental-Mendía & Guerrero-Romero, 2019). However, several studies have not observed any cholesterol lowering effects (Dash et al., 2013; Haghighatdoost & Hariri, 2018; Sahebkar, 2013; van der Made et al., 2015; Javid et al., 2017). Importantly though, many of these studies were conducted in those with metabolic disease such as diabetes and hypertriglyceridemia. Therefore, it is difficult to predict a lipid response to resveratrol supplementation in healthy individuals and additional research should be conducted to examine the effect of resveratrol supplementation in isolation on lipid profiles in individuals without metabolic disease.

To date, no beneficial cognitive effects have consistently been observed in healthy adults and so it seems that we can conclusively determine that 500 mg resveratrol supplementation has no cognitive enhancing abilities within this cohort. Instead, recent evidence appears to suggest more positive findings in more compromised demographics, when supplemented over an extended period (>10 weeks) (Anton et al., 2018; Evans et al., 2017; Zaw et al., 2020a, 2020b). As such, it seems probable that resveratrol supplementation is likely to be most effectively when administered over a longer period to older participants. It may well be the case here that this model reflects a truer picture of inflammatory activity and damage which resveratrol could be called upon to ameliorate.

More, generally a potential limitation to the current study is the compliance calculations employed; here in line with standard procedure within the Brain, Performance and Nutrition Research Centre and other similar research centres, participants were included within the data analysis providing their treatment compliance was in the range of 80-120%. As detailed within Section 2.2.3., the use of this criteria meant that the analysis included participants who under consumed (by less than 9 capsules total) or over-consumed (by more than 11 capsules total) the intervention, compared to that set out within the protocol (56 capsules total). However, it could be argued that a smaller compliance criterion should be employed to reduce the amount of over- and under- consumption here and ensure that the data included within analysis was more reflective of the 28-day supplementation period outlined within the protocol. A secondary option here, would be to employ a placebo run-in to the design of future trials, in an effort to

pre-empt compliance issues. Here, participants would be required to complete an initial placebo run in into the trial and providing their compliance reached a predetermined criterion, they would then be eligible to continue with the trial intervention period. This addition would potentially improve compliance within the intervention period, by only randomising participants engaged and compliant with the trial procedures.

A further potential limitation to the current trial, and relatedly, the data presented within Chapter 4, is in the statistical approach. Here, the current approach was chosen as it allowed for analysis of: Acute treatment effects within Day 1 (40 minute post-dose assessment, using Day 1 pre-dose baseline data as a covariate); Acute treatment effects within Day 28 only (40 minute post-dose assessment, using Day 28 pre-dose baseline data as a covariate); to explore the chronic effects of resveratrol following 28 days administration, the pure chronic analysis which assessed the Day 28 data (pre-dose, 40 minutes post dose, using Day 1 pre-dose baseline data as a covariate). Upon reflection, Linear mixed models could be a more advantageous approach rather than ANCOVAs as they have both the ability to model non-linear data and also account for missing data points (Krueger & Tian, 2004), often encountered in chronic intervention trials and particularly relevant in this trial with the inclusion of mobile assessments.

Nevertheless, there are a number of strengths of this research which should be noted. The first is the interdisciplinary nature of the trial design and the number of participants achieved in order to assess the interrelationships between quite a significant number of outcome measures. The current study also addressed several limitations of previous RCTs; including supplementation duration, increasing diversity in age and weight status in participant demographics, as well as measuring a range of blood biomarkers at each testing visit and measuring cognitive performance and mood weekly during the supplementation period. It is also beneficial to this field of research, which encompasses wider phenolics, that null effects were observed in this cohort on these measures. In line with the historical lineage of resveratrol studies here, this adds more support to the finding of null effects in young, healthy humans irrespective of any perceived biological compromise and adds support for the investigation of genuinely compromised models.

In this vein, future research within this area should investigate the potential cognitive enhancing effect of resveratrol in older individuals; who likely have higher inflammatory status due to age, and to focus also on obesity within this model. Investigations of supplementation administered over ≥10 weeks, and more cognitively demanding paradigms (both in terms of length of assessments and number of repetitions throughout the visit) would also be

warranted. Additionally, based on a recent change in focus in the literature and the rapid increase in interest in the gut microbiota; specifically, the individual differences in metabolism and impact of polyphenols on gut profile, the effect of resveratrol on gut microbiota composition must be considered. Here, modulation of the gut microbiota via resveratrol supplementation may have the ability to improve cognitive performance via the gut-brain-axis. It is imperative that this link is investigated further, to develop this field of work.

CHAPTER 3.
PHENOLIC MODULATION OF GUT MICROBIOTA

3.1. Gut microbiome

The following chapter presents data, specific to the rationale of this thesis, on the ability of phenolic compounds to affect the gut bacterial environment. To support this, some tangential dietary data will be drawn upon but this is by no means exhaustive and is incorporated to support the specific hypothesis that polyphenols may be regulated quite significantly by the gut environment, that they, in turn, can significantly influence the gut environment, and the impact that this may have on brain function in particular.

3.1.1. Background

The gastrointestinal (GI) tract, contains a microbial community populated by approximately 100 trillion microorganisms (primarily bacteria), which is collectively known as gut microbiota (Peterson & Artis, 2014). The diversity and quantity of bacteria vary along the gastrointestinal tract and are influenced by differences in host genetics and by interactions with the external environment, resulting in fluctuating inter-individual differences in composition (Brown, Sadarangani, & Finlay, 2013; Serra et al., 2018).

A close symbiotic relationship between humans and their microbial systems has been created over thousands of years of coevolution (Gowd et al., 2019); meaning that the composition and stability of the gut microbiome tightly regulates the physiological homeostasis of the human body. Importantly, the microbiota regulates many physiological processes including protecting against pathogens, maintaining the immune system and intestinal barrier integrity, food digestion, nutrient uptake and metabolism and producing short-chain fatty acids (SCFAs) (Barko, McMichael, Swanson, & Williams, 2018; Festi et al., 2014; Gérard, 2016; Sekirov, Russell, Antunes, & Finlay, 2010).

It seems axiomatic that this interaction with such a broad range of mechanisms would result in significant health effects. However, whilst the interrelationship between the gut microbiome and health was first hypothesised over 100 years ago by Elie Metchnikoff, it is not since the past 20 years that there has been a resurgence in interest (Mackowiak, 2013). Now, abnormalities in the microbiota are widely recognised to play a critical role in the aetiology and development of many chronic diseases (Patterson et al., 2016). However, what constitutes an 'abnormality' and, conversely, what a healthy gut looks like is less well known. With the

relatively recent increased interest in the gut microbiota and human health, the aim is to understand what bacterial composition can be classified as a 'healthy gut' and, subsequently, how this increased knowledge can be utilised to improve human health; including via targeted therapies for diseases (Chaplin et al., 2018) and, of importance here, whether diet and dietary supplementation could represent such a therapy.

3.1.2. Microbial composition

The number of microorganisms within the gut microbiome outnumber human cells, initially estimated at a ratio of 1:10 (Luckey, 1972) although more recent approximations suggest that the ratio of host-to-bacterial cells are much closer to 1:1 (Thursby & Juge, 2017). To date, most research on microorganisms and the interaction with the host has concentrated on the intestinal lumen, as this is the most densely colonised, hosting approximately 100 trillion bacteria (Frank & Pace, 2008).

The microbiome ecosystem comprises small concentrations of archaea, funghi, protozoa and viruses, alongside much larger quantities of bacteria (Dinan, Stanton, & Cryan, 2013). The thousands of species and strains of bacteria which form the microbiome are classified according to phyla, classes, orders, families, genera and species (Rinninella, Cintoni, et al., 2019). The most dominant of the gut microbial phylum are Firmicutes and Bacteroidetes, which represent approximately 70-90% of the community (Qin et al., 2010; Rinninella, Cintoni, et al., 2019), together with smaller concentrations of Actinobacteria, Fusobacteria, Proteobacteria and Verrucomicrobia (Arumugam et al., 2011). The Firmicutes phylum consists of more than 200 different genera, with *Clostridium* representing the largest proportion. This phylum also includes *Lactobacillus, Enterococcus, Ruminococcus* and *Faecalibacterium* (Healey, Murphy, Brough, Butts, & Coad, 2017). The phylum Bacteroidetes predominant genera includes *Bacteroides, Alistipes* and *Prevotella.* The phylum in lower abundance is comprised of the following genera: Actinobacteria (*Bifidobacterium),* Verrucomicrobia (*Akkermansia)* and Proteobacteria *(Escherichia)* but it is important to note that lower abundance does not necessarily denote lower importance or research interest; indeed some of the latter bacterial species like Akkermansia are more well-known than some of those with relatively greater abundance.

Initially it was thought that by assessing the proportion of specific bacterial taxa within the gut microbiome, humans could be divided into three main enterotypes that focus on the particular genera's: *Bacteroides, Prevotella* and *Ruminococcus* (Arumugam et al., 2011). Interestingly, these groupings have been associated with certain dietary lifestyles. For example, the

Bacteroides enterotype is linked to a diet rich in protein and animal fat, whereas the *Prevotella* enterotype is related to a diet high in carbohydrates (Wu et al., 2011). However, more recently it has been suggested that these broad enterotypes are not representative of the differing microbial communities within the human population and it has been proposed that the use of "biomarkers" is a more accurate term to overcome this (Gorvitovskaia, Holmes, & Huse, 2016).

More recently, it has been shown that gut bacteria composition can be modified by many external factors, specifically method of birth, diet, lifestyle and medication use (Lankelma, Nieuwdorp, de Vos, & Wiersinga, 2015), meaning that the composition of an individual's microbiome varies across the lifespan (Knights et al., 2014). Due to the aforementioned inter-individual variability and the malleability of the gut microbiota, to date, it is still debated as to what defines a "healthy" or optimal gut microbiota composition. Generally, however, markers of microbial stability, which includes richness and diversity are agreed as indicators of gut health (Cotillard et al., 2013a; Rinninella, Raoul, et al., 2019).

3.1.3. Origin and development across the lifespan

For many years the gut of a foetus *in utero* has been presumed to be sterile, with the initial colonisation occurring during vaginal birth (Gowd et al., 2019). However, more recently the relatively controversial idea of a prenatal microbiome has been suggested; as specific microbiota relating to the condition of the infant has been identified from human meconium (stool which forms in the foetus *in utero*) (Moles et al., 2013; Stout et al., 2013), indicating that the maternal microbiome may have an impact on the development of the foetus' prenatal microbiome.

In terms of evolution, humans have experienced a sudden shift in lifestyle, in a relatively short time-frame, in recent history. Here, the impact of an excessively hygienic, modern developed world, introduction of industrialised diet, high levels of sedentarism and modern medicine such as antibiotics, have been shown to be detrimental to the composition of the microbiome. Specifically, medical advances that are used to reduce infant mortality, including caesarean sections, formula-feeding and early-life exposure to antibiotics has been examined. There are distinct microbial differences from rectal swabs between vaginally-delivered infants and those born via caesarean section (Adlerberth et al., 2006). This disparity has been presumed to be due to the lack of initial exposure to the vaginal microbiome during birth, after the lack of microbial exposure in the womb, although this does not consider the recent evidence indicating a prenatal microbiome. Similarly, breastfed individuals have lower concentrations of *Escherichia coli* and *Clostridium difficile* (Penders et al., 2005) and higher concentrations of

Bifidobacterium concentrations (Fallani et al., 2010), when compared with formula fed infants. The use of antibiotics in early life, particularly in the first month, has been shown to have detrimental effects on the colonisation of the microbiome and, specifically, reductions in concentrations of Bifidobacterium and *Bacteroides fragilis* groups have been shown here (Healey et al., 2017). Antibiotic-induced microbial disruption has been linked to implications on the metabolic functionality of the host with evidence suggesting that this is associated with overweight and obesity in childhood (Bailey et al., 2014), alongside an increase in likelihood of developing allergies, eczema and asthma in childhood (Loewen, Monchka, Mahmud, & Azad, 2018).

There appear to be critical periods of microbial development in early life which is thought to play a substantial role in the maturation of the immune system; including the development of autoimmune and inflammatory diseases (Healey et al., 2017). Following birth, dramatic changes initially occur with lactation and then again after the introduction of solid foods (Tanaka & Nakayama, 2017). During these initial stages in the first 2-3 years of life, the infants microbial composition is highly malleable and has a low bacterial diversity (Voreades, Kozil, & Weir, 2014). It has been shown that disturbances in this period are linked to a higher risk of autoimmune disease and metabolic disturbances in later life (Francino, 2014). The microbial colonisation becomes relatively stable by age 3-5 years, where it becomes similar to an adult microbiome and, at this point, the composition is harder to disrupt; although environmental factors such as diet and antibiotics can still impact it (Uhr, Dohnalová, & Thaiss, 2019). As the human body ages, the diversity and stability of the microbiota declines and larger inter-individual variations are observed in the elderly; hence it is suggested that the impact of the gut microbiome on health and disease is more apparent in older age groups (Borre et al., 2014; Claesson et al., 2012). This reduction in microbial diversity has been consistently associated with unhealthy aging and frailty (Jackson et al., 2016; O'Toole & Jeffery, 2015). Suggesting that it is crucial to maintain a healthy gut microbiota to support healthy aging (Dinan & Cryan, 2017).

3.2. Gut microbiome and health

3.2.1. Dysbiosis

Although becoming an increasingly contentious term, dysbiosis is understood to denote an alteration in the composition and homeostasis of the microbiota. It is not thought to follow a definitive pattern, but is instead categorised as a change from the norm which results in the loss of microbiota stability (Zaneveld, McMinds, & Thurber, 2017). In particular, it is suggested that there is a disturbance between the balance of the beneficial and pathogenic bacteria

(Chan, Estaki, & Gibson, 2013). Dysbiosis is believed to include three different elements, which can occur alone or simultaneously: loss of beneficial organisms; growth of potentially harmful bacteria; and loss of overall microbial diversity (Peterson, Sharma, Elmén, & Peterson, 2015).

Clinical evidence suggests that a stable, rich and diverse microbial composition is essential for optimal human health and, in contrast dysbiosis has been linked to many chronic diseases; including inflammatory bowel disease (IBD) (Walters, Xu, & Knight, 2014), colon cancers (Ohigashi et al., 2013), obesity (Ley, Turnbaugh, Klein, & Gordon, 2006) and type 2 diabetes mellitus (Wu et al., 2010). However, although consistent research supports this link, it is currently difficult to determine whether there is a casual link between the dysbiosis of the gut microbiota and the development of these diseases or, conversely, if the dysbiosis is a result of the disease states. There is also a potential that the dietary patterns and medications associated with these diseases may be a contributing factor (Healey et al., 2017).

3.2.2. Intestinal barrier and the immune system

The intestinal barrier has two crucial roles; to allow nutrient absorption and to protect and defend the body from the potentially dangerous microorganisms that constantly challenge the GI tract (Rinninella, Raoul, et al., 2019). The separation that the barrier provides between the body and the gut prevents the translocation of luminal contents into systemic circulation and also protects the mucosal tissues and circulatory system from microorganisms and toxins (Turner, 2009).

The intestinal mucosal barrier comprises both external physical and inner immunological elements, which both have different roles (Bäckhed, Ley, Sonnenburg, Peterson, & Gordon, 2005; Neish, 2009). The outer anatomical barrier is made up of commensal gut microbiota, the mucus layer and the intestinal epithelial monolayer. The mucus layer is formed of mucin (protein cells which form cell barriers) molecules and provides a separation between the majority of the luminal contents and the intestinal epithelium, by allowing small molecules to pass through and preventing large particles from contacting the epithelial cell layer (Johansson et al., 2008).

The intestinal epithelial layer is considered the principal component of the intestinal mucosal barrier and serves several crucial roles. In particular, it acts as a barrier to prevent the invasion of harmful substances including microorganisms, toxins and antigens (Groschwitz & Hogan, 2009) and must be constantly alert to monitor the extracellular and intracellular environment

in order to maintain homeostasis (Cario, 2010). In addition to these protective roles, the intestinal epithelium also controls the uptake of dietary nutrients, electrolytes and other beneficial substances from the lumen into the body (Sánchez de Medina, Romero-Calvo, Mascaraque, & Martínez-Augustin, 2014). Increasingly, evidence indicates a role for gut microbial species in the intestinal mucosal barrier. They can influence the maintenance of the epithelial barrier both directly and indirectly by producing antimicrobial substances, preventing pathogen invasion and producing short-chain fatty acids; which are a crucial energy source for epithelial cells (Fava, Rizzetto, & Tuohy, 2019; Sánchez de Medina et al., 2014).

The inner layer comprises a network of immune cells which are organised within a compartmentalised system which is known as gut-associated lymphoid tissue (GALT). As one of the largest lymphoid organs, GALT contains approximately 70% of the body's immunocytes (Rinninella, Cintoni, et al., 2019) and provides immune tolerance to commensal bacteria. In addition, it has an abundant role in the response to pathogenic microorganisms and, as they have specific mucosal immune cells (such as dendritic cells and M-cells), GALT is able to interact with luminal antigens. These then present antigens to T-lymphocytes which encourages the production of cytokines and the activation of mucosal immune responses (Liévin-Le Moal & Servin, 2006).

Alterations in the intestinal barrier function can be caused by many factors, including dysbiosis of the gut microflora, modification of the mucus layer and damage to the epithelial layer by diet (Mu, Kirby, Reilly, & Luo, 2017). This impairment in gut barrier function increases intestinal permeability (often referred to as "leaky gut"), allowing bacteria and bacterial products to enter into systemic circulation (Kelly et al., 2015). Crucially, the immune system detects this potentially pathogenic bacteria and triggers pro-inflammatory responses; which if sustained, can contribute to the pathogenesis and development of intestinal and other chronic diseases (Serra et al., 2018).

3.2.3. Intestinal barrier and inflammation

Whilst, short-term inflammatory response offers a protective effect on the body; the intestine develops strategies to avoid the hyper-stimulation of pro-inflammatory signalling pathways, alongside maintaining the signals of the gut microbiota to maintain intestinal homeostasis, in order to protect the intestinal tissue from irreparable damage (Abreu, Fukata, & Arditi, 2005; Peterson & Artis, 2014). Host intestinal epithelial cells and immune cells have specific receptors called pattern-recognition receptors (PRRs) which allow them to recognise microbe-associated molecular patterns (MAMPs), toll-like receptors (TLRs) and nucleotide

oligomerization domain (NOD)-like receptors (Maynard, Elson, Hatton, & Weaver, 2012). These receptors are able to trigger responses in the "self" and "non-self" recognition, to protect the integrity of the intestinal barrier and also maintain the microbiota composition.

Alteration of the intestinal barrier also causes the release of lipopolysaccharide (LPS; also known an endotoxin), into the bloodstream. LPS is released by the Gram-negative bacteria in the outer membrane and promotes macrophage recruitment and polarisation in white adipose tissue, and is transported into intestinal cells by binding to its toll-like receptor 4 (TLR4) (Cani, Osto, Geurts, & Everard, 2012; Neal et al., 2006). The stimulation of TLRs causes activation of the signalling cascade nuclear factor-kappa B (NF-κB), eliciting a pro-inflammatory response (Biasi, Leonarduzzi, Oteiza, & Poli, 2013). Specifically, when LPS enters into circulation, metabolic endotoxemia is elicited and causes the production of inflammatory cytokines and mediators; including C-reactive protein (CRP) which contributes to the chronic low-grade inflammation of the host (Libby, Okamoto, Rocha, & Folco, 2010). Importantly, LPS is able to disrupt the endocannabinoid system and this further increases the intestinal barrier permeability; leading to more LPS entering the bloodstream and exacerbating this process.

The mechanisms underlying the increased intestinal permeability and inflammation are unclear, but the gut microbiota is thought to play a critical role. Evidence suggests that the intestinal epithelium uses signalling form the gut microbiota, through TLR activation, to increase the production of mucus and the transportation of immunoglobulin A (IgA); which strengthens the barrier function and maintains the immune tolerance against the gut microbiota (Baumgart & Carding, 2007; Peterson & Artis, 2014). Further, evidence suggests that the microbial shifts that occur during aging may predispose individuals to inflammation. Age is correlated with increased intestinal permeability and cytokine expression; resulting in chronic systemic inflammation (Deleidi, Jäggle, & Rubino, 2015; Tran & Greenwood-Van Meerveld, 2013).

A recent systematic review of 14 human studies (comprising a total of 1418 individuals) has investigated the role of the gut microbiome in chronic low-grade inflammation (Van den Munckhof et al., 2018). Several studies stratified participants based on the number of genes within their gut microbiota and therefore the richness and diversity of the microbial communities; classifying them as 'low gene count' and 'high gene count'. Here a low gene count was associated with increased levels of a single inflammatory marker; CRP (Cotillard et al., 2013a; Le Chatelier et al., 2013). Studies have identified specific bacterial genera associated with levels of CRP; specifically lower levels of *Faecalibacterium, Ruminococcus, Faecalibacterium prausnitzii, Lactobacillus, Bifidobacterium* and *Spretococcus* have been

correlated with higher levels of CRP in a range of populations, which includes older and overweight adults (Claesson et al., 2012; Furet et al., 2010; Martínez et al., 2013; Rajkumar et al., 2014). Additional studies have indicated that the abundance of specific gut microbial species is related to pro-inflammatory cytokines; with total bacterial cell count positively related to circulating TNF-α (Tiihonen, Ouwehand, & Rautonen, 2010). Furthermore, lower levels of *Ruminococcus, Prevotella* and *F. prausnitzii* coincided with higher IL-6 levels (Claesson et al., 2012; Furet et al., 2010; Martínez et al., 2013) and, finally, both positive and negative correlations between cytokines IL-6 and IL-8 and LPS and specific microbial species have been observed (Biagi et al., 2010; Clemente-Postigo et al., 2013; Radilla-Vázquez et al., 2016).

Whilst the underlying mechanisms are still unclear, it is apparent that gut microbiome composition has an effect on the expression of inflammatory adipokines resulting in potentially chronic inflammation which, if prolonged, has been linked to the development of several chronic diseases.

3.2.4. Inflammatory bowel disease (IBD)

One such chronic disease, with a relatively greater abundance of research interest, is Inflammatory bowel disease (IBD). IBD is a group of idiopathic and chronic inflammatory disorders of the gastrointestinal tract; including ulcerative colitis and Crohn's disease, which are characterised by uncontrolled and exacerbated inflammatory and oxidative responses that lead to serious intestinal injury (Biasi et al., 2013). At present, the cause and development of IBD is unknown. However, it is suggested to be an interplay of genetic predisposition and environmental triggers including stress, diet and antibiotic intake.

The role of the gut microbiome seems axiomatic here and, indeed, research shows that there are clear differences in microbial composition between IBD patients and controls; with lower concentrations of Firmicutes and Bacteroidetes and increased abundance of Proteobacteria and Actinobacteria in those with the conditions (Buttó & Haller, 2016; Sartor & Mazmanian, 2012). In those with IBD, it has been shown that intestinal epithelial cells have a higher expression of some TLRs at their apical (facing the outer 'space' or lumen) side, which has the potential to increase the interaction with the gut microbiota (Abreu, 2010; Lavelle, Murphy, O'Neill, & Creagh, 2010). Due to this, intestinal epithelial cells potentially upregulate the expression of TLRs during intestinal inflammation upon stimulation with cytokines, such as TNF-α and IFN-ɣ (Abreu, 2010). It is suggested that this sustained dysregulation of TLR signalling and, consequently, the over-activation of NF-kB cascade, contributes to chronic

intestinal inflammation, leading to pathogenesis of IBD (Biasi et al., 2013; Maloy & Powrie, 2011).

3.2.5. Obesity

Over recent years, the role of gut microbiota composition on body weight control and the pathogenesis of obesity has been investigated (Bäckhed et al., 2004; Gomes, Hoffmann, & Mota, 2018). A key area of focus here is the investigation of the composition of gut microbiota associated with obesity, and specifically differences in composition between obese and lean subjects. Findings indicate that those with low microbial richness are more likely to develop obesity than those with a higher microbial richness (Erejuwa, Sulaiman, & Wahab, 2014; Le Chatelier et al., 2013; Tremaroli & Bäckhed, 2012).

Considerable, early research indicated that an obese individual profile, in animal models, can be characterised by a greater abundance of Firmicutes and less Bacteroidetes (Ley et al., 2005; Murphy et al., 2010; Turnbaugh, Bäckhed, Fulton, & Gordon, 2008). Whilst some work in humans supports the association with this ratio (Ley et al., 2006), other work does not (Annalisa et al., 2014; Kocełak et al., 2013; Koliada et al., 2017; Schwiertz et al., 2010). Indeed, one of these studies observed that overweight and obese participants had a Firmicutes/Bacteroidetes ratio that favoured Bacteroidetes (Schwiertz et al., 2010). The ratio between these two phyla and the causal relationship on the pathogenesis of obesity has been repeatedly challenged (Cani & Van Hul, 2020) and is often critiqued for being too simplistic; as these phyla account for 85-90% of the overall microbial community. Additionally, evidence suggests that not all bacteria within these phyla are found in all subjects (Castaner et al., 2018). It is likely that the influence of gut microbiome on obesity is much more complex than simply an imbalance in the proportion of these phyla of bacteria (Harley & Karp, 2012). Despite this, there is some evidence to indicate that some microorganisms in these phyla have a causal relationship with obesity; specifically *Anaerobutyricum soehngenii* and *Lactobacillus reuteri* in the Firmicutes phylum, as well as *Bacteroides acidfaciens* and *Bacteroides thetaiotaomicron* in the Bacteroidetes phylum (Shetty et al., 2018) as detailed in Li et al. (2021).

A recent systematic review of 32 human trials aimed to cut through this noise and determine if there are differences between gut microbiota profiles of lean and obese individuals (Crovesy, Masterson, & Rosado, 2020). They conclude that obese individuals had higher counts of *Firmicutes, Fusobacteria, Proteobacteria* and *Lactobacillus,* and lower counts of *Bacteroidetes, Akkermansia muciniphila, Faecalibacterium prausnitzii, Lactobacillus*

plantarum and *Lactobacillus paracasei.* Interestingly, their findings also supported the *Firmicutes/Bacteroidetes* argument.

These discrepancies imply that supplementary work is necessary to understand the obese microbiota composition. Moreover, whilst there is certainly evidence to indicate a link between microbiota dysbiosis and obesity, it is important to recognise that, at present, it is unclear if this is a cause or consequence of obesity (Ravussin et al., 2012). However, obesity is argued to be a transmissible trait, with specific microbial compositions causing fat deposits, as initially shown when germ-free mice who were colonised with an "obese gut microbiota" had a greater increase in body fat in comparison to those colonised with a "lean microbiota" (Turnbaugh et al., 2006). Similarly, it was shown that an obesity phenotype could be transmitted from humans into germ-free mice (Ridaura et al., 2013). Most recently, a study using faecal microbial transplantation to treat an intestinal infection, indicated that the obesity phenotype could be passed from the obese donor to the lean receiver; with the receiver gaining 16 kg in 16 months (Alang & Kelly, 2015). This has, however, been the first study to show obesity as a transmissible trait between humans and, therefore, suggests that additional studies are required to support this.

Alongside excessive accumulation of body fat, obesity is associated with chronic low-grade inflammation (Emanuela et al., 2012). Here, an increase in adipose tissue (fat) is associated with the release of proinflammatory adipokines, therefore promoting inflammation and macrophages (Calder et al., 2011). Research has indicated that this obesity-related systemic inflammation is at least partly driven by an alteration in the gut microbial composition and function (Bäckhed et al., 2004). Much of the work into obesity and inflammation has focussed on the role of a high-fat diet (HFD) due to the findings of epidemiological data which consistently shows that eating a HFD increases the development of obesity (Golay & Bobbioni, 1997). Reports have shown that a HFD in both humans and animals could promote the growth of LPS-producing microbiota including *Escherichia, Enterobacter* and *Desulfovibrionaceae* (Amar et al., 2011; Xiao et al., 2014) and high circulating levels of LPS are consistently shown in obese rodents and humans (Cani et al., 2008; Creely et al., 2007). The consumption of a HFD is further thought to lead to the increased intestinal permeability that is often seen in obesity (Rainone et al., 2016); which allows bacterial components to enter the blood circulation more easily and leads to the production of proinflammatory cytokines and, if sustained, low grade systemic inflammation. Indeed, patients with obesity are often shown to have higher circulating pro-inflammatory cytokines including IL-6 and TNF-α (Creely et al., 2007; Weisberg et al., 2003).

As the gut microbiota can actively interact with the intestinal epithelium, it has an important role in the regulation of fat storage and increasing energy harvesting (Bäckhed et al., 2004; Turnbaugh et al., 2006). It is thought that the altered gut microbiota of an obese individual increases energy extraction from non-digestible dietary components and increases food utilisation efficiency; leading to increased energy harvesting which is likely to impact other pathways, including inflammation (Cooper, Martin, & Keim, 2015; Mulders et al., 2018). A key role of the microbiota is in the fermentation of indigestible carbohydrates into short chain fatty acids (SCFA) which provide an energy source to the host. Some studies have shown that overweight and obese individuals have higher concentrations of SCFAs compared to lean individuals (Schwiertz et al., 2010) and may also have a greater abundance of bacteria that is capable of fermenting carbohydrates; leading to an increase in SCFA biosynthesis. Further, the obese gut microbiota has been shown to decrease the expression of the circulating lipoprotein lipase inhibitor (LPL), fasting-induced adipose factor (FIAF), which results in increased enzymatic activity and leads to an increase in the storage of excess energy as white fat and glucose; further leading to the development of obesity (Bäckhed et al., 2004; Muscogiuri et al., 2019).

Above discussions allude to the contrary findings often observed in microbiota studies and here too we find that, conversely, several studies have observed lower levels of SCFA in obese individuals in comparison to lean (Nishitsuji et al., 2017). This may be due to the high level of processed foods in a HFD, which are typically low in indigestible carbohydrates, resulting in a lower production of SCFA. In addition, *Bacteroides* have a high capacity for digesting dietary polysaccharides and, whilst the evidence is mixed, obese-model microbiota tend to have lower levels of that phyla when compared to lean individuals (Muscogiuri et al., 2019). Higher levels of SCFA are seen to positively impact gut hormone secretion and satiety regulation that is not typically seen in an overweight individual; particularly when consuming a HFD which promotes low levels of satiety. SCFAs are able to interact with G-protein-coupled receptors (GPCRs), specifically GPR41, which stimulates the release of satiety peptides (including peptide tyrosine tyrosine (PYY) and glucagon-like peptide 1 (GLP-1)). This induces satiety and reduces food intake which may combat the development of obesity (De Silva & Bloom, 2012; Samuel et al., 2008; Wren & Bloom, 2007).

As research to date shows mixed findings, it is difficult to determine the role of the gut microbiota on SCFAs and energy metabolism in individuals with obesity. It is likely that this is due to individual differences within the gut microbiota composition of both overweight and lean individuals and this must be considered when conducting future work; particularly when

looking at modulation of the microbiota as a method in treating obesity, which has been recently suggested (Li et al., 2017).

3.2.6. Metabolic diseases and metabolic syndrome

Recent evidence suggests that alterations in the gut microbiome may contribute to the development of metabolic disorders including hypertension, oxidative stress, insulin resistance, hyperglycaemia and dyslipidaemia; leading to the progression of metabolic diseases such as diabetes, obesity, cardiomyopathy, osteoarthritis and neurodegeneration (Gowd et al., 2019; Hansen, Gøbel, Hansen, & Pedersen, 2015; Karim, Jia, Zheng, Cui, & Chen, 2018). Clinical evidence indicates that dysbiosis of the gut microbiota contributes to the development of hypertension in mice (Karbach et al., 2016) and humans (Li et al., 2017), and also impacts the specific compound molecules that increase the risk of arterial thrombosis and worsen stroke outcomes (Benakis et al., 2016; Koeth et al., 2013).

Where several of these risk factors occur simultaneously it is classified as metabolic syndrome and there is an associated increased risk of developing chronic diseases such as cardiovascular diseases (CVD) (Meigs et al., 2006). Importantly, evidence shows that as the prevalence of obesity increases, so does that of cardiovascular risk factors, leading to a consistent increase of the occurrence of metabolic syndrome. The prevalence is estimated to increase up to 53% of the population by 2035 (Engin, 2017).

Increasing evidence suggests that the gut microbial impact on inflammation is likely to be one of the major contributing factors to the development of metabolic syndrome and consequently CVD (Cani et al., 2008). It is likely that LPS plays an important role in this process, as its stimulation of TLRs is associated with the accumulation of macrophages in white adipose tissue, induction of insulin resistance and triggering of inflammatory responses; all of which are associated with metabolic syndrome (Caesar, Fåk, & Bäckhed, 2010; Cani, Amar, et al., 2007; Cani et al., 2012). Research has also indicated that TLR-signalling-deficient mice have both reduced adiposity and improved glycaemia (Saberi et al., 2009; Vijay-Kumar et al., 2010); further indicating the role of TLR signalling in the development of metabolic diseases. Similarly, it has been shown that by blocking pro-inflammatory pathways, for example by using a human monoclonal antibody that inhibits IL-1β (Canakinumal), a reduction in cardiovascular death, non-fatal stroke and non-fatal myocardial infarction in patients with atherosclerotic cardiovascular disease can be observed (Ridker et al., 2017). This suggests that the prolonged activation of pro-inflammatory signalling pathways, caused by increased LPS

circulation, is a contributing factor influencing host metabolic syndrome and subsequent cardiovascular disease risk (Hotamisligil & Erbay, 2008).

As illustrated below in Figure 3.1, increasing evidence indicates that the gut microbiome may have a key role in the pathogenesis of obesity. Whilst one of the roles of the microbiota is to protect the integrity of the intestinal barrier, this can be altered by various factors including by microbial dysbiosis and consumption of a high-fat diet, both of which tend to be observed in those who are obese (Mu et al., 2017). This increase in intestinal permeability allows translocation of LPS, resulting in elevated systemic levels of LPS which, in turn, triggers a pro-inflammatory response. This then leads to chronic low-grade inflammation which is consistently observed in obese patients (Emanuela et al., 2012). It seems apparent that there is a synergistic relationship between these factors; where an increase in inflammation contributes to the pathogenesis of obesity which further exacerbates the production of proinflammatory cytokines. It is therefore suggested that therapeutic approaches to reducing systemic inflammation, may have a subsequent impact on obesity and other related health factors.

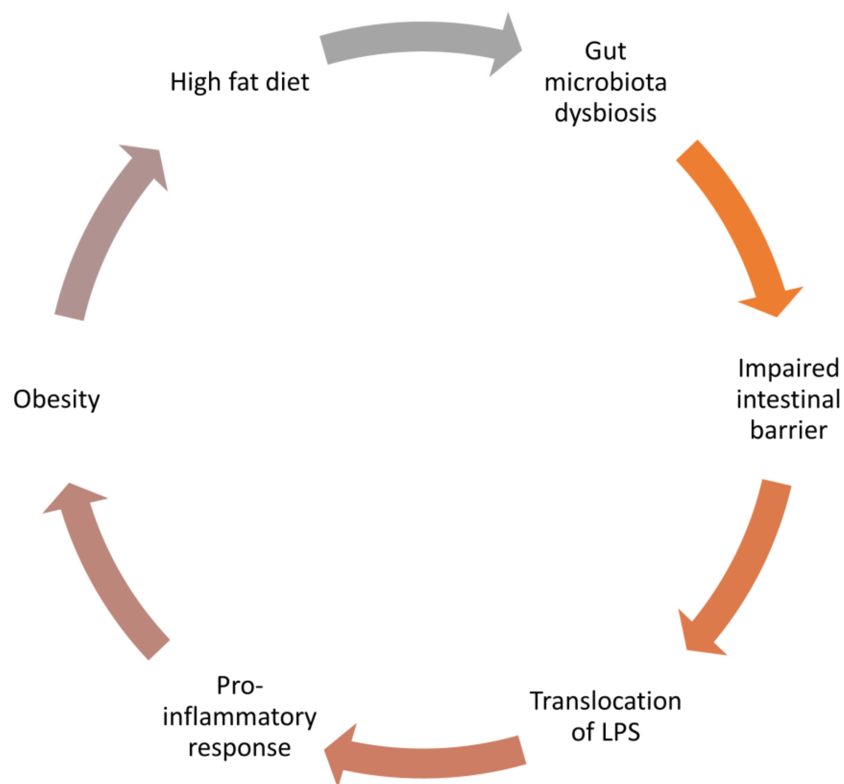

Figure 3.1. Illustrates the circular relationship between gut microbial dysbiosis and obesity. Where pathogenesis of obesity and the detrimental health outcomes associated with

this, are contributing factors in the development of metabolic diseases. Diagram adapted from (Rosca et al., 2020).

3.3. Microbiota-gut-brain axis

3.3.1. Background and mechanisms

Whilst the gut microbiota is not known to have any direct communication with the brain, it is believed to be involved in the bidirectional communication axis termed the gut-brain-axis. This is known to include components of the central nervous system, the neuroendocrine and neuroimmune systems and elements of the autonomic and enteric nervous systems (Dinan & Cryan, 2012). Signalling occurs through the vagus nerve, inflammatory and endocrine molecules and microbial metabolites (de Weerth, 2017; König et al., 2016). The concept of the gut-brain-axis has been recognised for some time, but more recently the importance of the bacteria within the gut has been acknowledged and, in response, it is now termed the microbiota-gut-brain axis (Mayer, Tillisch, & Gupta, 2015). Whilst many of the mechanisms which underly the axis remain unknown; the microbiota-gut-brain axis is key to maintaining host homeostasis and alterations in this are associated with the pathogenesis of chronic intestinal diseases. It is now also accepted that, whilst direct effects of the gut microbiome on the brain are still in question, modifications of gut microbiota and intestinal inflammation are correlated with the development of neurological and neuropsychiatric disorders, including Alzheimer’s disease, Parkinson’s disease, Autism spectrum disorder (ASD), schizophrenia, anxiety and major depressive disorder (Bruce-Keller, Salbaum, & Berthoud, 2018; Houser & Tansey, 2017; Moos et al., 2016).

Whilst research has identified numerous mechanisms that likely underpin the transmission of information between the gut and brain; it is most probable that they do not operate in isolation and instead are closely interrelated and work simultaneously. Research, to date, has primarily focussed on endocrine, metabolic, neural and immune pathways as likely mechanisms. These include various information carriers that signal information from the gut to the brain including gut hormones, immune mediators, spinal afferent neurons, gut microbiota-derived molecules and spinal afferent neurons (Farzi, Fröhlich, & Holzer, 2018; Holzer et al., 2017).

Due to the vast amount of sensory afferent neurons in the gut, neural pathways are considered to play a vital role (Blackshaw, Brookes, Grundy, & Schemann, 2007). The vagus nerve in particular has evinced the most convincing evidence here as the site for bidirectional communication between the gut and the brain (Bonaz, Bazin, & Pellissier, 2018). As illustrated within Figure 3.2., this links the viscera with the brain with the vagus nerve consisting of

sensory (afferent) and motor (efferent) neurons (Fülling, Dinan, & Cryan, 2019). Vagal afferent fibres are located within all layers of the digestive wall, but are unable to cross the epithelial layer (Wang & Powley, 2007). As such, without direct contact with the microbiota, the fibres rely on indirect microbiota signals through diffusion of metabolites or bacterial compounds (Bonaz et al., 2018). As vagus afferents respond to various stimuli, including nutrients, gut peptides, cytokines and hormones, enteroendocrine cells interact with vagal afferents either directly through the release of serotonin, activating 5-HT3 receptors (Li, Hao, Zhu, & Owyang, 2000) or via gut hormones including glucagon-like peptide-1 (Strader & Woods, 2005). Enteroendocrine cells detect signals from the gut microbiota through toll-like receptors which have the ability to recognise bacterial products like LPS or receptors for microbiota metabolites (Abreu et al., 2005; Samuel et al., 2008). Communication between the microbiota and the brain via the vagus nerve may additionally be facilitated via production of SCFAs by the microbiota, as this has the ability to activate vagal afferent fibres via numerous mechanisms (Lal, Kirkup, Brunsden, Thompson, & Grundy, 2001).

Several studies have demonstrated the ability of specific bacterial strains in the production of various neurotransmitters and neuropeptides, including serotonin, gamma aminobutyric acid (GABA), catecholamines and cytokines, which are used for neuronal and neuroendocrine signalling (Lyte, 2013). For example, evidence indicates that *Streptococcus, Enterococcus* and *Escherichia* species produce serotonin; GABA can be produced by *Lactobacillus* and *Bifidobacterium* species; and dopamine by *Bacillus* species (Barrett, Ross, O'toole, Fitzgerald, & Stanton, 2012; Lyte, 2011; Wikoff et al., 2009). These can then directly act on vagus nerve endings and information from the vagus nerve is then relayed into the brainstem, where gut vagal afferents mostly synapse onto neurons in the nucleus tractus solitarius (NTS). Evidence also indicates that vagal afferents from different areas of the GI tract are projected to different areas of the NTS; indicating that the location of microbiota within the GI tract will determine how the information is relayed (Fülling et al., 2019). From the NTS, information is relayed to various parts of the forebrain including the hypothalamus, amygdala and parabranchial nucleus. Administration of certain bacterial strains have been demonstrated to make use of vagus nerve signalling to alter behaviour via communication with the brain. Specific examples include that the anxiety and depressive-like beneficial effects of administration of *Lactobacillus rhamnosus* JB_1 are not observed following vagotomy (surgical removal/reduction of the vagus nerve) (Bravo et al., 2011) and similar effects are observed on cognition following prebiotic (2'-fucosyllactose) administration (Vazquez et al., 2016). Importantly, however, this effect of vagotomy has not been consistently observed in bacteria-brain communication (Bercik et al., 2011), suggesting that additional communication mechanisms are likely to occur either independently or in conjunction with the vagus nerve.

Stimulation of the vagus nerve has also been shown to exert anti-inflammatory effects, termed as the cholinergic anti-inflammatory pathway (CAP) (Borovikova et al., 2000). Where the release of acetylcholine from vagal efferents prevents the release of TNF-α by macrophages (Wang et al., 2003); indicating that the vagus nerve may also be involved directly or indirectly in inflammatory pathways, potentially also impacting upon microbial dysbiosis. Additionally, the vagus nerve offers a protective role through decreasing intestinal permeability; where vagus nerve stimulation increases the expression of tight junction proteins, therefore decreasing intestinal epithelial permeability (Van Houten, Wessells, Lujan, & DiCarlo, 2015; Zhou et al., 2013).

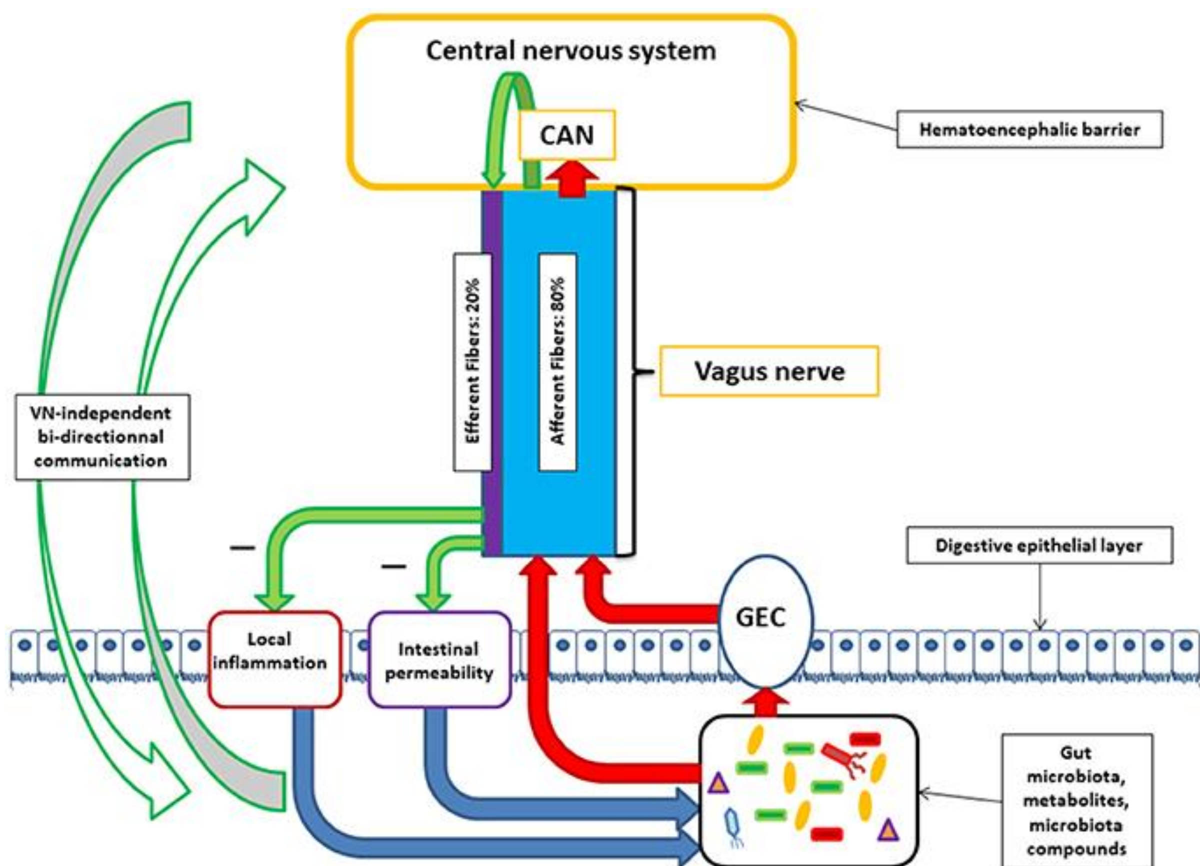

Figure 3.2. Illustrates the communication between the central nervous system and gut microbiota via the vagus nerve. Diagram from (Bonaz et al., 2018).

As previously mentioned, the bi-directional communication between the brain and the gut likely involves multiple mechanisms working in conjunction; one being the neuroendocrine system. Bacterial strains can produce various neurotransmitters, some of which can directly influence brain function and physiological activity by acting on neuroactive metabolites production (Cryan & Dinan, 2012). However, as the majority of neurotransmitters lack the ability to cross

the blood-brain barrier (BBB), it is unlikely that they can communicate directly with neuronal cells in the central nervous system (Spohn & Mawe, 2017). This suggests that the impact of gut microbiota on the production of neurotransmitter precursors, which do have the ability to cross the BBB, may be more significant. Particular focus has been placed on the essential amino acid, tryptophan, the precursor to serotonin, which can be produced by *Bifidobacterium infantis* and several other gut bacteria (O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015). The balance between the processing of tryptophan into its metabolites kynurenine and serotonin, has gained particular interest in the role of bacteria-brain signalling (O'Mahony et al., 2015). Tryptophan is metabolised by enzymes in the microbiota and intestinal mucosa, of which up to 95% is metabolised along the kynurenine pathway (Gao et al., 2018). Importantly, increases in proinflammatory cytokines triggers the enzymes tryptophan dioxygenase and indoleamine 2,3-dioxygenase which regulates this process; resulting in elevated kynurenine levels. This is associated with reduced neuroprotection, depression and anxiety-like behaviour via the resulting impact on a number of central neurotransmitter systems (Myint et al., 2007; Ruddick et al., 2006; Schwarcz, Bruno, Muchowski, & Wu, 2012). It has further been shown that, depending on the gut bacteria involved, kynurenine biosynthesis can be either increased or decreased, as certain probiotics have been shown to reduce levels (Desbonnet, Clarke, Shanahan, Dinan, & Cryan, 2014). In addition, the production of tryptophan may protect the intestines and CNS from inflammation, via activation of the acyl hydrocarbon receptor, which enhances production of the anti-inflammatory interleukin-22 (Marsland, 2016; Zenewicz et al., 2008).

As one of the most studied microbial-derived metabolites, short-chain fatty acids (SCFAs) have also been considered as a potential modulator of neurotransmitter production; likely because of their ability to act on the BBB, mucosal and immune cells, gastrointestinal endocrine and the cerebral microglia (Erny et al., 2015; Rooks & Garrett, 2016). SCFAs have the ability to release gut hormones like peptide YY (PYY), glucagon-like peptide-1 (GLP-1) and GLP-2 (Bindels, Dewulf, & Delzenne, 2013). Through the production of these peptides, the enteroendocrine cells are able to circulate information from the gut microbiota throughout the body, including to the brain. Not only are PYY and GLP-1 able to facilitate satiety, inhibit gastric motility and modify glucose homeostasis, there is evidence of altering mood and cognitive behaviour; a process which is thought to be dependent on modification of the activity of afferent vagal neurons (Farzi et al., 2018; Holzer, Hassan, Jain, Reichmann, & Farzi, 2015).

In summary, there are numerous underlying mechanisms suggested which may explain the communication between the gut microbiota and the brain; many which are likely to be altered by increased intestinal permeability and inflammation (Hsiao et al., 2013; Leclercq et al., 2014;

Soderholm & Perdue, 2001). However, due to the complexity and intricate connectivity of many of these mechanisms, it is possible that the proposed mechanisms work concurrently to communicate between the gut and the brain. Further studies are needed to better understand the impact that these microbial-brain communications have on the progression of brain disorders, brain structure, cognitive performance and mood. With this potential further understanding, it may therefore be possible to identify new approaches in the prevention and treatment of brain disorders and in the improvement of cognitive performance.

3.3.2. Gut, mood and stress

It has long been considered that changes in microbial composition could alter thoughts and emotions; potentially due to the effects on gastrointestinal discomfort and bowel habits, which may have a conscious impact on mood state (Loewenstein, 1996). As previously mentioned, the gut microbiota has the ability to effect the levels of various neurotransmitters and this microbial-induced dysregulation is thought to have a huge impact on the development of mood disorders (Umbrello & Esposito, 2016). Research has predominately examined depression, and this is likely because patients suffering with the disorder consistently present an altered microbial composition (Naseribafrouei et al., 2014; Zheng et al., 2016). Whilst the direction of the relationship between depression and gut dysbiosis in humans has yet to be elucidated, it has been suggested from rodent models that dysbiosis is a causal factor in the development of the disease; with several studies indicating that a microbial transfer from a depressed rodent can induce depression-like behaviours in an otherwise healthy rodent (Kelly et al., 2016; Zheng et al., 2016).

It has therefore been suggested that modulation of the microbiome, specifically via pre- or pro-biotic administration, may have a beneficial effect on depressive symptoms in animal models (Mangiola et al., 2016). However, to date, findings in humans remain inconsistent. Whilst, some studies have indicated reductions in anxiety-like behaviour in both rats and humans following supplementation with a probiotic formula (Messaoudi et al., 2011), supplementary studies have observed null findings in humans, despite consistent beneficial previous evidence in animal models (Kelly et al., 2017). These inconsistencies in findings, despite supplementing with similar probiotics over the same time period, fuel the confusion. For example, whilst one study in patients with major depressive disorder (MDD) observed positive effects on self-reported depression scores following an 8 week supplementation of a probiotic containing *Lactobacillus* and *Bifidobacterium* strains (Akkasheh et al., 2016), a second larger study found no effect on any psychological outcome measure (Romijn, Rucklidge, Kuijer, & Frampton, 2017). As detailed within Table 3.1 a potential explanation for the lack of consistent

findings within the above studies is the vastly differing study design; particularly the participant demographics. Here, some target healthy individuals, whereas others investigate those with mood disorders. Moreover, differing combinations of bacterial strains and the use of different methodologies to measure changes to psychological behaviour are additional potential explanations.

Table 3.1. Probiotic supplementation and behaviour in humans. Summary of study design and key findings of above studies investigating the effects of probiotic supplementation on psychological behaviour in humans.

Authors	N	Intervention	Duration	Key Outcomes	Key Findings
Messauoudi et al. (2011)	36 rats 66 humans (healthy participants)	Probiotic formula (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum R0175)*	Rats 2 weeks Humans 30 days	Rats – defensive burying test Humans – HSCL-90, HADS, PSS, CCL and urinary cortisol	Sig reduced anxiety-like behaviour in rats Alleviated psychological distress in humans. HSCL-90 score, HADS, CLL and UFC levels.
Kelly et al. (2017)	29 healthy male adults	*Lactobacillus rhamnosus* (JB-1)	8 weeks	Subjective measures of stress CANTAB cognitive battery Cold pressor test. Salivary cortisol	No effect on measures of mood, anxiety subjective stress or sleep quality No effect to cold pressor test
Akkasheh et al. (2016)	40 adults diagnosed with major depressive disorder	Probiotic formula (*Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium bifidum)*	8 weeks	Beck Depression Inventory	Sig decreased BDI total score
Romijn et al. (2017)	79 healthy adults with low mood	Probiotic formula *(Lactobacillus helveticus* and *Bifidobacterium longum)*	8 weeks	Subjective measures of depression, anxiety and stress	No sig difference on any psychological outcome measure

Recent systematic reviews have also observed these inconsistent findings, as detailed further in Table 3.2. In a systematic review and meta-analysis of 5 trials, Huang, Wang, and Hu

(2016), concluded that probiotic supplementation significantly reduced incidence of depression. Whilst the included studies were similar in terms of duration of supplementation and strain of bacteria, key differences in study design included sample size, participant age, outcome measures and current depressive status. The review also included the previously discussed study in participants with MDD (Akkasheh et al., 2016), when all remaining studies were conducted in healthy participants. Subgroup analysis within this meta-analysis indicated that probiotic administration was effective in participants aged <60 years, and was beneficial in both healthy participants and those with diagnosed MDD. Whilst they conclude that there is an observed potential beneficial role of probiotics in modulating depression, they note that additional work in larger sample sizes are necessary to draw further conclusions.

Similarly, a systematic review including 10 RCTs (6 in healthy populations and 4 in clinically diagnosed samples), also provided some limited support of probiotic supplementation in reducing anxiety and depression in humans (Pirbaglou et al., 2016). Five individual RCTs here observed beneficial results on anxiety or depression symptoms. However, this review details several shortcomings to the design of these studies, where assessment of risk of bias revealed issues including 2 studies failing to use placebo controls; failing to measure compliance and failing to disclose randomisation methods and group means. Additionally, as with the previous review (Huang et al., 2016), the authors noted substantial differences in sample size, population demographic, supplementation duration and probiotic formulation between studies. As such, they conclude the importance of future work to aim to use consistent probiotic formulation, to overcome inconsistencies potentially arising from variations in bacterial strains. Furthermore, the inclusion of studies assessing patients with health conditions (particularly those with GI disease such as IBS) makes it difficult to compare findings with healthy populations.

Despite the above reviews, tentatively suggesting improvements in various psychological outcomes following probiotic supplementation, an additional review of 10 RCTs, concluded no evidence of improvements to psychological outcomes (Romijn & Rucklidge, 2015). This review employed a stricter inclusion criteria to some previous reviews, where they only included studies that were double-blind, randomised and placebo-controlled; and that also used standardised, validated measures of psychological outcomes. However, they had no restrictions on the sample employed and therefore included studies in a range of ages and patients with IBS, schizophrenia, CFS and arthritis, and one study in children with autism. As such, again interpreting findings over such a wide population demographic is problematic; without also considering the differences in study design (dosage, duration, bacterial strain) previously mentioned.

Table 3.2. Systematic reviews investigating probiotic supplementation and behaviour in humans. Summary of review characteristic and key findings of above systematic- and meta- analyses investigating the effects of probiotic supplementation on psychological behaviour in humans.

	N	Intervention	Duration	Key Outcomes	Conclusions
Huang et al. (2016)	5 studies N range 40-278. Included studies in young & old healthy participants. And those with MDD	Probiotic formulas	Ranges from 4 – 20 weeks	Various depression scales and measures of subjective psychological outcomes.	Sig reduced incidence of depression. Most individual studies did not report sig results. Subgroup analysis showed beneficial effect on those <60 yrs. Those aged 65+ no effect observed. Probiotics beneficial in both healthy participants and those with MDD.
Pirbaglou et al. (2016)	10 studies N range 20-238 Included studies in healthy young & old adults. And MDD, CFS, IBS and cancer patients.	Probiotic formulation in varying forms (capsule, milk, powder, yogurt).	Ranges from 2-12 weeks	Various depression scales and measures of subjective psychological outcomes.	Limited evidence to suggest reductions in anxiety or depressive symptoms – observed in 5 RCTs.
Wallace & Milev (2017)	10 studies N range 25-710 Included studies in healthy young & old adults. Those suffering with stress and MDD and CFS patients.	Probiotic formulation. 1 study (n=710) no intervention, self-report of fermented food consumption	Ranges from 3 weeks-6 months	Various depression scales and measures of subjective psychological outcomes.	Daily supplementation could have a positive effect on mood and anxiety, particularly in MDD.
Romijn & Rucklidge (2015)	10 studies N range 39-362 Included studies in healthy young & old adults. And IBS, schizophrenia, CFS and arthritis patients. And children with autism.	Probiotic formulation in varying forms (capsule, milk, powder, yogurt).	Ranges from 20-98 days	Various depression scales and measures of subjective psychological outcomes.	No evidence of improved psychological outcomes.

Gut microbiota and stress

The link between the gut and negative mood has been well established; with the impact of negative affect, and particularly stress, well documented to be linked to gut dysbiosis and GI disease (Moloney et al., 2016). Specifically, studies have indicated that chronic stress is associated with increase in gut permeability (Soderholm et al., 2002); the development of IBS (Qin, Cheng, Tang, & Bian, 2014); and alterations to bowel function, including vomiting, nausea and changes in bowel habits (Molina-Torres, Rodriguez-Arrastia, Roman, Sanchez-Labraca, & Cardona, 2019). As such, importance has been placed on the potential role of the microbiota within this relationship, specifically how microbial changes can positively influence psychological mood and the mechanisms that this is predicated on.

Of these, the interaction between gut microbiota and the hypothalamic-pituitary-adrenal (HPA) axis, which is triggered in response to a stressor on the host, appears to be the most crucial. HPA axis activation leads to the release of corticotrophin-releasing factor (CRF) and adrenocorticotropic hormone from the hypothalamus and pituitary gland, respectively and cortisol from the adrenal gland (Carabotti, Scirocco, Maselli, & Severi, 2015). Examinations of models of intestinal bowel disease indicate that CRF1 has a crucial role in the stress-induced modulation of inflammatory responses, disruptions to gut permeability and modification of gut microbiota (Larauche, Kiank, & Tache, 2009; Tache, Larauche, Yuan, & Million, 2018). As these factors are intrinsically linked with the pathophysiology of IBD, this indicates that, in IBD patients, the disruptions of the HPA axis caused by microbial dysbiosis could intensify symptoms by further modifying the microbial homeostasis (Distrutti, Monaldi, Ricci, & Fiorucci, 2016). Germ-free mice[1] have been observed to have an abnormal and exaggerated HPA axis response when compared to controls, indicated by elevated concentrations of adrenocorticotropic hormone and corticosterone (Sudo et al., 2004). This elevated response has been shown to be ameliorated by administration of *Bifidobacterium infantis* in early life (Evrensel & Ceylan, 2015), suggesting the importance of microbial composition in regulating HPA response to stress. However, it has been suggested that there may be a critical period to normalise this response via bacterial transplantation; as introduction of microbiota at a later developmental stage had no effect on correcting the HPA axis response (Neufeld, Kang, Bienenstock, & Foster, 2011; Sudo et al., 2004). Therefore, microbial colonisation later may not be an efficient method to modulate the HPA response and alleviate stress and it is probable

[1] Germ-free mice are bred and raised specifically to have no exposure to microorganisms, therefore keeping them free of detectable viruses and bacteria (E. A. Kennedy, King, & Baldridge, 2018). The use of germ-free animal models allows the study of behaviour in the complete absence of microbes; or alternatively intentional colonisation by administration of specific microbes (Luczynski et al., 2016)

that additional mechanisms are involved. Specifically, the role of the vagus nerve; which can interact with the pro-inflammatory pathways activated during a stressor response. Release of CRF and subsequent activation of CRF_{1-2} receptors results in the release of cytokines (Overman, Rivier, & Moeser, 2012; Theoharides & Cochrane, 2004). A stress response, inhibits the vagus nerve and activates the sympathetic nervous system, therefore increasing the bodies defence through pro-inflammatory reaction (Bonaz, Sinniger, & Pellissier, 2016; Taché & Bonaz, 2007). A single acute stress episode results in prolonged increase of pro-inflammatory cytokines (Marsland, Walsh, Lockwood, & John-Henderson, 2017) which continues beyond exposure to the stressor, during the recovery period. Exposure to chronic stress continually disrupts the parasympathetic tone recovery, resulting in allostatic overload and diminishing the anti-inflammatory ability of the vagus nerve (Bonaz et al., 2018; McEwen, 2008). As such, chronic stress could interrupt the protective effect the vagus nerve has on the epithelial barrier, resulting in the "leaky gut" phenomenon and further exacerbating dysbiosis (Bonaz et al., 2018; Maes, Kubera, & Leunis, 2008). This also supports the importance of development of therapeutic approaches, such as dietary intervention, to target restoration of gut microbial communities reducing the inflammatory response exacerbated by stress and inflammatory bowel disease.

3.3.3. Social behaviour and neurodevelopmental disorders

Given the established link between the gut and brain and specifically the ability of gut microbes to interact with and produce neurotransmitters, increasing interest has been placed on the impact of microbial composition on behaviour. Social behaviour plays an essential role in the survival of an individual; and many of the earlier mentioned neurological disorders including anxiety, depression and stress results in disruptions to normal social behaviour (Parashar & Udayabanu, 2016). Similar to humans, rodents have a complex social system and naturally seek security from a healthy social environment. Given this, and the relative ease of investigating animal models here, much of the work to date has been conducted in rodents. Utilising the germ-free model, animals have consistently been observed to display altered social behaviour than control animals, specifically reduced socialisation with other rodents (Crumeyrolle-Arias et al., 2014; Degroote, Hunting, Baccarelli, & Takser, 2016; Desbonnet et al., 2014; Hsiao, 2014). However, the recurrent theme of contrary findings is also apparent here with some reports indicating that GF mice actually display increased sociability (Arentsen, Raith, Qian, Forssberg, & Heijtz, 2015). It has been suggested that evolutionary hypotheses may explain this increase in sociability with close-proximity acting as a means to acquire microbes from others in order to develop a normal microbial composition (Sarkar et al., 2018). Taken together, this suggests that whilst gut bacteria is integral for development and

maintenance of social behaviour, the relationship between these is complex and requires further understanding.

These observed differences in social behaviour of GF mice are thought to be potentially due to changes in brain function and structure. In particular, studies have detected lowered levels of brain-derived neurotropic factor (BDNF) in the cortex and hippocampus (Bercik et al., 2011; Heijtz et al., 2011; Sudo et al., 2004), which induces depressive-like behaviour (Taliaz, Stall, Dar, & Zangen, 2010). They have also been shown to display increased levels of various synapse-related proteins, which may result in alteration of synaptic plasticity, implicating motor control and developing anxiety-like behaviour (Braniste et al., 2014; Govindarajan et al., 2006; Möhle et al., 2016). Additionally, GF mice have been shown to display altered hippocampal neurogenesis (Möhle et al., 2016; Ogbonnaya et al., 2015), which may potentially lead to impaired spatial and object recognition (Jessberger et al., 2009).

As in humans, a rodent's early microbiome develops during pregnancy and birth, with the mothers' gut, vaginal and skin microbiota inoculating the new-borns microbial composition (Gomez de Agüero et al., 2016; Perez-Muñoz, Arrieta, Ramer-Tait, & Walter, 2017). Prenatal exposure to maternal high-fat diet and antibiotic use have both been shown to have a distinct impact upon microbial communities and also reduced social interaction in offspring (Buffington et al., 2016; Degroote et al., 2016; Leclercq et al., 2017). Further suggesting that actual composition of gut microbiota, particularly dysbiosis, has a profound impact on social behaviour. As such, the research area has progressed with the aim to understand if colonising of the microbiota can reverse these negative effects and potentially even be used in future as a therapeutic option in neurodevelopmental disorders.

Indeed, within the above study, Buffington et al. (2016) observed that when offspring of mothers fed a high fat diet (HFD) were colonised with gut microbiota from offspring of mothers with a normal diet, this restored normal social behaviour. Within the same work, those offspring from the HFD had reduced hypothalamic oxytocin levels which was reversed with treatment with the most reduced strain in their microbiome *(Lactobacillus reuteri)*, alongside normalising their social deficit in comparison to control animals. Similar effects have been observed previously when concurrently supplementing with antibiotics and *Lactobacillus rhamnosus* (JB-$_1$) prevented the decrease in sociability observed in those treated with antibiotics alone (Bravo et al., 2011). Likewise, 28-day administration of *Lactobacillus rhamnosus* (JB-$_1$) decreased anxiety-like behaviour in an induced stress state and also prevented deficits in social interactions (Bharwani, Mian, Surette, Bienenstock, & Forsythe, 2017). Taken together

these findings indicate the potential for intervention with *Lactobacillus* strains to positively impact social behaviour.

Interestingly, it has been shown that bacterial colonisation only restores certain elements of behaviour though. For example, whilst increases in sociability were observed, social novelty wasn't; indicating that different aspects of social behaviour have varying sensitivity to bacterial colonisation (Desbonnet et al., 2014). Crucially however, these discrepancies in findings may be due to methodological differences in the studies; including the age of the mice used. Here, those of greater age would have a more developed social neurocircuitry and this may lead to greater behavioural variations when compared with younger mice. Therefore, whilst evidence indicates that gut bacteria has a role in social behaviour, the direction of these effects it is as yet unclear and this may be impacted by a number of factors including the age and development of the animal utilised.

When investigating the causal relationship between gut bacteria and social behaviour, the neurodevelopmental disorder, Autism spectrum disorder (ASD) is most commonly studied. ASD is characterised by difficulties in social interaction and communication, repetitive behaviour and stereotyped interests (Baron-Cohen & Belmonte, 2005; Serra et al., 2018). Consistently, murine models of autism and humans with ASD have displayed altered microbial profiles, when compared to controls (Coretti et al., 2017; De Theije et al., 2014; Finegold et al., 2010; Kang et al., 2013; Son et al., 2015). In particular, studies in autistic children indicates vast abnormalities in microbial composition; most notably less abundance of *Firmicutes* and *Bacteroidetes* in severely autistic children (Finegold, Downes, & Summanen, 2012), with related dysregulation of gastroenterological and immunological processes (Adams, Johansen, Powell, Quig, & Rubin, 2011; Ashwood et al., 2011; Gorrindo et al., 2012). It has been suggested that neuroinflammation is a potential contributing factor of the behaviour observed in patients with ASD and that this may be the result of dysbiosis of the microbiome (de Theije et al., 2011; Hsiao, 2014). In addition, the imbalance in microbial composition may also underpin the increased levels of serotonin observed in patients with ASD; which has been linked to the GI symptoms and the mood and cognition profiles specific to these individuals (Berding & Donovan, 2016; Muller, Anacker, & Veenstra-VanderWeele, 2016).

Due to the apparent close relationship between gut microbial composition and the development of ASD, it is unsurprising that recent research has proposed a strategy of modulating the gut microbiota as a method of preventing and treating ASD (Hsiao, 2014). Within a rodent model of ASD, supplementation with *Bacteroides fragilis* was shown to reduce gut permeability, normalise gut bacterial composition and decrease ASD-like behaviours,

including abnormal stereotyped, communicative and anxiety-like behaviours (Hsiao et al., 2013).

Although limited, work in humans has also indicated that probiotics may be a potential therapeutic option for ASD. In a study of children with autism, 4-month administration of a supplement containing strains of *Lactobacillus, Bifidobacteria* and *Streptococcus,* normalised the *Bacteroidetes/Firmicutes* ratio and quantities of *Bifidobacterium* spp. and *Desulfovibrio* spp similar to those levels observed in healthy controls or non-autistic siblings (Tomova et al., 2015). This study however did not measure any changes in autistic behaviour following probiotic supplementation. Similarly, 12-week supplementation with *Lactobacillus plantarum* WCFS1 in autistic children, resulted in changes to microbial composition including increased counts of *Lactobacilli* and *Enterococci* groups, but no clear behavioural effects were observed (Parracho et al., 2010). Additional research, however, has indicated positive impacts on behaviour, including a case study on a 12-year-old boy with severe ASD. Here, supplementation for 4 weeks with a multi-strain mixture of 10 probiotics, resulted in an improvement in autistic core symptoms as measured by the Autism Diagnostic Observation Schedule (ADOS) (Grossi, Melli, Dunca, & Terruzzi, 2016). Most recently, a six-month trial supplementing a multi-strain mixture of 8 probiotics in children with ASD, observed significant improvements in ADOS scores only in subjects without gastrointestinal symptoms; despite observing improvements in GI symptoms in the gastrointestinal symptoms group (Santocchi et al., 2020). Whilst research in this area is still in its infancy, it seems plausible for future research to continue to consider the effect of microbial colonisation as a therapeutic treatment for ASD.

3.3.4. Neurodegeneration and neurological diseases

Recently, an increasing body of evidence has considered the role of the gut microbiome in the pathogenesis of neurodegenerative disorders. To date, much of the research has been conducted in animal models, with very few studies reported in humans (Bercik et al., 2011; Sampson et al., 2016). However, several studies have indicated that not only does dysbiosis of the gut microbiota play an important role in the brain structure and cognitive function (Fernandez-Real et al., 2015; Heijtz et al., 2011; Savignac et al., 2013), but also a potential role in the development of neurodegenerative diseases including Huntington’s disease, Alzheimer’s disease (AD) and Parkinson’s disease (Di Meo et al., 2018).

Research has indicated differences in the microbial composition of patients with AD when compared with controls, with AD sufferers exhibiting less abundance of *Firmicutes* and

Actinobacteria, an increase in *Bacteroidetes* and overall a less diverse microbiota (Vogt et al., 2017). Importantly this alteration in the gut microbiota may impact upon its ability to produce protective neurotransmitters, and dysfunction of these systems may impact upon the development and disease progression of AD and similar neurodegenerative disorders. In particular the GABA system is thought to play a critical role in AD, with supporting evidence indicating that GABA treatment can decrease beta-amyloid fibre- induced toxicity (Sun et al., 2012). Further, BDNF has been shown to play an important role in the growth and plasticity of synapses and, in AD, BDNF is decreased in the cerebral cortex (Connor et al., 1997). Therefore, several studies have investigated the therapeutic effect of BDNF in different neurological diseases (Nagahara & Tuszynski, 2011). Recent research has focussed on modulation of the gut microbiota as a method of prevention of disease progression (Bonfili et al., 2017), which may result in an increase in gut produced hormones such as ghrelin and leptin, which have been shown to decrease amyloid-β deposits (Niedowicz et al., 2013). Modulation may also cause a reduction in pro-inflammatory cytokines, which have the ability to reach the brain via systemic inflammation, resulting in neuroinflammation and impacting neuronal homeostasis, which would be detrimental within a neurodevelopmental disorder (de Theije et al., 2011; Dinan & Cryan, 2017).

Similarly, research has considered the link between the gut microbiota and Parkinson's disease. It has been suggested that patients suffering from neurological diseases often also report gastrointestinal problems, which are theorised to be caused by increased inflammation (Houser & Tansey, 2017). This is particularly apparent in this disease model, as Parkinson's patients often report GI problems in the decades prior to the appearance of typical Parkinson's symptoms (Houser & Tansey, 2017). Further, microbial analysis has indicated differences in composition when compared with controls, where Parkinson's patients showed an increase of *Enterobacteria* and decrease in *Prevotella* strains (Scheperjans et al., 2015), with the former associated with the severity of gait difficulty and postural instability. Whilst the mechanisms behind this association are still relatively unknown, it has been suggested that as a main characteristic of Parkinson's is accumulations of Lewy bodies (clumps of misfolded alpha-synuclein), these misfolded alpha-synuclein can be transferred from enteroendocrine cells to neural circuits through gut-brain communication pathways, therefore contributing to the pathogenesis of Parkinson's disease (Chandra, Hiniker, Kuo, Nussbaum, & Liddle, 2017).

3.3.5. Cognition

As established above, the gut microbiota and brain are able to communicate bidirectionally via numerous mechanisms, which has the potential to modify aspects of brain function including mood and behaviour. Given this, it is probable that said mechanisms would be able to impact upon cognitive function directly or indirectly. However, currently, only a small area of literature has considered the impact of microbial bacteria on cognition and the potential to modulate this via dietary intervention.

Early work in germ-free models have indicated some evidence of impairments to areas of cognitive performance including short term recognition and working memory (Gareau et al., 2011). This was evidenced by GF mice spending less time exploring novel environments and objects during task performance (Gareau, Sherman, & Walker, 2010). These authors concluded that a gut microbiome is imperative for normal cognitive functioning; with similar social impairments observed in another GF mice model (Desbonnet et al., 2014).

Considerable interest has focussed on the hippocampus and its potential as a neurobiological mediator within the gut-brain-axis communication, where reduced hippocampal activity may explain these cognitive impairments. Indeed, research indicates that it plays a key part in the generation and maintenance of spatial maps in rodents (O'Keefe & Dostrovsky, 1971) and GF mice have less c-Fos-positive CA_1 hippocampal cells following memory testing (Luczynski et al., 2016). It is likely that brain-derived neurotropic factor (BDNF; a protein that is closely related to neuroplasticity, learning and memory (Yamada, Mizuno, & Nabeshima, 2002)) may be involved here. Specifically, evidence shows that GF mice shown impairments in memory tests alongside reductions in hippocampal BDNF when compared to controls (Gareau et al., 2011). In support of this mechanism, several studies have indicated improvements in a range of learning and memory tasks, such as working memory and spatial learning, alongside increased concentrations of BDNF in rodents supplemented with the prebiotic human milk oligosaccharides (Vázquez et al., 2015). Similar increases in BDNF expression have been observed following administration of fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) combination prebiotics (Burokas et al., 2017; Savignac et al., 2013; Williams et al., 2016); indicating that the microbial interaction with BDNF may underpin the interaction with cognitive function.

Although the majority of work in this field is based on findings from animal models, there is some emerging, limited research on bacterial-cognition communication within humans. For example, evidence suggests that microbial composition, specifically the abundance of

Actinobacteria phylum is associated with performance on a task of motor speed, attention and cognitive flexibility, in obese adults (Fernandez-Real et al., 2015). Not only was the microbial composition shown to affect cognition here but also a greater bacterial diversity was shown to be associated with greater variation in brain microstructure; particularly within the hippocampus, hypothalamus and the caudate nucleus. Despite this, contradictory results have been observed in infant children where, lower gut bacterial diversity was associated with better cognitive performance (Carlson et al., 2018). Whilst studies have frequently suggested that increased bacterial diversity is associated with desirable outcomes, these results indicate that higher levels of diversity may not always be correlated with benefits. These contrasting results are likely to be due to the vastly differing age ranges utilised, particularly as the human microbiome isn't believed to fully develop and stabilise for the first 2-3 years of life (Voreades et al., 2014). Likewise, with work in different demographics at completely different stages of cognitive development, it is difficult to provide direct comparison in cognitive functioning. However, this work certainly highlights the potential associations between the microbiome and human cognition and warrants further investigation.

Despite the obvious limitations in extrapolating findings from animal models here, research utilising rodents have very useful in terms of identifying specific probiotic bacterial strains which may be key in the gut-cognition communication. Specifically, supplementation with *Bifidobacterium longum* strains has been shown to improve task performance on object recognition and maze learning in mice (Savignac, Tramullas, Kiely, Dinan, & Cryan, 2015). The same bacterial strains have also been shown to reduce anxiety-like behaviour in mice (Savignac, Kiely, Dinan, & Cryan, 2014). Similarly, research in humans has observed the same effects. Allen et al. (2016) investigated the effect of 4-weeks supplementation with a single strain of *Bifidobacterium longum 1714,* in healthy adults. They found that it can ameliorate both the physiological and psychological response to an acute stressor, as well as longer-term daily self-reported psychological stress, and observed a subtle improvement in visuospatial memory performance. Additionally, they noted changes in EEG mobility typical of prefrontal cortex activity and associated with learning memory performance (Asada, Fukuda, Tsunoda, Yamaguchi, & Tonoike, 1999; Hales, Israel, Swann, & Brewer, 2009).

Similar beneficial cognitive effects have been observed following supplementation with different strains. A study in a mouse model fed a Western diet and *Lactobacillus helveticus* R0052 for 21 days, observed that diet-induced impaired spatial memory and anxiety-like behaviour was corrected by probiotic ingestion (Ohland et al., 2013). Likewise, beneficial effects of this strain has been observed in human work. One study in healthy older adults indicated that 12-week supplementation with fermented milk containing *Lactobacillus*

helveticus had the potential to improve elements of cognitive performance (Chung et al., 2014). Here, participants performed better following probiotic supplementation on RVIP and stroop tasks; suggesting improvements to sustained attention and working memory performance.

However, despite some promising human findings, supplementary studies have failed to consistently and convincingly illustrate cognitive enhancing effects following probiotic supplementation. Notably, a relatively large (n=124) trial in healthy adults investigated the effect of 3-week supplementation of a probiotic milk drink containing *Lactobacillus casei* (Benton, Williams, & Brown, 2007). They observed significantly declines in long-term memory and episodic memory tasks, in comparison to placebo consumption. Similarly, a recent study, in which rats were fed a Western-type diet, reported impairments in object memory (despite some positive findings on other forms of memory) following supplementation with a multi-strain probiotic, irrespective of the diet consumed (Beilharz, Kaakoush, Maniam, & Morris, 2018). This may suggest that modulation of the microbiome has different effects depending upon the bacterial strain used and area of cognition studied.

A recent systematic review of 30 studies investigated the effects of probiotic supplementation on cognitive function across the human lifespan (Eastwood, Walton, Van Hemert, Williams, & Lamport, 2021). The included studies consisted of 5 studies in infants and children, 17 in an adult population and a further 8 in ageing adults; and included numerous clinical populations including MDD, CFS, AD and mild cognitive impairment. Cognitive performance was assessed across numerous cognitive domains, devised from 41 individual task measures and composite scores. The authors report that of the 30 studies included, 21 reported an improvement in at least one cognitive outcome; however, they noted that no beneficial effect was observed in infants and children. This could again be explained by methodological discrepancies and weaknesses in the said studies; including varying supplementation length, and supplementation vehicle, specifically when presented with breast milk vs. formula milk. Moreover, the vast cognitive development in early childhood may mean that it's difficult to observe subtle improvements by probiotic intervention.

When considering just the adult studies, they indicate that probiotic supplementation may have beneficial effects in clinical populations (specifically MDD, fibromyalgia and CFS) and this may speak to the benefit of microbial modulation in those with particularly compromised environments where a significant improvement can be made and, crucially, observed. It is however crucial to recognise methodological issues associated with some of these trials, including non-randomised and non-blinded trials. Therefore, whilst there is some promise,

these results must be interpreted with caution and require further investigation, with more sound methodological design. Pertinent to this thesis, evidence in healthy adults is less clear. Whilst six of these studies report limited positive effects, no consistent effects were observed on specific areas on cognition; and limited reporting of individual task performance makes it difficult to draw firm conclusions from current literature.

This is supported by a recent meta-analysis of 22 studies (n = 1551) investigating the effects of probiotics (11 studies, n = 724), prebiotics (5 studies, n = 355) and fermented foods (6 studies, n = 472) on cognitive performance (Marx et al., 2020). They included trials in healthy individuals and also clinical populations including AD, MDD and mild cognitive impairment. Fourteen of these included studies reporting selective benefits to cognitive performance, however lack of consistency in findings meant that these findings were not observed when pooled together. Separate meta-analyses were conducted for the three intervention types; where they observed no significant effects on global cognitive performance. Additionally, no significant effects were observed on individual cognitive domains, including working memory, attention and executive function. Again, these null findings may be explained by methodological flaws in the included studies. Specifically, inconsistencies in cognitive domains targeted in the trials resulted in low numbers of studies included in the meta-analyses of many of the cognitive domains, therefore these analyses may lack in statistical power. Moreover, of the 22 studies included, just 5 reported adequate statistical power necessary to detect cognitive differences. Whilst a smaller sample size may be sufficient to detect shifts in gut microbial species, it is unlikely that this would be adequately powerful to assess cognitive function; emphasising the need for well powered future work that might be more capable of detecting changes. As with previous reviews, these authors also concluded that, based on existing literature, it is too early to conclusively determine the effects of psychobiotic[2] intervention on cognitive performance.

The lack of conclusive and consistent results in this area are likely to be due to vast differences in the study designs including different ages of participants, areas of cognitive performance and bacterial strains used. Moreover, as reported within the above reviews, the majority of human studies have not employed fine-grained measures of psychological function, which may not be sensitive enough to detect the subtle, bacteria-induced changes in cognition. It is

[2] The term 'psychobiotic' was originally defined as a probiotic with a positive impact on mental health (Dinan et al., 2013) but has since broadened to encompass any microbiome-mediated strategy that can modify mental health and psychological performance (Sarkar et al., 2016).

suggested that sensitive cognitive tasks, may be more conducive to detecting subtle bacterial induced changes, which may otherwise have been missed, resulting in false negative results. Similarly, it has been proposed that bacterial-cognition interactions may be more likely to be observed in tasks which require sustained attention performance over a prolonged period of time, during which performance is likely to deteriorate (Allen & Smith, 2012; Verster & Roth, 2013).

Whilst still in its infancy, the research linking the gut with the brain in animal models, and the relatively smaller amount of data from human trials, certainly provides some promising evidence that a relationship exists. This is supported by mechanistic data evidencing the role of direct and indirect pathways between the two regions of the body which would be expected to, when activated (either by neurotransmitter production, vagus nerve stimulation and/or anti-/inflammatory activity) exert effects on brain function. The extent to which this can improve cognitive function it yet to be fully elucidated, with additional work clearly warranted to determine this.

3.4. Dietary modulation of the gut

3.4.1. Introduction

Based on the potential for microbiota composition to influence host health and cognition, an emerging field of nutritional psychiatry investigates the effects of dietary modulation of the microbiota; and the impact that may have on mental health and specifically, cognition (Sarris et al., 2015). Here, it is thought that dietary alteration of the microbiome could result in the growth of bacterial strains which impacts host health, cognition and behaviour, via the aforementioned mechanisms (Blanchflower, Oswald, & Stewart-Brown, 2013; Dash, Clarke, Berk, & Jacka, 2015).

Although it has been shown that the gut microbial composition is relatively stable in humans from late childhood through adulthood (Faith et al., 2013), dietary intake is considered one of the key modulators of microbial composition (David et al., 2014; Doré & Blottière, 2015; Zarrinpar, Chaix, Yooseph, & Panda, 2014). Research indicates that, in mice, diet changes (e.g. a high-fat diet) accounted for 57% of the total structural variation in the gut microbiota, whereas genetic mutation accounted for only 12% (Zhang et al., 2010). This is further supported when looking cross-sectionally across human populations, where it has been consistently observed that individuals from different countries have distinct bacterial populations, which correspond with differing dietary patterns (Wu et al., 2011; Yatsunenko et al., 2012). One of the first studies to observe these differences showed that children in a rural African village showed low levels of Firmicutes and high levels of Bacteroides, when compared

with Italian children, who presented higher levels of Enterobacteriaceae (De Filippo et al., 2010). Disregarding the many additional interacting variables which could influence these differing levels culturally, this data suggests that long-term dietary patterns and habitual diet intake play a key role in shaping the individual's stable microbial profile.

Following on from this, a symbiotic relationship exists between the gut microbiota and diet where dietary intake has the ability to modify the composition and function of the microbiota. In turn, the microbes then influence the metabolism, absorption and storage of nutrients from the diet (Gentile & Weir, 2018). In essence, diet has the ability to create an environment which is hospitable to its own nutritional profile, and this also presents an opportunity to affect that environment positively, through dietary intervention. This has been evidenced in several disease models, including neurological disorders, where dietary therapies have been shown to treat or ameliorate symptoms in ASD, and attention deficit hyperactivity disorder (ADHD) (Bos et al., 2015; Cooper, Tye, Kuntsi, Vassos, & Asherson, 2016; Ruskin, Murphy, Slade, & Masino, 2017). Additionally, dietary intervention has been shown to be effective in the prevention and treatment of chronic health conditions, including reducing CVD risk by 60% (Kris-Etherton, Etherton, Carlson, & Gardner, 2002) and reducing GI symptoms in some, but not all, patients with IBS (Staudacher & Whelan, 2017). However, these studies indicate a high degree of individual variability in the response to dietary intervention treatment for diseases, which has been indicated in clinical trial results in obesity (Seganfredo et al., 2017) and CVD (McMillan-Price et al., 2006). As such, the field is moving towards the development of "personalised nutrition", which enables dietary advice to be tailored specifically to the individual, with the aim of improving dietary habits to prevent and treat chronic disease (Biesiekierski, Livingstone, & Moschonis, 2019). This approach aims to determine the effects of specific dietary compounds on an individual, by utilising information from a range of host-specific variables including demographic information, health status, current diet, biological pathways and epigenetic characteristics (Biesiekierski, Jalanka, & Staudacher, 2019; Perez-Martinez, Garcia-Rios, Delgado-Lista, Perez-Jimenez, & Lopez-Miranda, 2012). However, this is area is in its infancy and to date much of the evidence in support is from observational studies. Nevertheless, this is an exciting development to this field of work.

3.4.2. Food components

As it is apparent that habitual dietary habits and baseline gut composition has at least some impact upon the response of the gut to dietary intervention; it is important to understand the influence of individual food components and dietary styles on gut composition in terms of richness and diversity (Rinninella, Cintoni, et al., 2019). A rich and balanced diet is associated

with the diversity and gene richness of the microbiota (Heiman & Greenway, 2016). In particular, the overall balance of macronutrients (protein, carbohydrate and fat) is known to influence the composition of the microbiota (Gentile & Weir, 2018; Madsen, Myrmel, Fjære, Liaset, & Kristiansen, 2017). The below sections will detail the food components and dietary styles relevant to the demographic group and dietary intervention utilised within this thesis. However, it is important to acknowledge that due to the compositional nature of diet, it can be difficult to clearly define the individual roles of each dietary component on the gut microbiota (Danneskiold-Samsøe et al., 2019), therefore the following subdivisions should be regarded in this light.

3.4.2.1. In-digestible carbohydrates - Dietary fibre

Indigestible carbohydrates (dietary fibre), are defined as carbohydrates that are resistant to digestion in the small intestine and go on to reach the large intestine, where they serve as substrates for the colonic microbiota and have a major impact on microbial composition and function (DeVries, 2003; Healey et al., 2017; Rinninella, Cintoni, et al., 2019). These include non-starch polysaccharides, lignin, resistant starches and non-digestible oligosaccharides (Mudgil & Barak, 2013). Of this, non-starch polysaccharides include cellulose and hemicellulose, including glucans, gums and pectins (Rinninella, Cintoni, et al., 2019). Non-digestible oligosaccharides consist of raffinose, stachyose, oligofructose and inulin and resistant starch is found within whole or partly milled grains or seeds (Rinninella, Cintoni, et al., 2019). Indigestible fibres are further categorised based on their fermentability; whether they are able to be fermented by bacteria in the colon or not (fermentable or non-fermentable) and solubility in water (soluble or insoluble) (Galanakis, 2019).

Considered beneficial to the gut, fermentable soluble dietary fibres include inulin, pectin, beta-glucan, fructo-oligosaccharides (FOSs) and galacto-oligosaccharides (GOSs). Fermentation by gut bacterial species results in a cascade of events including a promotion of growth of beneficial bacteria including *bifidobacteria* and *lactobacilli*, production of short-chain fatty acids (SCFAs), lowering of intestinal pH, reinforcement of tight junction and intestinal epithelial integrity (Chassard & Lacroix, 2013; Fava et al., 2019; Macfarlane & Macfarlane, 2003). Of central importance, fermentable dietary fibres are the primary energy source for most intestinal bacteria; directly affecting the bacterial species which rely heavily on consumption of these substrates (David et al., 2014). They also can indirectly alter microbial composition through 'cross feeding' where some types of bacteria provide metabolites for the growth of other bacteria (Holscher, 2017). In comparison, non-fermentable and insoluble dietary fibres include cellulose, hemicellulose, lignin and resistant starch (Galanakis, 2019). These mostly pass

through the digestive system, but likely influence microbial composition through their impact on aiding bowel regularity and gut transit rate and can have a laxative-like effect (Anderson et al., 2009; McRorie Jr, 2015; Tottey et al., 2017).

Consumption of a high-fibre diet provides the microbiota with a large range of fermentable substrates to use as energy sources and is consistently associated with greater microbial richness and diversity (Schnorr et al., 2014; Yatsunenko et al., 2012). This diet is also associated with beneficial health effects, including reduced inflammation, maintenance of healthy body weight and improved cognitive performance (Kaczmarczyk, Miller, & Freund, 2012; Kim et al., 2016). In particular, soluble dietary fibre is consistently shown to have beneficial cardiometabolic effects by reducing cholesterol levels, controlling blood glucose and regulating body weight (Hartley, May, Loveman, Colquitt, & Rees, 2016; Tosh, 2013). This could be predicated on the viscous gel, which forms in the stomach and intestine after consuming soluble fibre (Gunness & Gidley, 2010), which inhibits and slows down the absorption of carbohydrates and reabsorption of bile acids; leading to increased synthesis of bile acids from cholesterol in the liver (Gunness & Gidley, 2010). In contrast, low dietary fibre consumption results in microbial deprivation of fermentable substrates, which, if prolonged, can result in a depletion of microbial diversity and potential extinction of beneficial bacterial species which are unable to be restored through increased fibre consumption (Han et al., 2017; Healey et al., 2017; Sonnenburg et al., 2016). Diets low in fibre are also associated with increases in the penetrability of the inner mucus layer and increased abundance of mucin-degrading bacteria (Desai et al., 2016).

It is crucial to recognise that, whilst current UK dietary recommendations suggests 30 g daily consumption of non-digestible fibre, data indicates that very few adults meet this; with average daily intake just 19.7 g (Public Health England, 2020). Given the apparent importance of dietary fibre intake, recent intervention studies have aimed to investigate the effects of dietary fibre intake on both microbial composition and related health outcomes, including inflammation, in participants with low dietary fibre intake. Within this demographic, a 6-week intervention with whole grains resulted in a significant increase in the short-chain fatty acid producer *Lachnospira* and reductions in pro-inflammatory *Enterobacteriaceae,* when compared with refined grain intervention (Vanegas et al., 2017). No effects were observed in plasma cytokines. Most recent research has indicated that the inclusion of resistant starch in a weight maintenance diet (following a 21-day weight loss diet) positively altered the gut microbiota composition, whilst also reducing fasting blood glucose, compared to participants who consumed a diet with no resistant starch (Johnstone et al., 2020). Similar effects have been observed in a HFD mice model supplemented for 16 weeks with banana pulp dietary

fibres, resulting in a suppression in body weight, epididymal fat mass gain, improved serum lipid profiles, liver lipid profiles and intestinal profiles (Wei et al., 2020). They also observed significantly improved fecal short-chain fatty acid formation; which impacted on the intestinal microbiota on taxonomic levels by increasing the proportions of beneficial *Lactobacillus, Bacteroidales* and decreasing *Streptococcaceae.* The authors indicate that these results may suggest that banana pulp dietary fibre is a functional ingredient for preventing obesity, metabolic syndrome and intestinal microorganism imbalance (Wei et al., 2020).

3.4.2.2. SCFAs

As previously established, SCFAs are the main microbial end products from saccharolytic fermentation of fermentable dietary fibres in the large intestine (Cummings & Macfarlane, 1991; Macfarlane & Macfarlane, 2003). The type and quantity of SCFAs are mainly determined by the composition of intestinal microbiota and by how much carbohydrates are consumed (Nylund, Kaukinen, & Lindfors, 2016). However, the main SCFAs produced in the human colon are acetate, propionate and butyrate, in a molar ratio of about 3:2:2 (Fava et al., 2019).

Locally, SCFAs are important energy sources for the gut microbiota itself and for intestinal epithelial cells (Farzi et al., 2018). Here, they strengthen the integrity of the intestinal epithelium, increase mucus production, modulate gut motility and exert anti-inflammatory effects such as inactivation of nuclear factor kappa B and the promotion of regulatory T cells (Cotillard et al., 2013b; Furusawa et al., 2013; Umoh et al., 2016; Usami et al., 2008). The ability for SCFAs to regulate inflammatory response is well documented. For example, the interaction of butyrate with GPR109A, expressed in the gut, reduces the inflammation mediated by interleukin (IL) 8 and IL-10 and promotes lipolysis in adipose tissue (de Velasco, Ferreira, Crovesy, Marine, & do Carmo, 2018). Butyrate has been shown to hold widely acting effects by inducing mucin synthesis, decreasing bacterial transport across the epithelium, and improving gut integrity by increasing tight junction assembly (Peng, Li, Green, Holzman, & Lin, 2009). These changes can help reduce intestinal permeability and impede the pathogenic microbiota and their metabolites in the intestinal lumen from reaching the circulation (Bernardi et al., 2019), aiding the suppression of colonic inflammation (Elinav et al., 2011; Salcedo et al., 2010).

In addition to their ability to modulate inflammation, SCFAs are associated with various improvements to host health, including enhancing nutrient absorption, promoting satiety and potential anti-obesogenic effects (Duncan et al., 2007; Fava et al., 2019; González Hernández, Canfora, Jocken, & Blaak, 2019). Through their ability to release hormones and neuropeptides, including GLP-1 and PYY from intestinal endocrine cells (Rooks & Garrett,

2016), SCFAs can promote satiety via endocrine and vagus-dependent pathways (De Silva & Bloom, 2012). Propionate interacts mainly with GPCR41 and GPCR43 in enteroendocrine cells, stimulating the secreting of PYY and GLP-1 hormones, which has anorexigenic effects, thus contributing to the reduction of food intake (de Velasco et al., 2018). SCFA interaction with GPCR41 and GPCR43 in adipose tissue also stimulates the secretion of leptin, which suppresses adipogenesis, the interaction with GPCR41 can enhance energy expenditure by increasing the activity of the sympathetic nervous system (X. Li, Shimizu, & Kimura, 2017). Acetate has also recently been shown to have an important role in the browning of white adipose tissue, thermogenesis, and protection from obesity (Jocken et al., 2018; Weitkunat et al., 2017).

Pertinent to this thesis, there is some evidence to indicate that SCFAs concentrations differ between obese and normal weight individuals, with obese individuals shown to excrete more SCFAs in their faeces than lean subjects (Fernandes, Su, Rahat-Rozenbloom, Wolever, & Comelli, 2014). Whilst higher faecal levels of SCFAs may suggest that the "obese microbiome" could harvest more energy from the diet through increased SCFA production (Anhê, Varin, et al., 2015), it might also suggest decreased SCFA absorption or utilisation by colonocytes (den Besten et al., 2013). An increasing body of literature supports a protective effect of SCFA against obesity and metabolic disturbances by modulating energy metabolism, gut barrier integrity and inflammatory responses (Cox & Blaser, 2013). This further supports the body of research indicating the important relationship between dietary fibre intake, and as such SCFA production, and improved host health.

3.4.2.3. Probiotics

The modulatory effect of probiotics has dominated the literature in recent years. Probiotics are defined as a living microorganism, which when ingested in sufficient quantities can enhance the health of the host (Allen, Dinan, Clarke, & Cryan, 2017). The most common probiotic strains include *Bifidobacterium* spp., *Lactobacillus* spp., and *Enterococcus* spp. (Fredua-Agyeman, Stapleton, Basit, & Gaisford, 2017), and good dietary sources include dairy products such as fermented milk, yogurt and cheese (Fontana, Bermudez-Brito, Plaza-Diaz, Munoz-Quezada, & Gil, 2013). Recently fermented foods (such as kimchi and sauerkraut) made through controlled microbial growth and enzymatic conversions of major and minor food components (Marco et al., 2017), have been referred to as probiotics, when they meet the aforementioned definition (Hill et al., 2014). These probiotics sourced in food products must have the capacity to survive and resist the gastric juices and bile, also being able to colonise the digestive tract (Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013).

In addition to potential 'psychobiotic' effects detailed within Section 3.3.5; probiotic consumption has also been associated with various beneficial health outcomes; including anti-inflammatory and use in the application of chronic diseases including IBS, diabetes and arthritis (as detailed further in Roobab et al. (2020)). It is suggested that there are several potential mechanisms underpinning these beneficial effects, including immune system interaction, vagus nerve activation, tryptophan metabolism and microbial metabolites (Cryan & Dinan, 2012). Probiotic bacteria can promote the production of neuroactive substances such as serotonin (Yano et al., 2015) and GABA (Barrett et al., 2012), impacting psychological health, although these are unlikely to cross the blood-brain barrier and affect the brain directly. Probiotics can also reduce inflammation by enhancing the integrity of the gut bacteria, thereby preventing further bacterial translocation (Ait-Belgnaoui et al., 2012; Zareie et al., 2006).

Several groups have reported increased total bacterial load after regular consumption of fermented milk or yogurt (Goossens, Jonkers, Russel, Stobberingh, & Stockbrügger, 2006; He et al., 2008; Matsumoto et al., 2010; Zhong, Huang, He, & Harmsen, 2006). As such, several RCTs have aimed to investigate the effect of specific probiotic strains on gut microbial composition. One trial in overweight, healthy adults, administered one of four interventions for 6 weeks: placebo, omega 3 fatty acids, probiotic containing three strains of *Bifidobacteria,* four strains of *Lactobacilli,* and one strain of *Streptococcus,* or a combination of omega 3 and the above probiotic (Rajkumar et al., 2014). They observed that probiotic supplementation (alone and in combination with omega 3) resulted in improved health outcomes including significant reduction in total cholesterol, triglycerides, and LDL. Alongside this, improved hsCRP and significant increase in concentration of aerobes, total anaerobes *Lactobacillus, Bifidobateria,* and *Streptococcus were observed,* compared to placebo. This supports the ability of a multi-strain probiotic in modulating microbial composition and benefiting aspects of host health.

Similar beneficial effects have been observed in disease models. As an example, within patients with chronic liver disease, 14-day supplementation with a probiotic yogurt containing *Bacillus bifidus, Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophilus,* resulted in a reduced microbial imbalance and lower count of *Escherichia coli,* as well as improvements in clinical symptoms (Liu et al., 2010). Similarly, in children with *Helicobacter pylori* infection following 4-week intervention with a probiotic yogurt containing the same bacteria types, improved *Bfidobacterium* spp./ *E. coli* ratio and reduced *H. pylori* counts, as well as reduced IL-6 levels (Yang & Sheu, 2012); indicating a potential therapeutic use of probiotics in the treatment of disease.

There is also limited evidence that probiotic modification of the gut microbial community may exert weight controlling effects. Generally, whilst rodent work indicates that *Lactobacillus* and *Bifidobacterium* reduces diet-related weight gain and insulin resistance, in humans there is relatively weak evidence for the efficacy of probiotics in reducing weight and improving glycaemic control (Diamant, Blaak, & De Vos, 2011; Park & Bae, 2015; Samah, Ramasamy, Lim, & Neoh, 2016; Wang et al., 2015). Indeed, it has been reported that *Bifidobacterium* supplementation may exert beneficial metabolic effects in rodents fed a high-fat-diet, mainly by improving gut barrier integrity, bacterial LPS translocation, endotoxemia and inflammation (Cano, Santacruz, Trejo, & Sanz, 2013; Kondo et al., 2010; Núñez, Galdeano, de LeBlanc, & Perdigón, 2014). Recent studies have also suggested that *Lactobacillus gasseri* may decreased abdominal adiposity and postprandial lipid responses in Japanese overweight subjects (Ogawa, Kadooka, Kato, Shirouchi, & Sato, 2014). However, most studies testing probiotics in humans have not reported anti-obesity effects. For example, a study reported that probiotic treatment (*Lactobacillus* and *Bifidobacterium*) had no effects on BMI, body fat and waist-to-hip ratio in obese and overweight people (Zarrati et al., 2013). Although some studies have shown that probiotics may play a role in the regulation of body weight, information from human intervention trials is extremely limited and there are no conclusive data to support that probiotic intake may contribute to a decrease in body weight (Park & Bae, 2015; Sanz, Rastmanesh, & Agostonic, 2013).

3.4.2.4. Prebiotics

Despite the historical focus on pro-biotics, a recent shift in the literature focusses on dietary fibres that are classified as a ‘prebiotic’. These compounds pass undigested through the upper intestinal tract, where in the colon they are selectively fermented by gut microbial species, resulting in specific changes to microbial composition and have a beneficial effect on host health (Ansari, Pourjafar, Tabrizi, & Homayouni, 2020b; Gibson et al., 2017; Holscher, 2017). Whilst it was originally thought that prebiotics selectively enhanced only the growth of *Bifidobacterium* and *Lactobacillus* (Gibson & Roberfroid, 1995), it is now recognised that they may enhance the growth of other bacterial species and can still be considered ‘prebiotic’ providing the effects on host health are beneficial (Gentile & Weir, 2018). Sources of prebiotics include soybeans, inulins, unrefined wheat and barley, raw oats and non-digestible oligosaccharides such as fructans, polydextrose, fructooligosaccharides (FOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS) and arabinooligosaccharides (AOS) (Pandey, Naik, & Vakil, 2015). Recent technological advances that allow for detailed examination of microbial responses to dietary components have led to an expansion of the prebiotic concept, resulting in a more inclusive list of potential substrates and microbial targets, which now includes polyphenols (Duda-Chodak et al., 2015; Gentile & Weir, 2018). As this is

a relatively new area of research, the definition of prebiotic and the compounds classified as such, will evolve further and expand as research uncovers the impact of specific bacterial strains on the gut microbiota and host health (Koç, Mills, Strain, Ross, & Stanton, 2020).

The potential beneficial effects of prebiotics are generally attributed to the following two pathways: 1) increased abundance of beneficial bacteria and SCFA production and consequently, improved barrier function and resistance to inflammatory stimuli (Furusawa et al., 2013) and 2) increasing levels of some beneficial species (such as *Bifidobacterium)* that could contribute to restore gut dysbiosis (Cani, Neyrinck, et al., 2007). Animal studies have provided strong evidence that prebiotics can modulate the gut microbiota composition, specifically GOS, FOS, and polydextrose are reported to increase the abundance of probiotic bacteria such as *Bifidobacteria* and *Lactobacilli* (Canfora et al., 2017; Savignac et al., 2016; Singh et al., 2017; Thongaram, Hoeflinger, Chow, & Miller, 2017). Indeed, studies have shown that a diet rich in whole grain and wheat bran are linked to an increase in *Bifidobacterium* and *Lactobacilli* (Carvalho-Wells et al., 2010; Costabile et al., 2008). Similar studies have observed increases in the abundance of *Ruminococcus, E. rectale* and *Roseburia* following intake of resistant starch and whole grain barley (Keim & Martin, 2014; Leitch, Walker, Duncan, Holtrop, & Flint, 2007). Said increased populations of these bacteria in the intestines antagonistically suppresses the activity and growth of pathogenic bacteria (Lamsal, 2012). This is supported by evidence that FOS-, polydextrose-, and AOS-based prebiotics reduce *Clostridium* (Costabile et al., 2012; Kedia, Vázquez, Charalampopoulos, & Pandiella, 2009; Liu et al., 2014) and *Enterococcus* species (François et al., 2012; Kapiki et al., 2007). This results in health promoting effects, including reducing metabolic endotoxemia and inflammation, and improvements in gut barrier function (Boulangé, Neves, Chilloux, Nicholson, & Dumas, 2016; Scheen & Paquot, 2009). The effect of prebiotics on the human gut microbiota results in an increase in the levels of beneficial bacteria belonging to the *Bifidobacterium* and *Lactobacillus* genera, and also of other commensal bacteria, such as members of the Bacteroidetes phylum (Flint, Scott, Duncan, Louis, & Forano, 2012). Considering the complexity of gut microbiota organisation, it is thought that prebiotic dietary fibres exert their effect at multiple levels and on the gut microbiota as a whole not just targeting individual bacterial genera, which are commonly associated with the final benefits coming from prebiotics (Fava et al., 2019).

Prebiotic intake appears crucial in developing a rich and diverse microbial community, which is imperative for host health. Indeed, a diet low in prebiotics has been shown to reduce total bacterial abundance (Halmos et al., 2015); whereas a high intake of these compounds has resulted in an increase in microbiota gene richness in obese adults (Cotillard et al., 2013a). Studies in animals and humans have indicated that prebiotic intake has a number of beneficial

health effects pertinent to this thesis; such as improving mental health (Ansari, Pourjafar, Tabrizi, & Homayouni, 2020a), and reducing intestinal inflammation (Lomax & Calder, 2008). Recently a number of randomised controlled trials have reported beneficial effects of prebiotics on obesity and associated diseases by reducing bodyweight and increasing satiety (Cerdó, García-Santos, Bermúdez, & Campoy, 2019). Prebiotic consumption has also shown notable shifts in metabolic and immune markers, for example several studies have observed reductions in proinflammatory cytokine IL-6, insulin resistance, reductions in concentrations of serum triglycerides, total cholesterol and LDL-cholesterol and peak post-prandial glucose associated with the intake of non-digestible carbohydrates in whole grains (Keim & Martin, 2014; Kim, Hwang, Park, & Bae, 2013; Martínez et al., 2013). These beneficial effects of prebiotics on immune and metabolic function is thought to involve increased production of SCFAs and strengthening of gastrointestinal-associated lymphoid tissue (GALT) from fibre fermentation (Schley & Field, 2002).

Despite these promising health related findings, recent intervention RCTs indicate these may be observed irrespective of distinct modulation to microbial communities. As an example, in one study, following a 2-week Western diet run in, healthy adults were provided with a six-week intervention of a weight management diet with either refined grains or whole grains (Vanegas et al., 2017). Their findings indicated small shifts in gut microbiota composition, including significant increases in *Lachnospira* (an SCFA producer) and a decrease in pro-inflammatory *Enterobacteriaceae* following whole grain consumption. Alongside this, some beneficial health outcomes, such as increased stool weight and frequency, and increased stool acetate and total SCFAs, were observed. The authors highlight that, to see more pronounced changes host health, likely requires a more prominent change in microbial composition, which they hypothesise may require a more prolonged intervention period or a study demographic who are compromised in terms of inflammatory status or chronic disease.

Another study in overweight and obese individuals at risk of developing metabolic syndrome, investigated the effects of an 8-week diet intervention period of either a whole grain diet, or a refined grain diet, on the microbiota and health markers (Roager et al., 2019). They found that the higher fibre whole grain intervention had no significant impact on the gut microbial composition, diversity or functional potential in comparison to a refined grain intake. However, despite this there were marked improvements in inflammatory biomarkers (IL-6 and CRP) and a concurrent decrease in body weight. Indicating that the health benefits observed from whole grain intervention were independent of microbial changes; prompting the need for supplementary work in human intervention trials to consider the role of the microbiota in beneficial effects of prebiotics and similar compounds.

3.4.3. Dietary styles

Whilst it is important to consider the individual food components, as humans eat mixed diets rather than single nutrients in isolation, there has been a shift in research focus to examine the health effects of diets and dietary patterns instead. Indeed, experimental manipulation of an individual macro-nutrient invariably alters intake of other macronutrients that may have metabolic effects themselves. For example, high-fat diets are commonly low in fibre, and it may be this latter feature, and its detrimental effects on the microbiota, that drives some of the metabolic consequences of the diet rather than the elevated fat content by itself (Gentile & Weir, 2018).

3.4.4.1 Western diet

Of particular relevance to this thesis, the Western diet (WD) is a dietary habit typically consumed by those in developed countries and increasingly in developing countries, associated with economic growth (Rinninella, Cintoni, et al., 2019). It is categorised by consumption of western style food products, which are usually highly processed, highly calorie dense containing high amounts of saturated fatty acids, trans fats, added sugar and salt and additives. They are also predominantly animal-based in terms of protein, whilst low in mono- and poly-unsaturated fats, fruit and vegetables and fibre (Mills, Stanton, Lane, Smith, & Ross, 2019; Moubarac et al., 2014). A diet high in processed foods may present a higher risk of developing deficiencies of some vitamins and minerals, as they have been shown to have a lower micronutrient content than whole foods (Monteiro, Moubarac, Cannon, Ng, & Popkin, 2013; Via, 2012). Importantly consumption of a WD is associated with an increased risk of developing obesity, metabolic diseases, type 2 diabetes, cardiovascular diseases and colorectal cancer, potentially by causing dysbiosis in gut microbiota composition (Bouvard et al., 2015; Miclotte & Van de Wiele, 2019; Minihane et al., 2015; Whitmer, Gunderson, Barrett-Connor, Quesenberry, & Yaffe, 2005; Zinöcker & Lindseth, 2018).

Research has shown that WD induces changes in intestinal microbial composition leading to decreased bacterial diversity, high numbers of *Firmicutes* and *Proteobacteria*, and low *Bifidobacteria* and *Lactobacilli* levels (Agus et al., 2016; Araújo, Tomas, Brenner, & Sansonetti, 2017; Beilharz et al., 2018; Singh et al., 2017; Wu et al., 2011). Cross cultural research is highly valuable when considering the long-term impact of WD consumption on microbial composition. Specifically, when comparing the microbiota composition of European children consuming a WD with Burkino Faso (BF) counterparts, we see that the latter children consume a diet rich in millet and local vegetables, with few lipids and animal proteins (De Filippo et al., 2010). Results showed that the BF microbiota was enriched with *Prevotella* and *Xylanibacter*

and SCFAs, whereas the European children were more abundant in *Proteobacteria* and potentially pathogenic bacteria *Shigella* and *Escherichia*. Similarly, a study investigated the microbiota composition of volunteers from Venezuela, Malawi and the United States, where they identified that those in the US had the least microbial diversity, and lower abundance of *Prevotella* (Yatsunenko et al., 2012). Similar effects have been observed in studies of other populations including hunter-gatherers in the Chihuahuan Desert (Leach & Sobolik, 2010), the Hazda of Tanzania (Schnorr et al., 2014) and the Asaro and Suasi people of Papai New Guinea (Martínez et al., 2015). This wide-spread data indicates that consumption of WD results in a less diverse and rich microbiota, with reductions in beneficial bacteria and potential stimulation of pathogenic bacteria, when compared with less processed dietary styles. It also provides evidence that socioeconomic status, or more specifically, the economic wealth of a country, does not necessarily dictate gut diversity and, therefore, general health of its population. Indeed, it may even suggest the opposite; that the economic prosperity of a nation has a negative impact on gut microbial health, and this may be one factor influencing the correlation between the wealth of a nation and its obesity rates (Popkin, Adair, & Ng, 2012; Szilagyi, Smith, Sebbag, & Xue, 2021).

A recent systematic review investigated the effects of ultra-processed very low-energy diets on the gut microbiota and metabolic outcomes in individuals with obesity. They found changes to taxa within the Firmicutes phylum, including reduced abundance of potentially beneficial butyrogenic microbes (eg. *Roseburia, Lactobacillus,* Bifidobacterium and *Lachnospiraeceae).* They also saw increased abundance of potentially pathogenic microbes from the Bacteroidetes phylum, including increases in *Alistipes* and *Bacteroides* taxa (Lane et al., 2020).

It is difficult to discern which features of the WD contribute to these negative effects, these could include low fibre content, or high fat content or a combination of a multitude of factors, including the low-microbial content observed in highly processed foods (Alemao et al., 2020; Miclotte & Van de Wiele, 2019). Indeed, studies indicate that supplementing high fat diets with dietary fibre can alleviate many of the negative effects on the mucous barrier by restoring bacterial load and microbiome composition (Schroeder et al., 2018; Shi et al., 2020; Zou et al., 2018). In addition, the WD based on animal protein increases the abundance of bile-tolerant microorganisms such as *Alistipes, Bilophila* and *Bacteroides* and decreases the levels of Firmicutes that metabolise dietary plant polysaccharides such as *Roseburia, Eubacterium rectale* and *Ruminococcus bromii* (David et al., 2014). As such, it is likely that various components of the Western diet results in the observed harmful modulation of the microbiota.

Western diets are depleted from beneficial substrates, such as *Lactobacillus* and *Bifidobacterium*, enabling gut microbial synthesis of SCFAs, resulting in poor availability of SCFAs and therefore increased risk of intestinal inflammatory pathologies and metabolic diseases (Anhê, Varin, et al., 2015; Falony & De Vuyst, 2009). In addition, the typical Western diet may cause an imbalance in the immune system by increasing potentially pathogenic microorganisms in the microbiota (Koç et al., 2020). The WD may cause increased LPS production which results in increased colonic permeability (Myles et al., 2013) and systemic inflammation (Heinritz et al., 2016). The well documented detrimental effects of the WD on microbiota composition, alongside links to the pathogenesis of metabolic diseases calls for the need for dietary intervention in the Western world. Specifically, therapeutic intervention with prebiotics, including polyphenols, is likely a promising method as we will see in the following section.

3.4.4.2. Mediterranean diet

In contrast to the WD, the Mediterranean diet (MedDiet) compromises high consumption of fruits, vegetables, nuts, legumes, whole grains and olive oil with moderate intakes of dairy products, eggs, white meat and dish and low intakes of red meat, potatoes and processed foods (Chiva-Blanch et al., 2014; Lopez-Legarrea, Fuller, Angeles Zulet, Martinez, & Caterson, 2014). Moreover, the MD is typically characterised by low energy intake, with energy intake largely derived from carbohydrates and less from fats and proteins. In particular, this diet has greater prevalence of complex-carbohydrates over simple-carbohydrates and of MUFA and PUFA over saturated fatty acids (Fava et al., 2019). Moreover, regular consumption of polyphenols and other antioxidants, a high intake of prebiotic fibre and greater consumption of plant proteins than animal proteins is observed (Rinninella, Cintoni, et al., 2019). Epidemiological evidence has indicated that consumption of the MD is linked to a large number of health benefits including reduced mortality risk, reductions of inflammation, prevention of CVD, diabetes and metabolic syndrome, as well as improvements in cognitive function and mental health (Bonaccio et al., 2017; de Lorgeril et al., 1999; Kastorini et al., 2011; Lourida et al., 2013; Psaltopoulou et al., 2013; Salas-Salvadó et al., 2014; Sofi, Abbate, Gensini, & Casini, 2010).

Several studies have investigated the effects of Mediterranean diet consumption on microbiota consumption, with studies indicating that adherence to the MedDiet increased total bacterial diversity; the abundance of *Bifidobacterium* and *Lactobacillus,* the *Bifidobacteria/E.coli* ratio, whilst decreasing the numbers of *Bacteroides* and *Clostridium perfringens* (Mitsou et al., 2017; R. K. Singh et al., 2017). Another indicated that lower adherence to the MedDiet was related

to a higher ratio of *Firmicutes/Bacteroidetes* ratio (Garcia-Mantrana, Selma-Royo, Alcantara, & Collado, 2018). In addition, increased abundance of *Prevotella* and fibre-degrading bacteria that ferment complex carbohydrates to produce SCFAs, have been observed (De Filippis et al., 2016). Indeed, higher levels of total SCFAs have been associated with better adherence to the MedDiet (Garcia-Mantrana et al., 2018). Within a recent observation study of Italian students, higher adherence to the MedDiet was associated with higher levels of lactic acid bacteria (Gallè et al., 2020), which is associated with modulating inflammation and enhancing metabolism (Pessione, 2012). Similar increases in lactic acid bacteria have been observed following a 3-month intervention of a MedDiet, enhanced with 40 g extra virgin olive oil, in normal-weight and overweight individuals (Luisi et al., 2019). They concurrently observed significant reductions in inflammatory markers (TNFα, IL-6 and myeloperoxidase), supporting the role of MedDiet in modulating the gut microbiota and having beneficial health outcomes.

Supplementary studies have highlighted the importance of obesity in the microbial response to MedDiet. For example, a short-term (15 day) intervention with a MedDiet in elderly obese women, resulted in a reduction in weight and the obese-related microbial dysbiosis was partially reversed, marked by a reduction in pro-inflammatory bacteria *Collinsella (*Cancello et al., 2019). These observed beneficial findings were amplified following an additional 15-day supplementation of the MedDiet combined with a multi-strain probiotic; supporting the potential for even short-term dietary modification to be effective within this demographic.

Similar effects have been observed following longer MedDiet interventions. Here, a 2-year MedDiet intervention in patients with metabolic syndrome, resulted in partial restoration of various bacterial strains including *F. prausnitzii* and *B. longum,* where each were reduced at baseline in the metabolic syndrome participants compared with the control group (Haro et al., 2016). They further note that they observed a positive correlation between the abundance of this bacteria and plasma levels of HDL, and a negative correlation with plasma glucose levels. Similar beneficial effects have also been observed following an 8-month intervention with and without a weight loss on the gut microbiota in obese older adults (mean BMI 35.8, mean age 64.8). Specifically, significant increases in richness, diversity and relative abundance of the genera Lachnoclostridium, Veillonella and Bifidobacterium and *Blautia caecimuris* were reported (McLeod, Bernabe, Schiffer, Fitzgibbon, & Tussing-Humphreys, 2020). Taken together, these findings suggest that MedDiet intervention in older, overweight adults has the ability to modulate microbial composition which may have positive implications for human health.

Whilst research into the impact of the MedDiet and the microbiota is still a relatively novel area; the work to date, suggests that it may be the most promising solution to optimally modulate microbiota diversity, and stability as well as regular permeability and activity of the immune functions of the human host (Rinninella, Cintoni, et al., 2019). Although the beneficial effects of the MedDiet on the gut microbiota are multiple and intertwined, importance has been placed on the high consumption of plant-derived foods and, therefore, of phenolics. Those in support of the role of phenolics argue that hese beneficial effects could potentially be explained by consumption of individual strains and species; or through multiple plant bioactive compounds acting synergistically on microbial composition (Fava et al., 2019).

3.5. Polyphenols and the gut

Research has indicated a beneficial reciprocal relationship between gut microbiota and polyphenols, where it produces more absorbable and potent phenolic metabolites; and also increases the abundance of beneficial bacteria and reduces harmful bacteria in the gut microbiome (Ozdal et al., 2016). This mutually beneficial relationship is considered to positively impact on overall human health (Anhê et al., 2013; Gowd et al., 2019).

3.5.1. Effects of microbiota on polyphenols

Due to the low absorption of polyphenols, where only 5-10% of total polyphenol intake is absorbed in the small intestine (Anhê, Roy, et al., 2015); the remaining 90-95% of ingested polyphenols may accumulate in the colon, where they are subjected to enzymatic activities of the gut microbiota (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013). As such, interindividual differences in microbial composition likely results in differences to the metabolism, bioavailability and bioactivity of polyphenolic metabolites.

During the long journey through the GI tract, polyphenols are exposed to large variations in Ph. As such, some polyphenols change their chemical structure accordingly (Fang, 2014; McGhie & Walton, 2007). These molecular re-arrangements are important, as the vast changes in pH may likely affect their bioactive properties. As detailed previously in Section 1.2.1, polyphenols may undergo hydrolysis in the small intestine and glucuronidation, methylation and sulfation during intestinal absorption and liver passage. Once reaching the colon, either after being metabolised, or in their original form, polyphenols may be transformed further by microbial bacteria (Manach et al., 2004). In the colon, unabsorbed polyphenols are metabolised by gut microbes to produce bioavailable metabolites via hydrolysis, cleavage and reduction (Bowey, Adlercreutz, & Rowland, 2003). These metabolites are then often more active, better absorbed and able to persist in plasma for a longer time than their precursors

(Espín et al., 2017; Filosa et al., 2018; Jamar, Estadella, & Pisani, 2017; Tomas-Barberan, Selma, & Espín, 2018).

Importantly, evidence has indicated that there may be specific bacterial strains that are capable of metabolising polyphenols extensively. For example, evidence indicates that just four bacterial strains (*Escherichia* sp.4, *Escherichia* sp. 34, *Enterococcus* sp. 45 and *Bacillus* sp. 46) are capable of converting flavonoids extensively when compared with other strains (J.-h. Tao, Duan, Jiang, Qian, & Qian, 2016). Similar observations have been made for specific polyphenol groups, including anthocyanins; where *Lactobacillu plantarum, Lactobacillus acidophilus* and *Bifidobacterium lactis* seem particularly important in their metabolism (Braune & Blaut, 2016; Marín et al., 2015; Rowland et al., 2018). Additional bacteria-phenolic metabolism links are detailed further in Corrêa, Rogero, Hassimotto, and Lajolo (2019).

Of importance to this thesis, evidence has indicated which bacterial strains are involved in the metabolism of resveratrol. Donated faecal samples of 7 healthy volunteers, were prepared into a faecal suspension and *trans*-resveratrol added; where various selected bacterial strains were tested for their ability to metabolise resveratrol (Bode et al., 2013). Their findings indicated that of these, only *Slackia equolifaciens* and *Adlercreutzia equolifaciens* (which both belong to *Coriobacteriaceae* family) were able to produce resveratrols main metabolite, dihydroresveratrol. This finding is in support of previous work that identified *Eggerthella lenta* ATCC 43055, which also belongs to the *Coriobacteriaceae* family, as capable of producing dihydroresveratrol (Jung et al., 2009). This study also identified the metabolising potential of *Bacteroides uniformis* ATCC 8492; suggesting that these families, specifically are involved in the metabolism of resveratrol. Crucially, dihydroresveratrol glucuronides and sulfates are consistently observed in higher concentrations in plasma, urine and tissue, than resveratrol glucuronides and glucosides following intake of grape or red wine polyphenols or resveratrol supplementation wine (Andres-Lacueva et al., 2012; Rotches-Ribalta et al., 2012). This indicates the importance of gut bacterial metabolism of resveratrol in producing resveratrol derivatives to potentially confer health benefits (Chaplin et al., 2018).

Whilst specific bacterial strains may be crucial in the metabolism of polyphenols, there is also evidence to suggest that the overall composition and diversity of the microbiome impacts the type and bioavailability of polyphenolic metabolites (Blaut & Clavel, 2007; Simons, Renouf, Hendrich, & Murphy, 2005). The presence and abundance of certain bacterial strains can result in specific microbial interactions here. For example, *Bacteroides, Enterococcus, Enterobacter, Lactobacillus* and *Bifiobacterium* can execute hydrolysis reactions, and *Coriobacteriuaceae. Eubacterium* and *Clostriduim* possess a cleavage activity, while

Gordonibacter urolithinfaciens and *Lactonifactor longoviformis* can catalyse the reduction of polyphenols (Espín et al., 2017).

The presence of specific bacterial strains also appears to dictate the efficiency of polyphenolic intervention, resulting in individual variations in related health outcomes (Filosa et al., 2018). For example, a pilot study of 6-week supplementation with the phenolic compound capsaicin reported stronger beneficial effects on metabolic and inflammatory markers in healthy subjects that belonged to the so-called *Bacteroides* enterotype in comparison to those individuals from the *Prevotella* enterotype (Kang et al., 2016). Similarly, research has indicated that in response to isoflavones, some people have the ability to produce equol and *O*-desmethylangolensin (termed "producers"), whilst others don't ("non-producers") (Cortés-Martín et al., 2018). Several bacterial strains including *Adlercreutzia equolifaciens, Slackia equolifaciens* and *Slackia isoflavoniconvertens* have been identified as being able to convert isoflavones to equol (Rowland et al., 2018). The lack of presence of these bacterial strains could explain why some participants do not respond to isoflavone intervention; or likewise other polyphenolic interventions.

There is also a high degree of interindividual variation in the production of polyphenol metabolites, as evidenced by Teixeira et al. (2017) who observed polarised differences in urinary excretion of polyphenolic metabolites following a single dose of grumixama (Brazilian cherry) juice. Within their sample of 10 healthy women, they were able to categorise participants as high and low urinary metabolite excretors. Whilst the mechanisms behind these individual variations require considerable further elucidation, it has been proposed that the differential health effects associated with consumption of polyphenols could be associated with differences in the production of bioactive compounds by the gut microbiota as a result of the variations in microbial ecology that colonises the colon of each individual (Tomas-Barberan et al., 2018). Whilst gut microbiota is considered important in terms of impacting polyphenol metabolism, polyphenols also can modulate microbial composition.

3.5.2. Effects of polyphenols on microbial composition

Recently, consumption of polyphenols has been considered to exert a prebiotic-like effect on gut microbial composition; specifically due to their ability to enhance the growth of probiotic bacterial family (such as *Bifidobacteriaceae* and *Lactobacillaceae*) and by providing an antimicrobial activity against pathogenic gut bacteria (such as *E.coli, Clostridium perfingens* and *Helicobacter pylori*) (Duda-Chodak et al., 2015; Morais, de Rosso, Estadella, & Pisani, 2016; Ozdal et al., 2016; Tomás-Barberán, Selma, & Espín, 2016). This is likely the result of

improving host health via enhanced production of SCFAs, reducing gut barrier permeability and inflammatory response, as detailed previously within Section 3.4.3.3. Additionally, polyphenolic-modulation of the gut microbiota promotes the growth of specific gut microbial species including *Faecalibacterium* spp., *Akkermansia* spp., and *Roseburia* spp., each of which may provide benefits to host health (Espín et al., 2017; Tomás-Barberán et al., 2016). The ability for polyphenols to modulate microbial composition has been investigated in *in vitro* assays using human microbiota and in preclinical and clinical studies utilising polyphenol-rich food. These studies indicate that individual polyphenol groups may modulate the gut microbiota differently, as recently reviewed by Loo, Howell, Chan, Zhang, and Ng (2020). Research to date, is summarised briefly below and within Table 3.3, with red wine and grape polyphenols and resveratrol polyphenols discussed in more detail in Section 3.5.3.

The 'prebiotic-like' effect of polyphenols is well documented, where polyphenols and polyphenolic-rich foods including cocoa, tea extracts, nuts and various fruits including blueberries and apple extracts have enhanced the growth of beneficial bacteria *Lactbocilli* and *Bifidobacteria* (Byerley et al., 2017; Fogliano et al., 2011; Molan, Lila, Mawson, & De, 2009; Sembries, Dongowski, Mehrländer, Will, & Dietrich, 2006; H. Sun et al., 2018; Tzounis et al., 2011). In addition to prebiotic like effects, differences have been observed in core microbiota composition, specifically *Firmicutes/Bacteroidetes* ratio (Anhê et al., 2019; Etxeberria et al., 2015; Henning et al., 2018; Parkar, Stevenson, & Skinner, 2008). Here, a higher ratio is observed in disease models such as obesity and metabolic syndrome; and a reduction in this ratio may be indicative of improvements to host health.

Moreover, consistent enhancements have been observed to supplementary beneficial bacterial species, with considerable interest placed on *Akkermansia muciniphili,* a mucin-degrading microorganism (Espín et al., 2017). Of particular interest here, *A. muciniphili* occurrence inversely correlates with body weight, with decrements observed in mice models of obesity and type 2 diabetes (Everard et al., 2013). Within this model, a 4-week administration of *A. muciniphili* reversed endotoxemia, weight gain, inflammation and insulin resistance (Everard et al., 2013); consequently suggesting its use in the prevention or treatment of metabolic disease. Several studies have observed increased abundance of *A. muciniphili* following polyphenolic intervention. One example here includes increased abundance following 8 weeks supplementation of cranberry extract (rich in proanthocyanidins) in C57BL/6J mice fed a high fat and high sucrose diet (Anhê, Roy, et al., 2015). Similar effects have been observed in rodents, with a few examples seen following supplementation with quercetin (Etxeberria et al., 2015); betacyanins from red pitaya (Song et al., 2016) and artic berry extract (Anhê et al., 2018). Likewise, in human work, 4-week supplementation of 1000

mg pomegranate extract increases *A. muciniphila* in healthy adults capable of producing urolithin A (Henning et al., 2017; Li et al., 2015). Despite this, 3 week supplementation of 656 g pomegranate extract did not result in any changes to *A. muciniphila,* in overweight and obese adults (González-Sarrías et al., 2018); suggesting that phenolic modulation of *Akkermansia* requires further understanding.

Whilst much of the work indicates polyphenol intervention results in beneficial modulation of the microbiota, some work has indicated potentially detrimental effects of black tea to human gut microbial composition in the stimulated intestinal microbial ecosystem (SHIME) (Kemperman et al., 2013). Researchers here observed increases in pathogenic bacteria *Klebsiella* and reductions in beneficial *Bifidobacterium.* However, the authors report the dominance of *Klebisella* could be a result of the artificial nature of the gut model system; indeed previous criticisms of gut modelling is that they can suffer from a high abundance of Enterobacteriaceae, which was also observed in this work (Saulnier, Gibson, & Kolida, 2008). Furthermore, contrary results from a more recent batch-culture fermentation study with human faecal bacteria observed beneficial effects of tea polyphenols from black, green and oolong tea (Sun et al., 2018). This includes increased beneficial bacteria *Bifidobacterium* spp., *Lactobacillus* spp. and *Enterococcus* spp., whilst reducing *Prevotella, Bacteroides* and *Clostridium histolyticum.* Results also indicated an enhanced production of SCFA, suggesting that consumption of tea polyphenols may modulate intestinal flora and contribute to improvements in human health. Whilst still within its primitive years, the literature to date certainly shows that polyphenol consumption has the ability to modulate microbial composition. However, with much of the work so far conducted *in vitro* or within animal models, it is necessary for supplementary work to be conducted in human intervention studies. Given the complexity of the relationship between polyphenols and microbial composition, that likely varies between polyphenol type, dosage, duration and host health considerably more research is necessary to understand this further.

Table 3.3. Summary of polyphenolic-modulation on gut microbiota. Including *in vitro*, animal and human intervention trials. Table adapted primarily from recent reviews (Aravind, Wichienchot, Tsao, Ramakrishnan, & Chakkaravarthi, 2021; Corrêa et al., 2019; Gowd et al., 2019)

Reference	Polyphenol	Study methodology	Time frame	Microbial modulation	Metabolic outcome
			In vitro		
Tzounis et al. (2008)	150 mg/mL (+)-catechin	Batch culture fermentation	/	↑ *Clostridium coccoides* ↑ *Bifidobacterium* spp. ↑ *Escherichia* spp. ↓ *Clostridium histolyticum*	/
Fogliano et al. (2011)	Cocoa flavanols	Three-stage culture system	/	↑ *Bifidobacteria* ↑ *Lactobacilli*	↑ Butyrate
Lee, Jenner, Low, and Lee (2006)	Chinese tea extract	Culture	24 hr	↓ *Clostridium perfringens* ↓ *Clostridium difficile* ↓ *Bacteroides* spp.	/
Sun et al. (2018)	Green tea, oolong tea and black tea	Fermentation	36 hr	↑ *Bifidobacterium* ↑ *Lactobacillus* spp. ↑ *Enterococcus* spp. ↓ *Bacteroides* ↓ *Prevotella* ↓ *Clostridium histolyticum*	↑ SCFAs
Kemperman et al. (2013)	1000 mg /day phenols from black tea	SHIME model	2 weeks	↑ *Klebisiella* ↑ *Enterococcus* ↑ *Akkermansia* ↓ *Bifidobacterium* ↓ *B. coccoides* ↓ *Victivallis* ↓ F/B ratio	/
Parkar et al. (2008)	25 mg/mL quercetin	Human cell culture	/	↓*Staphyloccum aureus* ↓ *E. coli* ↓ *Lactobacillus rhamnosus* ↓ *Salmonella typhimurium*	/

Animal studies					
Jiao et al. (2019)	200 mg/kg bw Blueberry extract	Obese C57BL/6J mice w/ high fat diet	12 weeks	↑ *Proteobacteria* ↑ *Bifidobacterium* ↑ *Helicobacter* ↓ *Actinobacteria* ↓ *Prevotella*	↓ Body weight gain ↓ Serum LDL-cholesterol ↓ Total cholesterol in liver
Henning et al. (2018)	320 mg/kg bw black tea and 240 mg/kg bw decaffeinated green tea	Obese C57BL/6J mice w/ high fat and high sucrose diet	4 weeks	↓ Firmicutes ↑ Bacteroidetes ↑ *Pseudobutyrivibrio* (black tea only)	↓ Body weight ↑ SCFAs (black tea only)
Lacombe et al. (2013)	24 mg anthocyanins/ day from lowbush wild blueberries	Rats	6 weeks	↓ *Lactobacillus* ↓ *Enterococcus* ↑ *Bifidobacteria* ↑ *Coriobacteriacea*	/
Anhê et al. (2018)	Arctic berry extract	Obese C57BL/6J mice w/ high fat and high sucrose diet	8 weeks	↑ *Akkermansia muciniphila* ↑ *Oscillibacter* ↑ *Turicibacter*	↓ Circulating endotoxemia ↓ Intestinal and hepatic inflammation
Anhê et al. (2019)	200 mg/kg Camu-camu extract	Mice w/ high fat and high sucrose diet	8 weeks	↓ F/B ratio ↑ Microbial richness	↓ Body weight gain ↓ Fat accumulation ↓ Metabolic inflammation ↓ Endotoxemia ↑ Glucose tolerance ↑ Insulin sensitivity
Song et al. (2016)	200 mg/kg/day betacyanins from red pitaya	C57BL/6J mice w/ high fat diet	14 weeks	↑ *Akkermansia* ↓ *Firmicutes* ↓ *Bacteroides*	↓ Body weight gain ↑ Insulin resistance
Sembries et al. (2006)	Apple juice	Male wistar rats	4 weeks	↑ *Lactobacillus* ↑ *Bifidobacterium*	↑ SCFAs
Molan et al. (2009)	4 mL/kg/day blueberry extract	Female Sprague Dawley rats	6 days	↑ *Lactobacillus* ↑ *Bifidobacterium*	/
Byerley et al. (2017)	11 g walnuts daily	Fischer 344 rats	10 weeks	↑ *Lactobacillus* ↑ *Ruminococcaceae*	/

Etxeberria et al. (2015)	30 mg/kg quercetin	Wistar rats w/ high fat diet	6 weeks	↑ *Roseburia* ↓ *Bacteroides* ↓ *Anaeotruncus* ↓ F/B ratio ↓ *Erysipelotrichaceae* ↓ *Bacillus* ↓ *Eubacterium cylindroides*↓	/
Anhê, Roy, et al. (2015)	200 mg / kg cranberry extract	C57BL/6J mice w/ high fat and high sucrose diet	8 weeks	↑ *Akkermansia* spp.	↓ Body weight gain ↑ Insulin sensitivity ↓ Triglycerides
		Human intervention trials			
Tzounis et al. (2011)	494 mg/day cocoa flavanols	Healthy adults	4 weeks	↑ *Bifidobacterium* ↑ *Lactobacillus* ↓ *Clostridia*	↓ Plasma triacylglycerol ↓ CRP
Brasili et al. (2019)	500 mL/day orange juice	Healthy adults	7 days	↑ Moglibacteriaceae ↑ Tissierellaceae ↑ Veillonellaceae ↑ Odoribacteraceae ↑ Ruminococcaceae	/
Vendrame et al. (2011)	25 g of wild blueberry powder	Healthy male adullts	6 weeks	↑ *Bifidobacterium* ↓ *Giardia duodenalis*	/
Liu et al. (2014)	10 g/ day Almond skin powder & 56 g whole almonds	Healthy adults	6 weeks	↑ *Bacteroides* spp. ↑ *Lactobacillus* spp. ↓ *E. coli* ↓ *C. perfringens*	/
Clavel et al. (2005)	100 mg / day isoflavones + pre- and probiotic groups	Post-menopausal women	2 months	↑ *Clostridium coccoides* ↑ *Eubacterium rectale* ↑ *Lactobacillus* ↑ *Faecalibacterium prausnitzii*	/
Li et al. (2015)	1000 mg pomegranate extract	Healthy adults	4 weeks	↑ *Akkermansia* spp. ↑ *Actinobacteria* ↑ *Lactobacillus* ↑ *Enterobacter* ↑ *Escherichia*	/

Henning et al. (2017)	1000 mg pomegranate extract	Healthy adults	4 weeks	↓ *Firmicutes* ↑ *Akkermansia* spp.	/
González-Sarrías et al. (2018)	656 mg of polyphenols from pomegranate extract	Overweight and obese adults with mild hyperlipidaemia	3 weeks	↑ *Odoribacter* ↑ *Bacteroides* ↑ *Faecalibacterium* ↑ *Butyricicoccus* ↑ *Butyricimonas* ↓ *Parvimonas* ↓ *Metanobrevibacter* ↓ *Metanosphaera*	↓ Lipopolysaccharide-binding protein

3.5.3. Effects of red wine and grape polyphenols and resveratrol on microbial composition

Whilst the impact of polyphenols on microbial composition has been summarised above, of importance to this thesis is the impact of resveratrol and related polyphenols from red wine and grapes. Which has gathered increasing interest in recent years. Research to date is summarised below and within Table 3.4.

Red wine and grape extracts contain vast amounts of polyphenols, predominantly flavonoids, specifically flavan-3-ols and anthocyanins; alongside lower abundance of flavonols, flavanonols and flavones (Monagas, Bartolomé, & Gómez-Cordovés, 2005); each of which have the ability to modulate microbial composition. Much early work was conducted in batch culture fermentation studies, which have observed that red wine and grape extracts increased beneficial bacteria including *Lactobacillus, Bifidobacterium, Bacteroides* and *Ruminococcus* spp. and reductions in *Clostridium* spp. (Cueva et al., 2013; Dueñas et al., 2015; Sánchez-Patán et al., 2012). Despite this, similarly detrimental changes to microbial composition (as those seen in the abovementioned black tea trial (Kemperman et al., 2013)) were observed in the SHIME model with red wine and grape extract. Again, an antimicrobial effect was observed here; noted as reductions in bacterial growth and numbers of present species. Moreover, they noted the reduced presence of *Bifidobacterium,* alongside growths of *Klebsiella* and *Akkermansia.* The authors do note that the effects of polyphenol intake on microbial composition is likely to be less severe *in vivo;* as here they utilised a high dose (1000 mg) of one single polyphenol mixture, rather than reduced levels in natural food sources, where these would interact with other dietary components and baseline microbial composition, likely reducing the availability of polyphenols to the gut microbiota.

Beneficial modulatory effects have also been observed in animal models. Specifically, 16-week administration of red wine polyphenols (50 mg/ kg daily) in F344 rats, resulted in increases in *Lactobacilli, Bifidobacterium* and *Bacteroides;* with concurrent decreases in *Clostridium* (Dolara et al., 2005). Moreover, increases in abundance of *Lactobacillus, Clostridales* and *Ruminococcus* was observed following 6-day grape seed extract supplementation in pigs (Choy et al., 2014). Further, within obese mice, a 30-day dietary supplementation with grape polyphenols significantly modulated the gut microbial community; indicated by a reduction in *Firmicutes/Bacteroidetes* ratio and a promotion of *Akkermansia muciniphila* (Roopchand et al., 2015). Taken together, these animal findings suggest promising effects of polyphenol intervention on microbiota composition; limited work in humans appears to also support this.

In a randomised, controlled crossover intervention 10 healthy male adults received red wine, de-alcoholised red wine or gin for 20 days (Queipo-Ortuño et al., 2012). Results indicated significant changes in microbial composition following red wine. Specifically, increased abundance of *Bacteroides, Bifidobacterium, Prevotella, Enterococcus* and *Blautia coccoides-Eubacterium rectale* groups were observed; indicating a beneficial, prebiotic-like effect on microbial composition (Dueñas et al., 2015). Alongside this, concurrent reductions in *Actinobacteria* and *Clostridium* spp. (although the *Clostridium* reduction was not significant), which are associated with gut dysbiosis and adverse health effects (Hungin et al., 2013), were reported. Taken together, this trial indicates a potential prebiotic like effect of red wine polyphenols on microbial composition, which was also associated with improvements in health outcomes including reduced triglycerides, total cholesterol, blood pressure and CRP.

Likewise, similar effects were observed following 30-day consumption of red wine or de-alcoholised red wine in male adults with metabolic syndrome (Moreno-Indias et al., 2016). These trials observed increases in *Bifidobacteria, Lactobacillus* and *Faecalibacterium prausnitzii* (both capable of producing butyrate), and concurrent reductions in *Enterobacter cloacae* and *Escherichia coli*. The authors indicate that these gut microbial changes may suggest polyphenol intervention as a promising approach in the treatment of metabolic syndrome. A recent systematic review of 7 trials aimed to investigate the effects of grape and wine extracts on gut microbiota in healthy adult participants (Nash et al., 2018). However, of the included studies, only one reported the effect of polyphenol intervention on gut composition (Queipo-Ortuño et al., 2012), whereas the remaining investigated effects on microbial mediated phenolic metabolites; emphasising the need for more human intervention trials to assess the impact of red wine and grape extracts on microbial composition.

The many additional compounds within the above beverages limits the extent to which findings can be extrapolated to polyphenols specifically, However, limited work has investigated the effect that isolated polyphenols, like resveratrol, can have as a potential prebiotic on gut microbial composition. At present, almost all work has been conducted in animal models. In rats fed a high-fat diet, 6 weeks of supplementation with 15 mg/kg resveratrol alongside 30 mg/kg quercetin observed some small changes in bacterial composition. Namely, these manifested in a reduction in *Firmicutes/Bacteroidetes* ratio and select bacterial species like *Erysipelotrichaceae* and *Eubacterium cylindroides* (Etxeberria et al., 2015). Similarly, high-fat diet fed mice, supplemented with 200 mg/kg resveratrol for 12 weeks, also observed a reduction in *Firmicutes/Bacteroidetes* ratio, and increased growth of beneficial bacteria *Lactobacillus* and *Bifidobacterium* (Qiao et al., 2014).

With such limited work to date, it is difficult to understand the effect that resveratrol supplementation may have on microbial composition. Specifically, due to vast changes in study design, including animal species, dosage and duration, it is challenging to directly compare the results above (Chaplin et al., 2018). Moreover, an important consideration within the research to date here is the high dosage utilised within many of the animal models. As noted within Table 3.4, resveratrol dosage is often calculated based on body weight of the animal up to a maximum of 450 mg/kg body weight daily. To put that into perspective, if we were to employ this approach in human research, calculations utilising the average weight of the healthy volunteers within Chapter 2 (71 kg), indicates that anything over 70.5 mg/kg body weight, would exceed the suggested upper safety level of human consumption (5 g/day) (Brown et al., 2010).

To the best of current knowledge, there is just one study investigating the microbial effects of resveratrol supplementation in humans (Most, Penders, Lucchesi, Goossens, & Blaak, 2017). However, this study employed a combined polyphenol supplement (epigallocatechin-3-gallate and resveratrol), so it is not possible to disentangle the effects of these two polyphenols. Within this trial, overweight adults received 282 mg EGCG and 80 mg resveratrol for 12 weeks. Their results showed reductions in *Bacteroidetes* and, interestingly, *Faecalibacterium prausnitzii* only in male participants. This would suggest the potential for sex differences in response to polyphenolic intervention; however, it is difficult to conclude this with any certainty without supplementary work to confirm this.

Table 3.4. Summary of red wine and grape polyphenol-modulation on gut microbiota. Including *in vitro*, animal and human intervention trials. Table adapted primarily from recent reviews (Aravind et al., 2021; Corrêa et al., 2019; Gowd et al., 2019)

Reference	Polyphenol	Study methodology	Time frame	Microbial modulation	Metabolic outcome
			In vitro		
Kemperman et al. (2013)	1000 mg / day polyphenols from red wine and grape extract	SHIME model	2 weeks	↑ *Klebsiella* ↑ *Alistipes* ↑ *Cloacibacillus* ↑ *Akkermansia* ↑ *Victivallis* ↓ *Bifidobacterium*	/
Barroso et al. (2014)	200 mg red wine polyphenols	SHIME model	2 weeks	↓ *Bifidobacterium* ↓ *Bacteroides* ↓ *E. rectale* ↓ *G. coccoides*	/
Cueva et al. (2013)	Grape seed extract	Fermentation	48 hours	↑ *Enterococcus* ↑ *Lactobaciullus* ↓ *Clostridium histolyticum*	/
			Animal studies		
Chen et al. (2016)	0.4% resveratrol	C57BL/6J mice	30 days	↑ *Bifidobacterium* ↑ *Lactobacillus* ↑ *Bacteroides* ↑ *Akkermansia* ↓ *Prevotella* ↓ *Ruminococcaceae* ↓ *Anaerotruncus* ↓ *Alistipes* ↓ *Helicobacter* ↓ *Peptococcaceae* ↓ F/B ratio	/
Zhao et al. (2017)	15 mg/kg bw resveratrol with 30 mg/kg bw quercetin	Obese rats w/ high fat diet	10 weeks	↓ Firmicutes ↓ F/B ratio	↓ body weight gain ↓ adipose tissue weight ↓ serum lipids

					↓ inflammatory markers (TNF-α, IL-6)
Larrosa et al. (2009)	1 mg/kg bw resveratrol	Rats	25 days	↑ *Bifidobacteria* ↑ *Lactobacilli* ↓ *Enterobacteria*	↓ Inflammatory markers (IL-6, haptoglobin and fibrinogen)
Roopchand et al. (2015)	Grape	Obese C57BL/6J mice	16 weeks	↑ *Akkermansia muciniphila* ↑ *Alistipes* spp. ↓ *Clostidiale* ↓ F/B ratio	↑ Glucose tolerance ↓ Metabolic endotoxemia ↓ Intestinal and systemic inflammation
Dolara et al. (2005)	50 mg/kg red wine polyphenols	F344 rats	16 weeks	↑ *Bacteroides* ↑ *Bifidobacterium* ↑ *Blautia coccoides*	/
Etxeberria et al. (2015)	15 mg/ kg resveratrol and 30 mg/kg quercetin	Wistar rats w/ high fat diet	6 weeks	↓ F/B ratio ↓ *Erysipelotrichaceae*	↓ body weight gain ↓ serum insulin levels
Choy et al. (2014)	1% Grape seed extract	Crossbred female pigs	6 days	↑ *Lachnospiraceae* ↑ *Clostridales* ↑ *Lactobacillus* ↑ *Ruminococcaceae*	/
Qiao et al. (2014)	200 mg/ kg resveratrol	Male Kunming mice w/ high fat diet	12 weeks	↓ F/B ratio ↑ *Lactobacillus* ↑ *Bifidobacterium* ↓ *Enterococcus faecalis*	↑ Fasting induced adipose factor
Sung et al. (2017)	450 mg / kg bw resveratrol	C57Bl/6N mice with heart failure	2 weeks	↓ F/B ratio ↑ *Akkermansia* ↑ Bilophila	↑ Insulin sensitivity ↑ Metabolic rate
Wang et al. (2020)	300 mg / kg bw resveratrol	C57BL/6J male mice w/ high fat diet	16 weeks	↓ *Desulfovibrio* ↓ *Lachnospiraceae* ↓ *Alistipes* ↑ *Bacteroides*	↓ Body weight ↑ Insulin resistance

Yang et al. (2019)	400 mg / kg resveratrol with 200 mg / kg sinapic acid	Wistar rats w/ high fat diet	8 weeks	↑ *Blautia* ↑ *Doria* ↓ *Bacteroides* ↓ *Desulfovibrionaceaesp*	/
Human intervention trials					
Moreno-Indias et al. (2016)	272 mL/day dealcoholized red wine	Adults with metabolic syndrome	30 days	↑ *Bifidobacterium* ↑ *Lactobacillus* ↑ *Faecalibacterium prausnitzii* ↑ *Roseburia* ↓ F/B ratio	/
Queipo-Ortuño et al. (2012)	272 mL/day red wine	Healthy male adults	20 days	↑ *Enterococcus* ↑ *Prevotella* ↑ *Bacteroides* ↑ *Bifidobacterium* ↑ *Enterococcus* ↑ *Bacteroides uniformis* ↑ *Eggerthella lenta*	↓ Blood pressure ↓ Triacylglycerol ↓Total cholesterol ↓ CRP
Yamakoshi et al. (2001)	500 mg/day grape extract	Healthy adults	2 days	↑ *Bifidobacterium* ↓ *Enterobacteriaceae*	/
Most et al. (2017)	282 mg EGCG and 80 mg resveratrol	Overweight and obese adults	12 weeks	↓ *Bacteroidetes* ↓ *Faecalibacterium prausnitzii* (in men)	↑ Fat oxidation
Walker et al. (2019)	2 g resveratrol	Obese male adults with metabolic syndrome	35 days	↑ *Akkermansia*	↑ Insulin sensitivity ↑ Glucose homeostasis

3.5.4 Effects of polyphenolic-gut microbiota interactions on metabolic disorders

Given the close link between microbial composition and metabolic disease, it is likely that polyphenolic modulation of microbiota contributes to improvements in host health. Indeed, microbial modulation via polyphenol intervention has been associated with beneficial metabolic outcomes, as detailed previously within Tables 3.3. and 3.4. As such, recent work has considered how phenolic intervention might be a therapeutic option in the prevention and treatment of various metabolic disorders.

Much work has considered the potential anti-obesity effects of polyphenols via microbial modulation, specifically by reducing the elevation in *Firmicutes/Bacteroidetes* ratio that is typically observed in obesity (Gowd et al., 2019). Variations in other bacterial species are also associated with obesity-related dysbiosis; including increases in *Lachnospiraceae* and reductions of *Akkermansia* (Everard et al., 2013; Kameyama & Itoh, 2014). Specifically, the abundance of *Akkermansia* is inversely correlated with body weight (Everard et al., 2013) and, therefore, increases in *Akkermansia* may be a key mechanism in improving host health. Indeed, supplementation with *Akkermansia* within obese mice, reversed high-fat diet induced metabolic disorders, including insulin resistance, inflammation, fat-mass gain and intestinal permeability (Everard et al., 2013). Whilst the elaborate mechanisms between dietary intervention, microbial composition and host health outcomes are yet to be fully elucidated; these findings indicate potential microbial targets for polyphenol administration to improve obesity-related dysbiosis and related health outcomes.

The majority of this work has been conducted in animal models of obesity, induced by high fat diets. Here, cranberry extract supplementation in mice fed a high fat and high sucrose diet, resulted in increased abundance of *Akkermansia* species and also improvements in multiple metabolic health outcomes; including reduced triglycerides, reduced inflammation and diet-induced weight gain (Anhê, Roy, et al., 2015). Likewise, administration of grape polyphenols in an obese mice model resulted in clear modulation in gut microbial species; including increased *Akkermansia* and reduction in the *Firmicutes* to *Bacteroidetes* ratio. Concurrent reductions in intestinal and systemic inflammation, and improved glucose tolerance, were also reported (Roopchand et al., 2015). Recent work has indicated the effectiveness of blueberry polyphenols here also. As an example, a 12-week supplementation with blueberry polyphenol extract, in a model of obese mice, resulted in reduced body weight gain and returned lipid metabolism to normal. They also observed modulation to specific gut bacteria, including increased *Bifidobacterium* and *Desulfovibrio* and decreased *Prevotella* (Jiao et al., 2019).

Moreover, exacerbated oxidative stress is associated with the pathogenesis of obesity-mediated metabolic syndrome (Santilli, Guagnano, Vazzana, La Barba, & Davi, 2015) and polyphenols may counteract this mechanism. For example, research indicates that 10-week supplementation of grape polyphenol extract, in mice fed a high-fat diet, is sufficient to increase *Akkermansia* and suppress the high-fat diet-induced generation of ROS. This indicates that a potential mechanism behind this effect is the ability of the gut microbiota to interact with oxidative stress response in metabolic syndrome (Gowd et al., 2019).

As chronic-low grade inflammation has a critical influence on the onset and progression of metabolic disease, the ability of polyphenols to interact with this via microbial modulation is also a key avenue for therapeutic approach (Gowd et al., 2019; Roopchand et al., 2015). Indeed, recent work has indicated that curcumin supplementation has the ability to modulate microbiota and suppress inflammatory response in high-fat diet fed mice (Islam et al., 2021). Here, 14-week supplementation of curcumin resulted in increased abundance of *Lactococcus, Parasutterella* genus; and a concomitant reduction in inflammatory genes including NF-κβ, IL-6 and TLR4.

Moreover, contributing to inflammation is the alteration to the intestinal barrier which results in the release of LPS into the bloodstream, further exacerbating the proinflammatory response by inducing the production of pro-inflammatory cytokines (Libby et al., 2010). Research indicates that polyphenolic intervention can modulate both gut microbiota and plasma lipopolysaccharide-binding protein (LBP), a marker of endotoxemia. In a randomised, placebo-controlled, crossover study, overweight and obese adults consumed two doses of pomegranate extract (low dose 450 mg, high dose 1.8 g) daily for 3 weeks, with a 3 week washout between condition (González-Sarrías et al., 2018). Their results indicated that, following the high dose, plasma LBP significantly reduced the increases in bacterial strains important for maintaining gut barrier function (including *Bacteroides, Faecalibacterium)*. They also observed decreases in *Parvimonas* and *Methanobrevibacter* which are associated with increasing inflammation. The authors concluded that pomegranate extract supplementation decreased endotoxemia in overweight and obese adults by modulating gut microbial composition. Taken together this body of work indicates that polyphenols can positively influence metabolic health outcomes, likely by modulating microbial composition. However, this likely varies greatly between animals and humans, as well as individual polyphenols, dosage and supplementation duration.

Resveratrol supplementation also has the ability to modulate obesity-related dysbiosis. For example, administration of 200 mg/kg resveratrol five days a week for 8 weeks, in mice on a high-fat diet, reduced body weight and fat deposition compared to controls (Jung et al., 2016). These authors also observed that high-fat induced increases in bacterial strains (*Lactococcus* and *Clostridium)* were reversed by resveratrol supplementation. They showed that these beneficial effects were caused by resveratrol-induced activation of the mTORC2 signalling pathway; which is known to be important in energy regulation. In further support of resveratrols beneficial effects, 12-week supplementation of resveratrol (200 mg/kg body weight) in mice fed a high-fat diet, resulted in a reduction in *Firmicutes/Bacteroidetes* ratio, whilst also increasing beneficial bacteria *Bifidobacterium* and *Lactobacillus* and inhibiting the growth of *Enterococcus faecalis* (Qiao et al., 2014). Concurrently, fasting-induced adipose factor (FIAF) expression increased, alongside decreased high-fat diet induced- adipogenesis and lipogenesis. Likewise, co-supplementation of resveratrol (15 mg/kg) and quercetin (30 mg/kg) in high-fat fed rats, led to reductions in *Firmicutes/Bacteroidetes* ratio, and inhibited growth of various bacterial strains associated with diet-induced obesity (Etxeberria et al., 2015). Simultaneous reductions in body weight gain and serum insulin levels were also reported.

More recent research suggests that resveratrol may exert beneficial effects without modulating the *Firmicutes/Bacteroidetes* ratio. In support of this, in rats fed a high-fat diet supplemented with resveratrol (400 mg/kg) and sinapic acid (200 mg/kg) for 8 weeks, reduction of fasting blood glucose levels and increased HDL cholesterol was observed (Yang et al., 2019) . Despite no change in *Firmicutes/Bacteroidetes* ratio, the authors did observe variations in bacterial strains, including increases of *Lachnospiraceae* strains (associated with butyrate production) and reductions of inflammation related species *Bacteroides* and *Desulfovibrionaceaesp.* This suggests that anti-obesity effects can be exerted by phenolic administration via specific bacterial species modulation and that this interaction is likely more complex than just an imbalance in *Firmicutes* and *Bacteroidetes* (Harley & Karp, 2012).

Despite promising work in animal models, to date very limited work in humans exists. One study co-supplemented obese adults (n=38) with resveratrol (80 mg/day) alongside epigallocatechin-3-gallate (282 mg/day) for 12 weeks (Most et al., 2016). Their findings indicated a reduction in *Bacteroidetes* and also in *Faecalibacterium prausnitzii,* although this was limited just to male participants. Within the male participants, they also observed an increase in fat oxidation. A recent pilot study aimed to investigate the effects of resveratrol supplementation on microbial composition and inflammation in men with metabolic syndrome (Walker et al., 2019). Here, participants (n = 31) received 2 g of resveratrol for 35-days and their results indicated improvements in insulin sensitivity and glucose homeostasis, alongside

concurrent increases in *Akkermansia* abundance. However, these findings were restricted to just the Caucasian participants (n = 11). With such limited work in humans it's difficult to understand why sex and race differences have been observed in these studies, moreover the small sample used makes it difficult to generalise these findings to other populations.

Whilst the work suggests a beneficial modulatory effect of resveratrol, which may confer beneficial health effects, the mechanisms behind this interaction is still yet to be fully elucidated (Chaplin et al., 2018). Nøhr et al. (2016) suggest that resveratrol may be able to decrease endotoxemia, via the aforementioned reduction of LPS, consequently reducing systemic inflammation and therefore improving metabolic health outcomes. A secondary hypothesis, is that resveratrol may exert beneficial effects via its antioxidant properties, specifically by counteracting ROS and inhibiting amine oxidase activities (Chaplin et al., 2018). In support of this, recent work indicated that high-fat diet fed mice who received resveratrol supplementation (300 mg/ kg/ day) for 16 weeks had significantly reduced diet-induced LPS, an increased abundance of *Bacteroidetes* and reduction of *Firmicutes* (Wang et al., 2020). Alongside this, an increase in bacterial strains capable of producing SCFAs was reported. The resveratrol group also had better intestinal permeability, reduced diet-induced ROS and malondialdehyde (MDA) production, indicating a reduction in oxidative stress. The authors conclude that resveratrol supplementation can improve diet-induced damage to the intestinal barrier in high-fat fed mice; likely through interacting with LPS and ROS. However, the specifics of these mechanisms require further elucidation.

Cumulative evidence indicates that polyphenol intervention, including resveratrol, can exert a prebiotic-like effect on gut microbiota, which may positively effect host health, particularly metabolic outcomes. To date, there is limited work to understand if these beneficial effects in animal models are transferable to humans, stressing the need for human intervention studies. Moreover, not only can modulations in bacterial composition improve host health via reductions in inflammation, oxidative stress and various other pathways, but they can also promote cognitive enhancement via the gut-brain-axis. As such, Chapter 4 aims to investigate the interrelated effects that resveratrol supplementation can have on cognitive performance and gut microbiota, alongside related metabolic health outcomes in an overweight and obese demographic sample.

CHAPTER 4

THE ACUTE AND CHRONIC EFFECTS OF RESVERATROL SUPPLEMENTATION ON COGNITIVE FUNCTION, GASTROINTESTINAL MICROBIOTA AND CEREBRAL BLOOD FLOW: A DOUBLE BLIND, PLACEBO CONTROLLED, PARALLEL-GROUPS STUDY IN HEALTHY, OVERWEIGHT HUMANS

4.1 Introduction

Whilst Chapter 2 indicated no clear effects of resveratrol supplementation on cognitive performance or inflammatory biomarkers; it is theorised that the null findings were due to the sample demographic utilised. Specifically, said participants were hypothesized to be 'too healthy' to benefit from resveratrol supplementation. Indeed, these null cognitive findings are consistent with previous work conducted in healthy, young adults (Eschle et al., 2020; Kennedy et al., 2010; Wightman et al., 2019). Here, trials consistently observed no effect on cognitive performance, following supplementation with 500 mg resveratrol, despite reliably demonstrating increases in cerebral blood flow during task performance. Similar effects have been observed in other polyphenols, most notably work in cocoa flavanols; where despite significant increases in CBF, no cognitive benefits were observed in young, healthy adults (Decroix et al., 2016; Francis et al., 2006). These results are supported by a recent systematic review, who concluded that whilst cocoa flavanol supplementation often results in improvements to blood flow, often no concurrent benefits in cognitive function is observed (Socci et al., 2017).

Shifts in the literature indicate that young, healthy adults, in particular, are unlikely to benefit from polyphenol supplementation. It is instead hypothesized that more cognitively compromised individuals, be that via aging or disease, are likely better candidates for response to intervention. In support of this, limited recent work has showed promise in older populations. Specifically, improvements in both cognitive task performance and CBF was observed following 12-week blueberry extract supplementation in healthy, older adults (Bowtell et al., 2017). Likewise, resveratrol supplementation appears more promising in more compromised demographics. Specifically, 90 day supplementation of 1000 mg resveratrol resulted in enhanced select aspects of cognitive functioning in older adults (Anton et al., 2018). Similarly, several studies in overweight, older adults has suggested positive effects of resveratrol supplementation when presented in combination with additional polyphenols. For example, 26-week co-supplementation of resveratrol and quercetin improved word recall and concurrently increased functional connectivity of the hippocampus (Witte et al., 2014). Likewise, acute ingestion of resveratrol-enriched red wine resulted in significant improvement

in serial 3 task performance (Scholey et al., 2014). Most recent work has investigated post-menopausal women, as the loss of oestrogen after menopause, may result in reductions in cerebral blood flow and accelerated cognitive ageing (Genazzani et al., 2007). Following 14-week resveratrol supplementation in post-menopausal women, improvements were observed on a range of cognitive tasks assessing various aspects of cognition, including executive function and verbal memory (Evans et al., 2017). Alongside this, improved cerebrovascular responsiveness, as measured by TCD, was observed; indicating resveratrol-modulation of CBF. Likewise, 12-month resveratrol supplementation in post-menopausal women resulted in improvements in cognitive flexibility and processing speed cognitive domains (Zaw et al., 2020a, 2020b); suggesting that resveratrol supplementation is likely more effective as a cognitive enhancer in older, more compromised demographics.

In addition, it is crucial to note that many of the above studies indicating promising findings employed chronic intervention designs (12 weeks+), whereas earlier work primarily used single acute doses. The suggestion that prolonged intervention might be more effective is also supported by a recent systematic review, which indicated that resveratrol supplementation appears most efficient in interventions >10 weeks in length (Asgary et al., 2019). Taken together the literature suggests resveratrol supplementation is most useful when presented over a prolonged period to older, compromised (for example overweight and obese) individuals.

Of increasing interest in the literature is the role of the gut microbiota, specifically the potential for dietary intervention to modulate microbial composition and subsequently benefit host health. This includes via reducing inflammatory response, improving metabolic outcomes and cognitive performance via the gut-brain-axis. Whilst microbial composition is considered relatively stable throughout adulthood, external factors, predominantly diet, lifestyle habits, antibiotic use and infection, can modify composition (Borre et al., 2014; Serra et al., 2018). Diet is considered one of the most important of these external modifiers of the microbiome (Graf et al., 2015); as the overall balance of the primary macronutrients, as well as consumption (or conversely the lack) of micronutrients, prebiotics, probiotics, food additives and other dietary components can modulate the microbiota (Roca-Saavedra et al., 2018) and subsequently lead to dysbiosis.

Dysbiosis describes an alteration of the gut microbiota and is linked to sustained intestinal inflammation, eventually contributing to chronic intestinal diseases, particularly inflammatory bowel diseases (Serra et al., 2018). Of interest, certain dietary patterns are particularly associated with microbial changes and dysbiosis (Herpertz-Dahlmann, Seitz, & Baines, 2017).

For example, the 'Western diet' (which is high in saturated fatty acids, sugar and protein (Tengeler et al., 2018)); is associated with a decrease in gut microbial diversity (Agus et al., 2016; Beilharz et al., 2018) and reduction in beneficial gut bacteria; including bifidobacteria and lactobacilli (Araújo et al., 2017; Sandhu et al., 2017; Singh et al., 2017). Research suggests that chronic low-grade systemic inflammation, that is consistently associated with obesity, plays a vital part in obese-related dysbiosis (Boulangé et al., 2016)

High-fat diet-induced alterations to the intestinal barrier results in the release of lipopolysaccharide (LPS) to the bloodstream; amplifying production of pro-inflammatory cytokines and consequently playing an important role in the onset and progression of low grade systemic inflammation and the pathogenesis of metabolic diseases (Cani, Neyrinck, et al., 2007; Rainone et al., 2016). Importantly, further research suggests that long-term systemic inflammation contributes to changes in brain morphology as well as cognitive and mood dysfunction in mice and humans (Fineberg & Ellman, 2013; Gainey et al., 2016; Jacka, Cherbuin, Anstey, & Butterworth, 2014; Jørgensen et al., 2014; Misiak et al., 2012; Young et al., 2014).

Most recent research here has investigated the composition of the gut microbiome with specific focus on identifying strains of bacteria that may be implicated in dysbiosis; contributing to chronic intestinal diseases, cognitive alterations, and neurological disorders. As an example, studies have suggested that participants with inflammatory bowel disease (IBD) had higher levels of *Proteobacteria* and *Actinobacteria*, but lower levels of *Firmicutes* and *Bacteroidetes* compared to healthy controls (Buttó & Haller, 2016). Whereas a strain of *Bifidobacterium longum* has been shown to alter cognition in both rodents and humans (Allen et al., 2016; Savignac et al., 2015). Vogt et al. (2017) have also demonstrated that patients with Alzheimer's disease (AD) have a reduced microbial diversity compared with controls, with a decrease in the abundance of Firmicutes and an increase in Actinobacteria abundance. However, despite these identified changes in specific bacterial strains, to date, there is a limited amount of research into what these changes mean and, more widely, what constitutes a 'healthy' microbiome. However, it is generally agreed that it is advantageous to have a greater diversity of gut bacterial populations (Fernandez-Real et al., 2015) and that decreased diversity is harmful (Beilharz et al., 2018).

Given the health promoting effects of polyphenols, specifically their ability to interact with inflammatory pathways and association with reduced disease incidence (Anhê et al., 2013; Serra et al., 2018), polyphenolic modulation of the microbiota has gathered considerable recent interest. Indeed, phenolics and phytochemicals have been argued to exert prebiotic-

like effects on microbial composition (Gibson et al., 2017). Recent research here suggests that polyphenols modulate the gut microbiota by promoting the growth of specific gut microbial species including *Akkermansia* spp., *Faecalibacterium* spp. and *Roseburia* spp. (Danneskiold-Samsøe et al., 2019). In positively promoting such species, polyphenols may also be beneficially modulating the inflammation which is the hallmark of dysbiosis. Here, previous findings also show that certain bacterial strains can both enhance or inhibit the secretion of pro- and anti-inflammatory cytokines (Sarkar et al., 2018) and it is proposed that prebiotic consumption can counteract systemic inflammation, caused by high fat diet, by physically binding to pattern-recognition receptors; thus inhibiting the release of proinflammatory cytokines and increasing the expression of anti-inflammatory cytokines (Eiwegger et al., 2010; Zhou et al., 2015).

Resveratrol has well-documented abilities of inhibiting pro-inflammatory signalling cascades and therefore decreasing the expression of many pro-inflammatory markers which subsequently reduce neuroinflammation in a number of *in vitro* and *in vivo* models (Renaud & Martinoli, 2014; Spencer et al., 2012). As such, it is of great interest as a therapeutic intervention; particularly, in reducing the neuroinflammation associated with obesity. Although polyphenolic compounds have been widely studied in relation to neurological disorders, the correlation between attenuation of neurological features and the modification of intestinal inflammation promoted by supplementation of these compounds has not yet been clearly established.

Previous work has, however, considered the effect of red wine extracts (with high resveratrol content) on gut microbiota populations and biomarkers of systemic inflammation. Here significantly increased abundance of *Bacteroides, Bifidobacterium* and *Prevotella,* were observed following 4-week consumption of red wine; alongside significant decreases in blood pressure, cholesterol and C-reactive protein concentrations (Queipo-Ortuño et al., 2012). Likewise, similar effects were observed in male adults with metabolic syndrome following 30-day red wine supplementation (Moreno-Indias et al., 2016). This resulted in increases in SCFAs producing bacterial strains (*Bifidobacteria, Lactobacillus* and *Enterobacter cloacae*) and reduced abundance of pathogenic strains (*Escherichia coli);* with concurrent reductions in blood pressure, total cholesterol, triglycerides and CRP.

Several animal trials have shown that resveratrol improves the gut microbiota dysbiosis in mice following consumption of a high-fat diet by increasing the *Enterococcus faecalis* and increasing the growth of *Lactobacillus* and *Bifidobacterium* (Qiao et al., 2014). Similar findings, also in rats fed a high-fat diet, after 8-weeks co-supplementation of resveratrol and sinapic

acid, showed reductions in bacterial strains related to inflammation (*Bacteroides* and *Desulfovibrionaceaesp),* increases in *Lachnospiraceae* and concurrent increases in HDL cholesterol (Yang et al., 2019). To date, limited human work exists that investigates the effects of resveratrol supplementation on microbial composition and related metabolic outcomes. However, reduced *Bacteroidetes* and *Faecalibacterium prausnitzii* and increased fat oxidation has been observed in male overweight adults following 12-week co-supplementation of epigallocatechin-3-gallate (282 mg/day) and resveratrol (80 mg/day) (Most et al., 2017). Likewise, 35-day resveratrol supplementation (2 g/day) increased *Akkermansia* abundance and improved glucose homeostasis and insulin sensitivity, in a pilot study of men with metabolic syndrome (Walker et al., 2019). However, with such limited work in humans it's difficult to understand the extent of beneficial effects that resveratrol intervention might have on gut microbial composition, host health and cognitive performance.

Of importance here, recent work has also indicated that obese individuals have reduced cerebral blood flow. Using data from the Irish Longitudinal Study on Ageing, cerebral blood flow data was collected from 495 adults (>50 years) using arterial spin labelling MRI, alongside measurements of BMI, waist circumference and waist-to-hip ratio (Knight et al., 2021). Their analysis indicated that increases in the physical weight measurements was associated with a reduction in CBF. Specifically, 0.43 kg/m^2 BMI increase, 0.01 increase of WHR and waist circumference increase of 1.3 cm were all associated with a decrease in CBF, that was equivalent to 1 year of ageing. Given resveratrol's well-known ability to increase CBF in young, healthy adults, it seems probable that resveratrol intervention in overweight individuals could mitigate the CBF deficits associated with weight gain.

To date, no research has directly examined the effects of polyphenolic supplementation on the interrelationships between an individual's gut microbiota, levels of systemic inflammation and effects on brain function. The current study aims to do so by investigating the effects of 12-week 500 mg resveratrol supplementation on cognitive function, cerebral blood flow, gastrointestinal microbiota and systemic inflammation in overweight and obese adults. Whilst previous research shows that polyphenol supplementation has a beneficial effect on cerebral blood flow and cognitive performance, this effect seems strongest in compromised demographic groups. As mentioned earlier, overweight and obese individuals are likely to suffer systemic inflammation and relatedly microbial dysbiosis, partly due to diet-induced alteration of the intestinal barrier. Here, resveratrol may be able to mediate the exacerbated inflammatory response via prebiotic-like promotion of inflammatory-reducing gut bacteria. Additionally, as chronic systemic inflammation is associated with compromised brain function, resveratrol may be able to mediate this via this anti-inflammatory, gut-brain axis pathway.

This study therefore aims to address the following research questions relating to the effects of 500 mg resveratrol supplementation in overweight and obese adults:

1. What are the acute (45- and 240-minute post dose) and chronic (84-day supplementation) effects of resveratrol supplementation on cognitive performance. Specifically, here, the effect on the performance on the following cognitive tasks:
    a. Immediate word recall
    b. Numeric Working Memory
    c. Choice Reaction Time
    d. Corsi blocks
    e. Serial subtraction of threes
    f. Serial subtraction of sevens
    g. Rapid Visual Information Processing
    h. Peg and Ball
    i. Delayed word recall
    j. Delayed Face to Name recall
    k. Delayed Picture Recognition
    l. Delayed word recognition
2. What are the acute (45- and 240- minute post dose) and chronic (84-day supplementation) effects of resveratrol supplementation on cognitive performance. Specifically, here, the effect on the performance on the following cognitive domains, with scores calculated using individual task performance:
    a. Accuracy of Attention
    b. Speed of Attention
    c. Working Memory
    d. Speed of Memory
    e. Episodic Memory
    f. Overall Accuracy
    g. Overall Speed
3. What are the acute (45- and 240-minute post dose) and chronic (84-day supplementation) effects of resveratrol supplementation on subjective mood. As assessed with Bond Lader Mood Scales, with the following outcome measures:
    a. Alertness
    b. Calmness
    c. Contentment

4. What are the acute (45- and 240- minute post dose) and chronic (84-day supplementation) effects of resveratrol supplementation on subjective mood, as assessed with Profile of Mood States (POMs).
5. What are the acute (~115/~155- minutes post dose) and (chronic (84-day supplementation) effects of resveratrol supplementation on cerebral blood flow (as measured using Quantitative Near-Infrared Spectroscopy) at rest and during cognitive demand. With the following outcome measures:
    a. Oxygen Saturation
    b. Total Haemoglobin
    c. Oxygenated Haemoglobin
    d. Deoxygenated Haemoglobin
6. What are the chronic (following 84-day supplementation) effects of resveratrol supplementation on gut microbiota composition, measured in stool samples:
    a. Overall diversity
    b. Alpha diversity
    c. Beta diversity
    d. Changes in abundant taxa between treatment groups, pre and post intervention
7. What are the acute (~300-minute post dose) and chronic (84-day supplementation) effects of resveratrol supplementation on the following blood biomarkers, related to inflammation, cholesterol and resveratrol supplementation:
    a. Total cholesterol
    b. C-Reactive Protein (CRP)
    c. Ferric reducing antioxidant power (FRAP)
    d. Glucose
    e. High-density lipoprotein (HDL)
    f. Interleukin-6 (IL-6)
    g. Low-density lipoprotein (LDL)
    h. Resveratrol-3-O-D-glucoside
    i. Resveratrol
    j. Resveratrol-3-O-sulfate
    k. Resveratrol-4-O-D-glucoronide
    l. Triglycerides
8. What are the chronic (following 84-day supplementation) effects of resveratrol supplementation on urinary metabolite profile:
    a. Identified mass spectral features that differ between intervention groups via both positive and negative mode ionisation analysis

9. What are the acute (45- and 240- minute post dose) and chronic (84-day supplementation) effects of resveratrol supplementation on blood pressure and heart rate.
10. What are the chronic (84-day supplementation) effects of resveratrol supplementation on body weight and Body Mass Index (BMI)

Based on the previous literature and above aims, it is hypothesised that acute (45 minutes post dose) and chronic (84-day) supplementation with 500 mg resveratrol will improve performance on cognitive tasks (with improvements measured as increased accuracy and/or decreased reaction time on individual tasks and cognitive domains), in overweight and obese, but otherwise adults. Recent literature suggests that the focus of resveratrol supplementation in a more 'compromised' demographic (here through obesity) is likely to exert more beneficial effects, than in the young, healthy populations that have primarily been studied thus far. Within this obese model, participants are hypothesised to have a dysbiotic gut microbiota and relatedly, exacerbated systemic inflammation, alongside elevation of related health markers including cholesterol and blood pressure. Here, the potential for resveratrol to impact upon gut microbial communities, alongside its ability to interact with numerous biological systems may underpin improvements in cognitive performance and the above health markers. Moreover, given previous evidence of resveratrol's ability to modulate cerebral blood flow within the pre-frontal cortex in healthy young adults following acute supplementation, it is hypothesised that the same effects will be observed in this older, overweight population following acute and chronic supplementation of resveratrol.

4.2. Materials and Methods

4.2.1. Study design and ethics

This study employed a randomised, double-blind, placebo-controlled, parallel groups design where participants were randomly assigned to consume one of two treatment groups – 500 mg Veri-te^{TM} resveratrol or placebo for a supplementation period of 84-days. As shown within Figure 4.1. participants were required to initially attend a training and screening visit, followed by two assessment visits on Day 1 and Day 84.

These testing visits comprised of participants attending the research centre fasted, providing a blood, urine and stool sample before being provided with a standardised breakfast. Following this participants completed a baseline cognitive assessment and a 50% subsample of participants completed a 5 minute baseline resting cerebral blood flow assessment using

quantitative near infrared spectroscopy. Following this participants consumed their treatment for the day and completed a second cognitive assessment (post dose assessment 1), 45 minutes later. Following this assessment the qNIRS subsample completed a post dose assessment of cerebral blood flow at rest and during cognitive demand. All participants were provided with a standardised lunch, completed a third cognitive assessment (post dose assessment 2) at 240 minutes post dose, followed by a final blood sample. With 500 mg resveratrol or placebo consumed daily at home. Participants also attended a 5-minute treatment exchange visit at the mid point of the trial, to check continued eligibility and collect the final six weeks of treatment. Further details of the procedure is detailed within Section 4.2.8, with the timeline of testing visits detailed further in Figure 4.4 within this section.

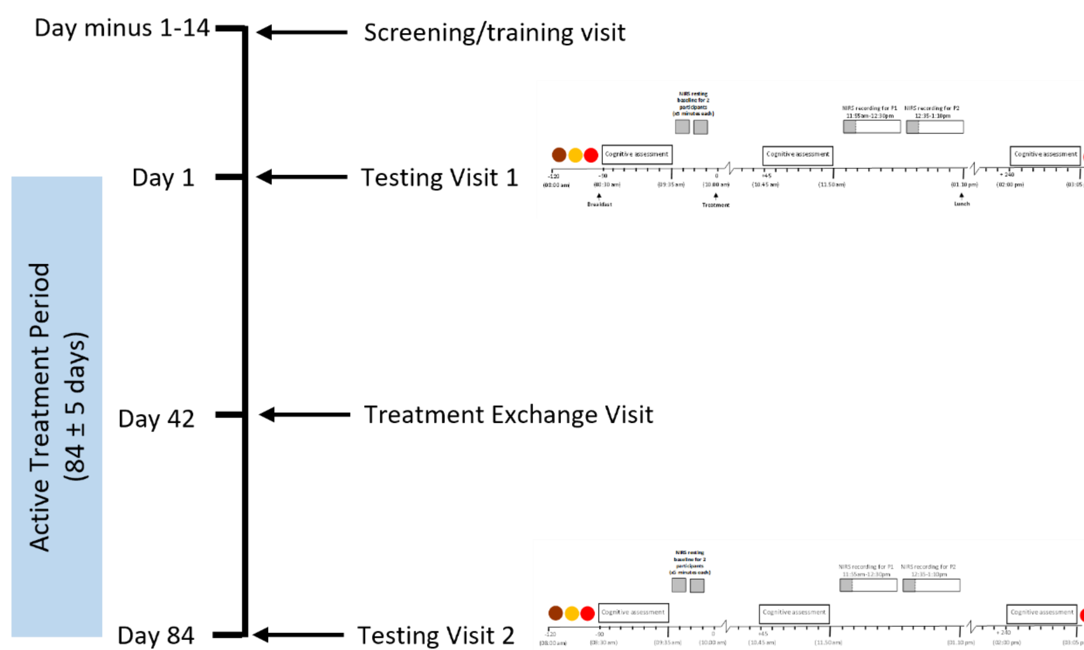

Figure 4.1. Overview of trial procedure. The figure depicts the overview of the trial. With participants assessed on the first (Day 1) and final (Day 84) day of their supplementation period, following a training session conducted prior to the initial, acute session. Participants also attended a treatment exchange visit at the midpoint of the trial, to check continued eligibility, compliance and collect the final six weeks of treatment.

Ethical approval was gained from Northumbria University's Psychology Department (submission reference: 1147) and was conducted according to the Declaration of Helsinki (1964). The study was registered on www.clinicaltrials.gov under the identifier NCT03448094.

4.2.2. Participants

One hundred and thirty-seven males and females aged 35-60 years were recruited, of these one hundred and ten were enrolled and randomised into the study. Participants were recruited

via advertisement posters within Northumbria University, on local transport services and within local businesses; emails sent to Northumbria University staff and students, and those signed up to the Brain, Performance and Nutrition Research Centre (BPNRC) participant database. In addition, paid adverts were used on social media and local newspapers and a mail shot sent to homes within the North East of England.

Participants were either overweight or obese (with a BMI between 25-42 kg/m^2) and were required to be in good health, as assessed by the following criteria. Participants were excluded from the study if they had high blood pressure (defined as systolic >159 mmHg or diastolic >99mmHg); currently taking any medication (with the exception of contraceptives and stable use of hormone replacements) which would contraindicate with the study; were smokers or used vapes; had any food allergies or sensitivities; were pregnant, planning to become pregnant or breastfeeding; had any sleep disturbances or took sleep aid medication; had any history of vascular, neurological or psychiatric illness; had a current diagnosis of depression or anxiety; had regular (>1 per month) migraines; any disorder of the blood or heart disorder; a respiratory disease that required regular medication; Type I or Type II diabetes; renal disease, hepatic disease or disease of the gastrointestinal tract; a visual impairment that couldn't be corrected with glasses or contact lenses (including colour blindness) and any learning difficulties. In addition, participants were excluded if they had used antibiotics, prebiotics or probiotics within the previous 8 weeks to enrolment; or used dietary or herbal supplements within the last 28 days or if they consumed >5 portions of fruit or vegetables per day or had an excessive caffeine intake (defined as >500 mg per day). As this study involved blood samples, participants were also excluded if they met any of the following criteria: had any known active infections; had a diagnosis or high risk of contracting syphilis, hepatitis or HIV; recent (minimum 3 years all clear) history of breast cancer or a mastectomy; haemophilia or any similar blood clotting disorder.

The sample size for this study was calculated based on a small effect size (d = 0.24) observed within Chapter 2 for effects on resveratrol-3-sulfate following 4-week supplementation with a resveratrol. Given this effect size, an a priori calculation of the size of sample required in order to detect a significant difference between the groups given 70% power and an alpha level of 0.05, is 110 participants. Power calculations were made using GPower 3.1.

All participants provided urine and blood samples (subject to a phlebotomist obtaining them) and a subsample of 96 participants provided stool samples. Of these, 78 participants provided samples on both study visits. A subsample of fifty-five participants participated in an optional element of the study, where cerebral blood flow data was captured using quantitative near

infrared spectroscopy (qNIRS). Eight participants withdrew from the study following randomisation, 4 due to adverse events, 1 due to medication change and 3 left the trial due to time commitments. This resulted in one hundred and two participants completing the study as planned. Participant disposition through the trial is displayed in Figure 4.1 and their demographic data in Table 4.1.

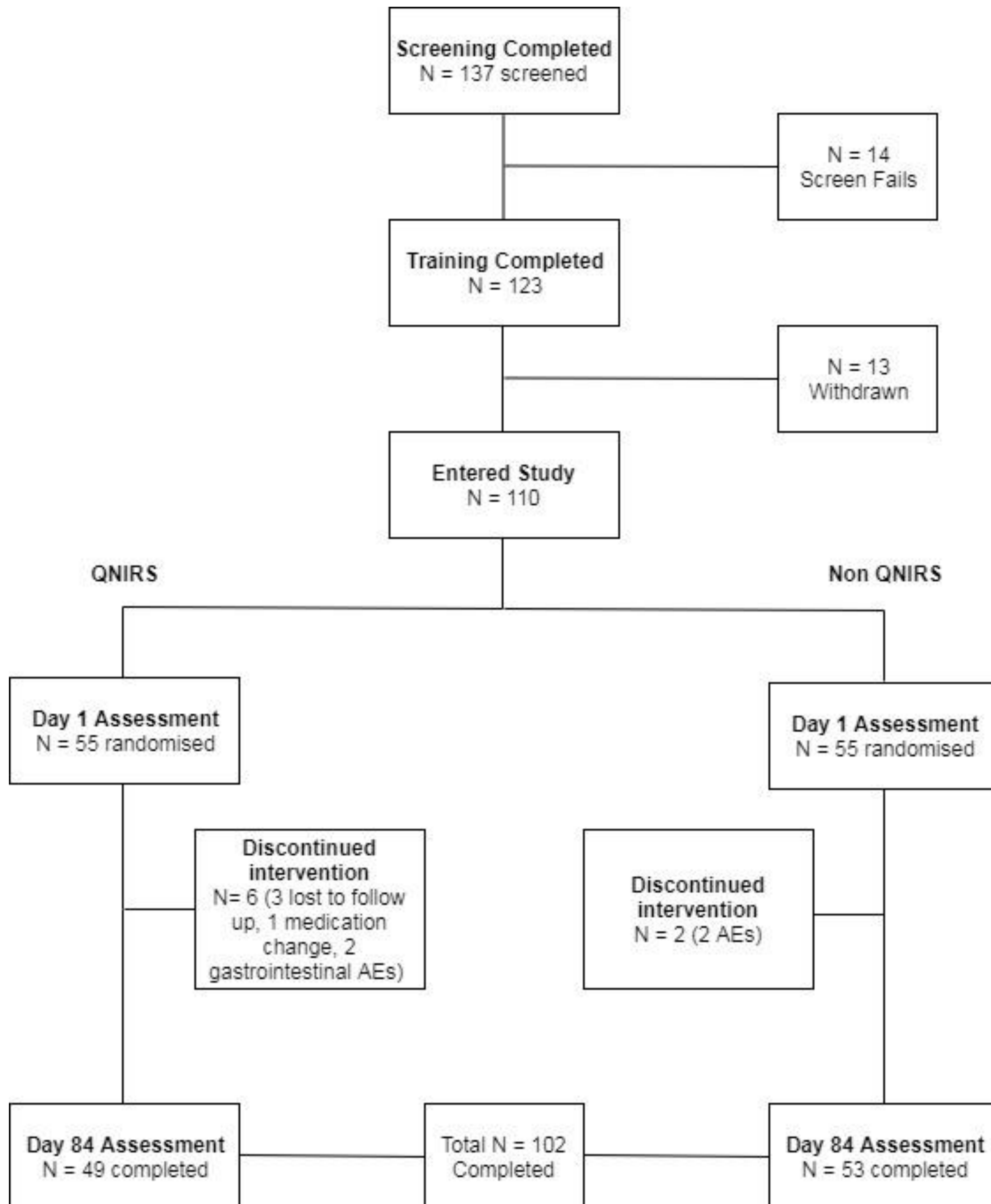

Figure 4.2. Participant disposition through the trial. The figure depicts the disposition of participants throughout the study, culminating in N=102 of the 110 who were randomised.

Table 4.1. Participant demographic information and characteristics. Means and Std. Deviation (sd) are presented where appropriate, with F and p values of the main effects from the one-way ANOVAs conducted on the baseline data by treatment group.

		Baseline		Main effects	
		Mean	SD	*F*	*P*
Age	Placebo	47.76	7.24	.21	.64
	Resveratrol	47.10	7.50		
Sex (Male/Female)	Placebo	13/42	-	-	-
	Resveratrol	13/42	-		
Years in Education	Placebo	16.32	3.19	2.07	.15
	Resveratrol	17.29	3.79		
Fruit and Vegetable (portions per day)	Placebo	2.95	1.17	6.67	.10
	Resveratrol	3.23	1.18		
Alcohol (Units per day)	Placebo	0.94	1.01	1.45	.23
	Resveratrol	0.73	0.80		
Caffeine consumption (mg/day)	Placebo	234.64	111.21	1.01	.31
	Resveratrol	210.93	134.90		
Systolic blood pressure (mmHg)	Placebo	131.10	14.18	.56	.45
	Resveratrol	129.22	11.77		
Diastolic blood pressure (mmHg)	Placebo	86.35	8.94	1.27	.26
	Resveratrol	84.3	10.08		
Heart Rate (BPM)	Placebo	73.95	10.63	.21	.64
	Resveratrol	72.89	13.10		
BMI (kg/m^2)	Placebo	30.59	4.35	.20	.65
	Resveratrol	30.22	4.31		
Waist to hip ratio	Placebo	0.90	0.07	.95	.33
	Resveratrol	0.89	0.06		

4.2.3. Treatments

Treatments are identical to those detailed in Section 2.2.3. Where all participants were randomly assigned to one of two treatment conditions, which each involved the consumption of two capsules daily:

1. 500 mg Veri-te^{TM} resveratrol
2. Placebo (cellulose microcrystalline)

The lead researcher reconstituted both treatments into identical white bottles containing 90 capsules in each. To ensure blinding was maintained throughout the trial, a third-party researcher coded the treatments as A and B and created a stratified randomisation schedule. Treatment bottles were labelled with a treatment randomisation number that corresponded to their inclusion in the study (Non qNIRS 101-155; qNIRS 201-255), assigning each participant to an A or B treatment. Treatment bottles were assigned to participants in a sequential order stratified by participation in the optional qNIRS measurement. Upon completion of all data analysis, the lead researcher and principal supervisor were unblinded to allow for interpretation of results.

Participants were randomly allocated to a single treatment for the duration of the trial, with treatments provided in two bottles containing 90 capsules (with the first bottle dispensed at testing visit 1 and the second at a 6-week check-up appointment). Participants consumed their first (Day 1) and final (Day 84 +/- 5 days) treatments in the research centre, where they consumed both capsules at once at approximately 10:00 am. During the 84-day supplementation period, participants were instructed to consume two capsules each day: one in the morning with breakfast and the second in the evening with their evening meal.

4.2.4. Treatment guess questionnaire and compliance

Compliance to the treatment regimen was primarily measured by a count of the returned capsules. A secondary compliance measure of the treatment diary (which required participants to note the time of each capsule consumption) (Appendix VI) was also used. As participants were provided with 180 treatment capsules throughout the supplementation period, a treatment compliance percentage was calculated by comparing the number of capsules that were actually returned by each participant at the mid-point and end of the study with the number of capsules that should have been returned. Compliance percentages were calculated as:

$$\text{Treatment Compliance (\%)} = \frac{\text{Number of capsules returned}}{\text{Number of capsules that should have been consumed (Days enrolled in trial x2)}} \times 100$$

To verify the blinding process, after completion of the study, participants completed a treatment guess questionnaire (Appendix III). They were asked to choose whether they'd received the active or placebo treatment and provide a reason for this. Responses to this questionnaire were analysed via Chi-square test to assess the number of correct and incorrect responses given by treatment group.

4.2.5. Physiological measures

4.2.5.1. Computerised cognitive assessments

Cognitive function was assessed using a configuration created and delivered using the Computerised Mental Performance Assessment System (COMPASS, BPNRC, Newcastle upon Tyne, UK). This is detailed previously within Section 2.2.4.1.1.

The selection of cognitive tasks were specifically chosen to provide a broad assessment across all cognitive domains, a technique also employed in Chapter 2. These cognitive domains included episodic memory, working memory, attention and executive function. In order to increase the cognitive demand of the paradigm, when compared with the paradigm employed in Chapter 2, supplementary tasks were utilized and the Cognitive Demand Battery was completed three times in succession.

Similar paradigms and selections of tasks (including the Cognitive Demand Battery) have been used previously and demonstrated sensitivity to a number of nutritional interventions, including work from the Brain, Performance and Nutrition Research Centre (Kennedy et al., 2019; Wightman et al., 2018; Wightman et al., 2021). The tasks which comprise each assessment are described below in order of completion. The cognitive assessment lasted 65 minutes in total and was completed three times over the course of each testing day. The

timelines of each assessment and the cognitive domains that individual tasks load upon are shown in Figure 4.2.

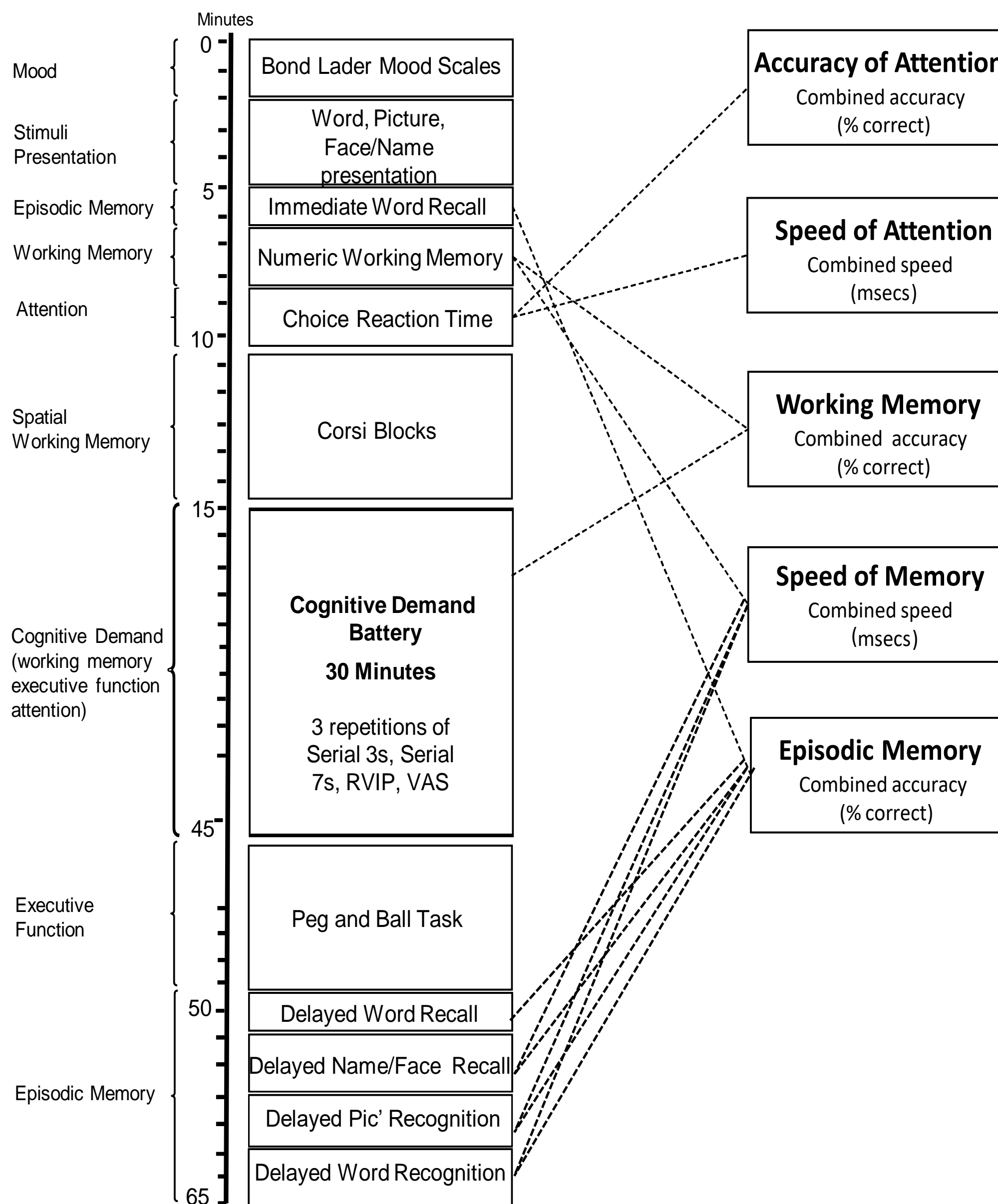

Figure 4.3. The running order of the individual cognitive assessments. Tasks are shown in order of completion with approximate timings. On the left the 'cognitive domain' assessed by the task is shown and the boxes to the right show global measures into which data from several tasks have been collapsed.

4.2.5.1.1. Word Presentation

Identical task as described in Section 2.2.4.1.1.

4.2.5.1.2. Picture Presentation

As described in Section 2.2.4.1.2.1. with the exception that this task was presented via COMPASS as part of the laptop-based configuration instead of the Cognim^{app} mobile phone assessment detailed in Chapter 2.

4.2.5.1.3. Face and Name Presentation

A set of twelve passport-style photographic images of people were presented sequentially, one at a time, in a random order to participants. A first and last name was assigned to each photograph and presented on the screen underneath the person's face. Stimulus duration was one second, with a 3-second inter-stimulus duration.

4.2.5.1.4. Immediate Word Recall

As detailed within Section 2.2.4.1.1.1.

4.2.5.1.5. Numeric Working Memory

As described in Section 2.2.4.1.2.2. with the exception that this task was presented via COMPASS as part of the laptop-based configuration instead of the Cognim^{app} mobile phone assessment detailed in Chapter 2. As such, participants responded using button response pad 'Yes' and 'No' buttons.

4.2.5.1.6. Choice Reaction Time (CRT)

As described in Section 2.2.4.1.2.3. with the exception that this task was presented via COMPASS as part of the laptop-based configuration instead of the Cognim^{app} mobile phone assessment detailed in Chapter 2. As such, participants responded using button response pad 'Left' and 'Right' buttons.

4.2.5.1.7. Corsi Blocks

As detailed within Section 2.2.4.1.1.2.

4.2.5.1.8. Cognitive Demand Battery

Participants completed three repetitions of a 10-minute computerised "Cognitive Demand Battery" (total completion time ~30 minutes); multiple consecutive completions of this battery of tasks reliably increase self-ratings of mental fatigue and has been shown to be sensitive to many natural interventions (Kennedy et al., 2008; Reay, Kennedy & Scholey, 2005; Wightman et al., 2018).

The battery comprises of: two minutes each of serial 3 and 7 subtractions, followed immediately by 5 minutes of Rapid Visual Information Processing (RVIP). Mental fatigue and task difficulty is self-rated after each completion of the three tasks.

4.2.5.1.8.1. Serial 3 Subtractions

As detailed within Section 2.2.4.1.1.3.

4.2.5.1.8.2. Serial 7 Subtractions

As detailed within Section 2.2.4.1.1.4.

4.2.5.1.8.3. Rapid Visual Information Processing (RVIP)

As detailed within Section 2.2.4.1.1.5.

4.2.5.1.8.4. Visual Analogue Scales (VAS)

As detailed within Section 2.2.4.1.1.6. Participants were also required to rate the task difficulty as this point, using the same anchored scale.

4.2.5.1.9. Peg and Ball

Participants were presented with two configurations of three coloured balls (blue, green and red) on three pegs, where each peg had the capability of holding three balls. The top diagram denoted the "goal" configuration of balls on pegs and participants had to rearrange the balls on the "starting" configuration below this to match the "goal". To do this, participants were required to use the mouse and drag and drop the balls in the "starting" configuration to the correct pegs to match the "goal" configuration. They had to do this in the least number of moves possible. Subjects randomly completed 5 trials each (15 in total) which could be solved

in 3, 4 and 5 moves. The task is scored for average thinking time (ms), average completion time (ms) and errors (total number of moves in excess of minimum required to complete all trials).

Task outcome measures: Average thinking time (msecs); Average completion time (msecs) and number of errors. Here, a lower (quicker) reaction time and lower number of errors is indicative of better performance on the task.

4.2.5.1.10 Delayed Word Recall

As detailed within Section 2.2.4.1.1.8.

4.2.5.1.11. Delayed Face to Name Recall

The 12 target faces presented at the beginning of the battery were displayed on the screen one at a time. Below each face was a list of 4 forename options and a list of 4 surname options. Participants used the mouse to select the forename and surname that they think were presented with each face at the beginning of the session. The task outcomes include percentage accuracy for overall correct forenames and correct surnames and reaction time (ms).

Task outcome measures: % Accuracy overall, % Accuracy Correct Forenames, % Accuracy Correct Surnames. Overall reaction time (msec). Here, a high accuracy score and a lower (quicker) reaction time is indicative of better performance on the task.

4.2.5.1.12. Delayed Picture Recognition

As described in Section 2.2.4.1.2.5. with the exception that this task was presented via COMPASS as part of the laptop-based configuration instead of the Cognim^{app} mobile phone assessment detailed in Chapter 2. As such, participants responded using button response pad ‘Yes’ and ‘No’ buttons.

4.2.5.1.13. Delayed Word Recognition

As detailed within Section 2.2.4.1.1.9.

4.2.5.2. Cognitive domain data

As detailed previously in Section 2.2.4.2. common practise within the area is to collapse individual task scores into relevant outcome measures, where often clearer cognitive effects are observed. Data from the current study lends itself to the analysis of the following global cognitive domains: accuracy of attention, speed of attention, working memory, speed of memory, episodic memory, overall accuracy and overall speed.

As previous, cognitive domains were calculated by changing individual task scores into standardised Z scores and then grouping these scores. The specific calculations for each of the cognitive domains are detailed below.

4.2.5.2.1. Accuracy of Attention

The accuracy of attention data was calculated from standardised values using the following calculations:

Accuracy of attention = (Zcrt accuracy + Zrvip accuracy)/2

4.2.5.2.2. Speed of Attention

The speed of attention data was calculated from standardised values using the following calculations:

Speed of attention = (Zcrt RT + Zrvip RT)/2

4.2.5.2.3. Working Memory

The working memory data was calculated from standardised values using the following calculations:

Working Memory = (Znwm accuracy + Zcorsi blocks)/2

4.2.5.2.4. Speed of Memory

The speed of memory data was calculated from standardised values using the following calculations:

Speed of Memory = (Znwm RT + Zpic recog + Zword recog)/3

4.2.5.2.5. Episodic Memory

The episodic memory data was calculated from standardised values using the following calculations:

Episodic Memory = (Zntf accuracy + Zpic recog accuracy + Zword recog accuracy + Ziwr + Zdwr)/5

4.2.5.2.6. Overall Accuracy

The overall accuracy data was calculated from standardised values using the following calculations:

Overall accuracy = (Znwm accuracy + Zcrt accuracy + Zss3 accuracy + Zss7 accuracy + Zrvip accuracy + Zntf accuracy + Zpic recog accuracy + Zword recog accuracy + Ziwr + Zdwr)/10

4.2.5.2.7. Overall Speed

The overall speed data was calculated from standardised values using the following calculations:

Overall speed = (Znwm RT + Zcrt RT + Zrvip RT + Zpab RT + Zntf RT + Zpic recog RT + Zword recog RT)/7

4.2.5.3. Mood assessment

4.2.5.3.1. Bond Lader Mood Scales

The current study employed the Bond Lader (Bond & Lader, 1974) mood scales to assess mood at the start of each computerised cognitive assessment. The Bond-Lader mood scales have been used in many trials and comprise a total of sixteen 100 mm lines anchored at either end by antonyms (for example. “alert-drowsy”, “calm-excited”. Participants are required to

place a cross on the line to indicate their current subjective position between the antonyms. Outcomes comprise three factor analysis derived scores: “Alertness”, “Calmness” and “Contentment”.

4.2.5.3.2. Profile of Mood States (POMs)

As detailed within Section 2.2.4.3.2. Participants completed POMs assessments at the start and end of each testing visit.

4.2.5.4. Blood pressure assessment

As detailed within Section 2.2.4.4.

4.2.5.5. Body Mass Index (BMI)

As detailed within Section 2.2.4.5.

4.2.5.6. Quantitative Near-Infrared Spectroscopy (qNIRS)

Cerebral blood flow was monitored using a frequency domain “quantitative” NIRS system (OxiplexTS Frequency-Domain Near-Infrared Tissue Oximeter; ISS, Inc., Champaign, IL, USA). NIRS has been previously been utilised to examine the haemodynamic response in humans during activated brain function (Villringer, Planck, & Hock, 1993). Recently the technique has been employed in the field of nutritional neuroscience and has been shown to be a sensitive measure of change in cerebral oxygenation in the prefrontal cortex following pharmacological supplementation, including of polyphenols (Kennedy et al., 2010; Wightman et al., 2012).

This system gives absolute measurements of absorption of near-infrared light emitted at two distinct wavelengths (691 and 830 nm), which allows for the quantification of oxygenated haemoglobin (HbO_2) and deoxygenated haemoglobin (HHb) (μmol/l), these values are then used to determine total haemoglobin (HbO_2 + HHb) and oxygen saturation (HbO_2/tHb x 100%). This system is ideal for quantifying acute changes in haemodynamic response over an extended period (i.e., with intermittent testing throughout one visit) and in a chronic context (comparing CBF between Day 1 and Day 84).

Light was emitted at 691 and 830 nm by optical fibres glued in pairs to four prisms (eight fibres in total) that were separated from the collector bundle, also glued to a prism, by 2.0, 2.5, 3.0 or 3.5cm. Each of the emitter and collector bundle prisms were embedded into a flexible polyurethane resin to form a sensor with the overall dimensions of 7.6cm x 2.5cm x 0.3 cm. Identical sensors were attached to either side of the forehead of participants and secured in place with a self-adhering bandage. The sensors were positioned so that the bottom edge was level with the top of the participants' eyebrows and the middle edge touching at the midline of the forehead. Data were collected at a rate of 5Hz.

4.2.7. Biological measures

4.2.7.1. Urine samples

Spot urine samples, avoiding the first morning void, were provided prior to treatment consumption on each testing visit in the laboratory. Samples were collected in sterile 30 ml tubes, refrigerated and 1 mL aliquots pipetted into sterilised microtubes and then stored at -20°C until analysis.

The following analysis was completed by Lewis Cuthbertson and William Cheung, within the Applied Sciences department at Northumbria University. Urine samples were defrosted on ice, vortexed and then equal volume (100 µL) of urine was mixed with chilled (-20°C) with LC-MS grade methanol. Samples were vortex mixed and chilled on ice for 30 minutes. Samples were then centrifuged at 13,000 rpm for 2 minutes. The top 150 µL was aliquoted, filtered and transferred to an LCMS vial for analysis. Quality control samples were prepared by aliquoting 5 µL of each sample together, vortexing and collecting 100 µL.

Hydrophilic interaction liquid chromatography (HILIC) based analysis was conducted using a Dionex 300 Ultra High Pressure Liquid chromatography (UHPLC) and Q-Extractive high resolution mass spectrometer system. Samples were analysed in random order, with pooled quality control samples and blank injections. The data was acquired on both Positive and Negative mode polarity (independently). Thorough analysis methodology is detailed within Langer, Kennel, and Lodge (2018).

4.2.7.2. Stool samples

The samples were collected at home by participants within 18 hours of attending the research centre using Fe-Col® Faecal Collection Kits (Alpha Laboratories). Upon arrival, samples were

immediately frozen at -80°C, until sample preparation and analysis upon completion of the study. The following analysis was completed by Lewis Cuthbertson with advice from Darren Smith, within the Applied Sciences department at Northumbria University. Here, each stool sample was partially defrosted, 100 mg weighed out and DNA extracted using Qiagen HTP Power Soil DNA extraction kit as per manufacturer's instructions. All DNA was quantified using a Qubit, fluorimeter with DNA purity > 1.8 A260/A280. Amplification of the V4 region of the 16S rRNA gene using the method set out by Kozich, Westcott, Baxter, Highlander, and Schloss (2013).

The QIIME2 bioinformatics pipeline (Bolyen et al., 2018), was utilised to transform raw data files to amplicon sequence variants (ASVs) as described by Callahan et al. (2019). Data decontamination, analysis and visualizations were carried out in R studio, using; decontam, phyloseq, vegan, ggplot2, gridextra, and scales packages.

A total of 8530597 reads made up of 7375 taxa were screened for non-bacterial sequences and reduced to a total of 8281908 reads containing 7029 bacterial taxa. Samples were then screened for contaminants based on the prevalence of bacteria in negative control samples, further reducing the total library size to 5035953 and total number of bacterial taxa to 6272 across 162 samples. Negative controls were then parsed from the dataset. Counts of bacterial taxa within samples were then normalised by conversion to relative abundance to account for variation in sequencing depth. The average read count for decontaminated samples was 31086.13. The SD was 17673.37 reads. Rarefaction curves were plotted to show sufficient sampling depth had been achieved.

Alpha diversity metrics were calculated and significance of multiple continuous variables determined using the Pairwise wilcoxon test with bonferroni adjustment. Beta diversity was assessed using weighted Bray-Curtis distance and displayed using PCoA analysis and PERMANOVA was used to determine significance of dissimilarity between groups. Differential abundance analyses were carried out in DeSeq2, which fits negative binomial generalized linear models between groups and tests for significant difference using the Wald test, and controls FDR using the Benjamini-Hochberg method.

4.2.7.3. Blood samples

Venous blood samples were collected using 10 ml serum vacutainers

Fasted venous blood samples were collected using 10ml serum and 6ml lithium heparin (LH) vacutainers to assess the following biomarker outcomes: total Cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, C-reactive protein (CRP), interleukin-6 (IL-6), ferric reducing antioxidant power (FRAP), glucose, resveratrol, resveratrol-3-O-D-glucoside, resveratrol-3-0-sulfate, resveratrol-4-O-D-glucoronide. Venous samples were collected on both testing visits before the administration of the day's treatment (fasted sample) and then 305 minutes post dose. Samples were inverted 6 times and allowed to coagulate at room temperature for 1-2 hours. Samples were processed within 2 hours of collection. The samples were centrifuged at 600 RCF for 20 minutes at 4°C to obtain plasma, which was then pipetted into eppendorfs and stored at -80°C until analysis. Analysis was conducted as detailed within Section 2.2.5.1, by Samantha Bowerbank, within the Applied Sciences department at Northumbria University.

4.2.8. Procedure

Participants were required to attend the laboratory at Northumbria University, UK, on four separate occasions. The first of the visits was an initial training/screening visit, where participants were briefed on the requirements of the study, provided informed consent and demographic information, were screened against the inclusion and exclusion criteria and trained on the cognitive and mood measures.

Following the introductory visit, participants attended the laboratory in an overnight fasted state at 8.00 am on two separate occasions (Day 1 and Day 84). Participants must have refrained from alcohol for 24 hours and caffeine for 18 hours; they were also required to document their diet for the four days prior to each testing visit. Participants also provided the researcher with a stool sample that was collected either the previous day (no more than 18 hours prior), or that morning. Testing took place in a suite of testing facilities within the Brain, Performance and Nutrition Research Centre, Northumbria University, with participants visually isolated from each other.

The assessment procedure of each testing day was identical: on arrival participants provided a blood sample and then consumed a standardised breakfast. This comprised 2 slices of toasted Hovis soft white bread: 186 kcal, 1.4g fat, 2.8g sugar, 7g protein; with Lurpak slightly salted spread 15 g: 106 kcal, 11.7 g fat, <0.1 g sugar, <0.11 g protein. Participants were also offered a decaffeinated tea or coffee with their breakfast (Sainsbury's Gold Roast Decaffeinated Instant Coffee: 1 kcal, 0 g fat, 0 g sugar, 0 g protein; Tetley's Decaffeinated

teabags: 1 kcal, 0 g fat, 0 g sugar, 0 g protein; with Semi Skimmed milk if desired ~10 ml: 5 kcal, 0.1 g fat, 0.4 g sugar, 0.3 g protein).

At 8:30 am participants began their baseline cognitive assessment, they first completed the paper and pencil POMs, followed by the computerised cognitive assessment and measurements of heart rate and blood pressure. A subsample of participants provided additional CBF measures at two points during the testing day and, at this point, those participants provided a baseline resting measure by wearing the NIRS headband for 5 minutes. Participants were required to provide a urine sample prior to consuming the days treatment (this was from the current morning, but not their first urination of the day). Following this all participants consumed their treatment for the day (~10:00 am). Two further cognitive assessments (followed immediately by single measurements of BP and HR readings) commenced at 45 (~10:45 am) and 240 (~2:00pm) minutes post dose.

The timings of assessments here were chosen based on previous literature, here 45-minutes was selected for the first post dose assessment, which is consistent with the body of literature from the Brain, Performance and Nutrition Research Centre which precedes this thesis (Kennedy et al., 2010; Eschle et al., 2020; Eschle, 2017). Whilst these trials failed to observe cognitive benefits, all observed clear haemodynamic modulation at this timepoint, suggesting that within this more compromised demographic we could anticipate to observe cognitive enhancements from 45-minutes post dose. Moreover, results of the bioavailability assessment within Kennedy et al. (2010) confirmed the presence of resveratrol metabolites in plasma from 45-minutes post dose, peaking at ~90 minutes post dose, which would coincide with participants completing the cognitive demand battery within this first post dose assessment.

The rationale behind choosing a 240-minute post dose assessment, was to add to the existing literature by incorporating an additional assessment to measure the acute effects of resveratrol. In the literature to date, most trials have been limited to acute trials with a single post dose assessment (as in Kennedy et al., 2010; Wightman et al., 2014; Wightman et al., 2015; Eschle et al., 2017; Scholey et al., 2014; Wong et al., 2013) or have only considered the chronic effects of intervention (as in Witte et al., 2014; Wong et al., 2013; Evans et al., 2017; Anton et al., 2018; Zaw et al., 2020a, 2020b). With the exception here of a single trial by Eschle et al. (2020) who investigated the cognitive effects of resveratrol at 45-, 120- and 180-minutes post dose; however here no effects on cognitive performance were observed at any timepoint. Within the present trial, in the absence of clear previous literature or pharmacokinetic data to dictate this; as an exploratory addition, the timing of this assessment was decided on off the basis of both the design of similar polyphenolic intervention trials and

also, the practical feasibility of running the testing day. Here looking at the designs of recent trials investigating products with similar bioactive phytochemicals, the Brain, Performance and Nutrition Research Centre have conducted two trials of note. The first, investigating the effects of a wild green-oat (*Avena sativa)* extract, which employed a similar design to the present trial, with post dose assessments at 1-, 2.5-, 4- and 6-hour timepoints (Kennedy et al., 2017); likewise a similarly designed trial investigated the acute effects of a sage extract at 120- and 240- minutes post dose (Wightman et al., 2021). Whilst undoubtedly here, the bioactive constituents within these interventions differ from resveratrol; in this situation with limited resveratrol work to base an acute design on, previous trials of similar design were invaluable in making this decision. Secondly, for practicality purposes, the 240-minute timing was chosen to allow incorporation of the measurements of cerebral blood flow in a subsample of participants and also ensure that all participants (including those within the subsample group) had an adequate break and time for a standardised lunch, before completing the final cognitive assessment of the day.

In between the second and third cognitive assessments of the day, the NIRS sub-sample provided a post-dose measure of CBF; during which time they completed a 5-minute post-dose baseline followed by three completions of the cognitive demand battery (30 minutes in total), as shown in Figure 4.4. Participants were provided with a standardised lunch between the second and third cognitive assessment at ~1:10pm. A final blood sample was collected at the end of each testing visit at ~3:05pm.

Lunch comprised a cheese sandwich (Hovis soft white bread x 2 slices: 186 kcal, 1.4 g fat, 2.8 g sugar, 7 g protein; Sainsbury's British Medium Grated Cheddar Cheese 30 g: 127 kcal, 10.5 g fat, <0.5 g sugar, 7.6 g protein; Lurpak slightly salted spread ~10 g: 72 kcal, 8 g fat, <0.1 g sugar, <0.1 g protein), one packet of ready salted flavours crisps (Walkers 25 g bag: 132 kcal, 8 g fat, 0.1 g sugar, 1.5 g protein) and one pot of custard (Ambrosia 125 g pot (due to an ordering error, some participants consumed the light versions (values in italics) for both visits): 124/*113* kcal, 3.5/ *2.3* g fat, 14.3/*13.8* g sugar, 3.6/*3.6* g protein). With total nutritional value of the meal calculated as (Ambrosia light values in italics): 641/*630* kcal, 31.4/*30.2* g fat, 17.8/*17.3* g sugar, 19.8/*19.8* g protein. This lunch was optional (as long as non/consumption of components was the same for both visits) to avoid the potentially more disruptive effects of eating items which were unpalatable to participants. This decision meant that of the 110 participants randomised into the trial there was the following deviations where participants did not consume the full standardised meals, as set out within the protocol: did not consume the custard pot (N=5); did not like cheese, so had plain bread and butter (N=2); did not like butter or cheese, had dry toast and bread (N=1); ate only 1 slice of toast (N=1); did not consume

breakfast as typically do not (N=2). Given the limited number of procedural deviations here and the negligible difference in study meal composition and nutritional value it seems unlikely that this will have had an impact on cognitive performance in the final post-dose assessment. Nevertheless, this is a potential limitation to the trial design that should be noted here.

At the end of Day 1 participants took away a six weeks supply of their daily intervention and a diary to record their consumption of the treatment. After six weeks, participants returned to collect a further six weeks supply of treatment and confirm continued compliance with the study procedures and inclusion/exclusion criteria with the researcher. At the end of Day 84 diary sheets and returned capsules were assessed to confirm compliance. The timelines and assessments of Day 1 and Day 84 are shown in Figure 4.3.

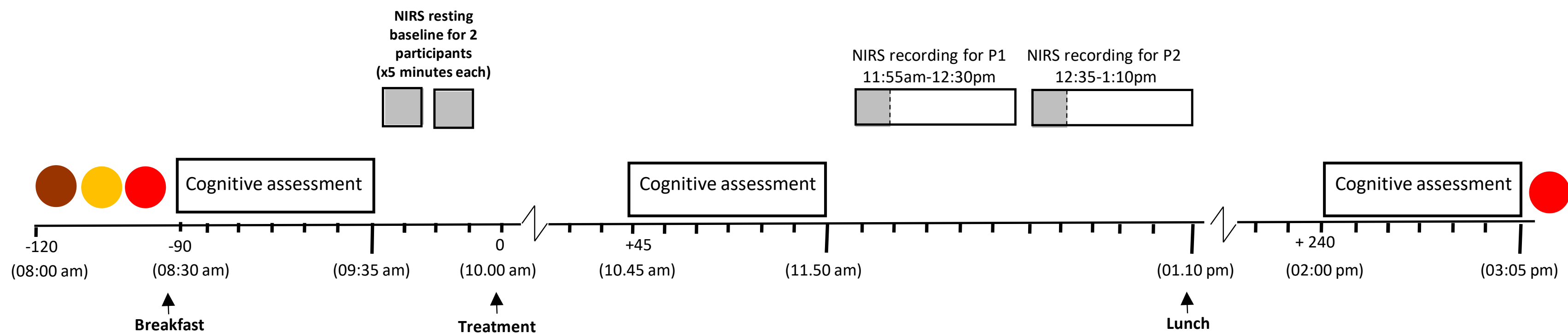

Figure 4.4. Testing session timeline of both the acute and chronic visit. Participants provided the researcher with a stool sample (obtained either the previous day (no more than 18 hrs previously) or that morning) and give a blood sample before consuming a standardised breakfast and completing a full cognitive assessment pre-dose. Participants were required to provide a urine sample (from the current morning but not the first of the day), prior to consuming their treatment at 10:00am. Post-dose cognitive assessments took place at 45- and 240- mins following treatment consumption. For the sub-sample of 50% of the recruited sample undertaking the qNIRS assessment cerebral blood-flow parameters will be measured at baseline (x5 mins resting) and at approximately 115-150 and 155-190 minutes post-dose (x 5 mins resting and during x30 mins task performance). A standardised lunch was provided at approximately 190 mins post-dose. A final blood sample was collected at the end of the day.

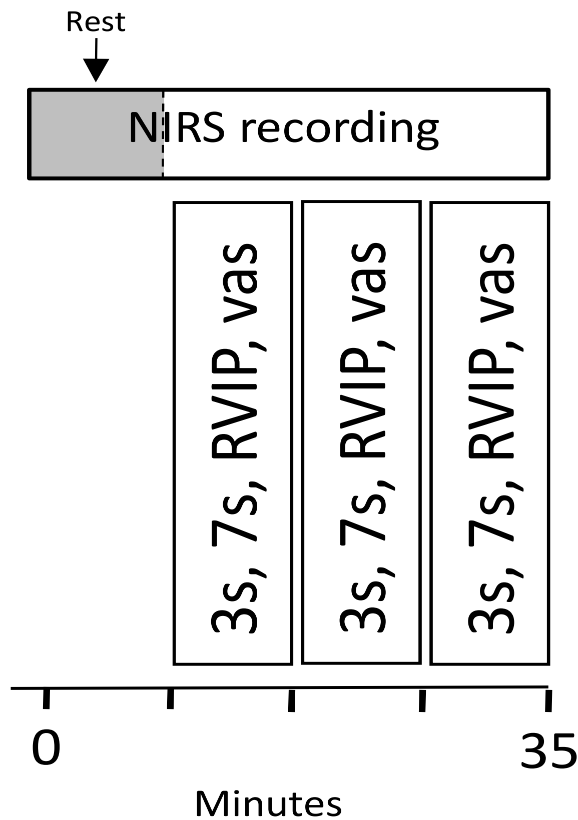

Figure 4.5. QNIRS Testing Paradigm. For the sub-sample of 50% of the recruited sample undertaking the qNIRS assessment cerebral blood-flow parameters was measured at baseline (x5 mins resting) and at approximately 115-150 and 155-190 minutes post-dose. During the post-dose assessment participants were fitted with the QNIRS headband and rested for 5 minutes. Following this they completed three rounds of the cognitive demand battery. With the assessment lasting approx. 35 minutes.

4.2.9. Statistical Methods

4.2.9.1. Data Cleaning Procedures

One hundred and ten participants were randomised into the study, of which 102 completed both study visits. Of the eight participants who did not complete the study, three participants were lost to follow up, one due to a new prescription of anti-depressant medication and four due to adverse events (as detailed within Section 4.3.1.).

Before conducting analyses, deviations from procedure were checked to identify the per protocol population, this resulted in exclusion of six additional participants from the Day 84

analysis. Each of these six were excluded due to antibiotic use during the supplementation period, therefore breaching the exclusion criteria. These were consumed by participants reporting shingles, urinary tract infection, ear infection, cellulitis, impetigo and helicobacter pylori infection. One additional participant was excluded from analysis due to low compliance (72%).

Following this the data was investigated for potential outlier data, following the same procedures are detailed within Section 2.2.7.1.

4.2.9.2. NIRS Data

NIRS data collection was attempted for a subset of N=55 participants, mean age 47.96 years, 49 right-handed, 6 left-handed; mean years in education 16.6 years, mean BMI 30.32.

Data cleaning resulted in the removal of N=6 datasets on Day 1 due to data variations which were outside of the x2 standard deviations of mean cerebral blood flow levels, resulting in a sample size of N=49 for Day 1 analyses. In terms of Day 84 analyses, the sample size was N=46, as N=3 participants withdrew during the supplementation period; N=3 were removed due to protocol deviations concerning medication use; N=2 were removed due to data variations outside of 2SD; and N=1 due to equipment recording errors.

Data was first averaged across the 2 hemispheres and converted to change-from-baseline (this being an average of the 5-minute recording taken after the pre dose cognitive task battery completion and just before treatment consumption). Post-dose data was split into two distinct periods: resting and active. This data was then averaged into 2 minute epochs for analysis (with the exception of the resting period and RVIP task which were split into 2.5 minute epochs). Resulting in 14 epochs during the post-dose assessment.

Analysis of data was conducted via a three-way ANOVA utilising treatment (resveratrol/placebo) x epoch (x 14) x day (Day 1 and Day 84) as factors. If significant main or interaction effects were observed, post-hoc planned comparisons (Sidak corrected) were conducted between the two treatment groups.

4.2.9.3. Cognitive Data

4.2.9.3.1. Individual task analysis

All cognitive task data, was analysed using analysis of covariance in SPSS (Version 26). All data was analysed for baseline differences via univariate ANOVAs with 'treatment' as a fixed factor. These are reported where they arise and pertain to subsequent effects on the main analyses.

The analysis of all COMPASS cognitive outcomes, blood biomarkers and blood pressure was conducted in three ways: acute effects within Day 1; acute effects within Day 84; and pure chronic effects within Day 84. To analyse this three ANCOVAs were conducted:

1. *Acute effects within Day 1 and Day 84*

To ascertain any acute treatment effects of resveratrol within Day 1, Post-dose data was analysed via ANCOVA with 'treatment' as a fixed factor and their baseline data from that day used as a covariate.

2. *Pure chronic effects on Day 84*

To ascertain if any pure chronic effects of resveratrol supplementation had taken place on Day 84, here, pre- and post-dose data from Day 84 were analysed with 'treatment' as a fixed factor and Day 1 baseline performance as a covariate.

NIRS post dose cognitive data was analysed using a one-way ANOVA with 'treatment' as a fixed factor.

4.3. Results

4.3.1. Compliance and adverse events

For participants who completed the study, mean compliance was observed to be very good for both treatment groups at 96% overall (96.64% Placebo, 96.10% resveratrol), with a one-way ANOVA identifying no significant differences for compliance between treatment groups [$F(1, 99)=.12$, $p = .729$]. The treatment consumption period was intended to last 84 +/-5 days, however participants supplementation period ranged from 76 – 99 days. The primary compliance measure was a capsule count upon return to the lab for visit 2. A secondary

measure; completion of a treatment diary noting the time of treatment consumption each day, was utilized to support this information.

Compliance ranged from 70 – 116%, meaning that n=4 was outside of the >80/<120% compliance range set for inclusion in analyses, N=1 (70%) was excluded anyway due to antibiotic use, n=1 (72%) was excluded for being outside the range, a further n=2 (76%) were decided to be included in the analyses irrespectively.

Initially, adequate compliance was assessed as >80% and <120% of the required supplementation as per the protocol (84 days supplementation). For this trial, a compliance of <80% would equate to missing >32 capsules during the supplementation period; whereas a value of >100% would be achieved if participants were in the trial >84 days (for example due to rescheduling testing visit 2). Here, if participants did not return any treatment, therefore consuming all 180 given capsules, their compliance would equate to 107%, to achieve a compliance score greater than this participants would need to be provided with additional capsules to those initially provided, for example if all 180 capsules were consumed during the supplementation period at home, an additional 2 capsules would be provided at testing visit 2 to consume within the laboratory. Here the decision to change the compliance inclusion criteria from 80% to 76% was based on the following, that in order to achieve a compliance of 76% this would equate to participants missing >39 capsules during the supplementation period, just 7 additional capsules (equivalent to 3.5 days full dose) than to achieve 80%. Here, due to the length of the supplementation period and the small difference in actual capsules consumed; it was decided that modifying the compliance inclusion criteria slightly would be a better option than removing these two participants from the analysis and thus further reducing the sample size.

Participants completed a treatment guess questionnaire at the end of their final testing visit and a Chi-Square test of these responses indicated no significant differences in the participants' ability to correctly identify which treatment they had taken for the duration of the study [$\chi_2(1) = .15$, $p = .690$].

Participants were required to report any adverse events throughout the duration of treatment administration, within their treatment diary. All symptoms in all cases resolved during the course of the study, with the exception of 4 participants who ceased participation due to AEs (x2 gastrointestinal issues, x1 migraine, x1 change in breast tissue), but follow up with these indicated that all issues had resolved and participants were in good health.

A chi-square test conducted on this data revealed no significant association between treatment and adverse event reporting [$\chi^2(1) = .15$, $p = .690$].

Table 4.2. Frequency of adverse events. Reported via treatment diary over the 12-week intervention period, reported by treatment group.

Adverse event	Treatment	
	Placebo	Resveratrol
Upset stomach	7	4
Headache	30	25
Shingles	1	0
Vitamin D deficiency	0	1
Heartburn	8	9
Blurred vision	1	0
Loose stools	0	2
Muscle/joint pain	3	5
Cramp	0	2
Migraine	2	1
Vomiting/nausea	2	1
Cold/flu	12	5
Haemorrhoids	1	0
Thrush	2	0
Hair loss	1	0
Helicobacter pylori	1	0
Rash	0	3
Eye pain	1	0
UTI	0	1
Dry stools	1	0
Hunger pains	1	0
Eye infection	0	1
Sciatica	1	0
Mouth ulcer	0	1
Cellulitis	1	0
Impetigo	0	1
Change to breast tissue	0	1
Total	76	63

4.3.2. Physiological Results

Due to the number of statistical analyses performed, only those with significant main or interaction effects including treatment will be reported.

4.3.2.1. NIRS

4.3.2.1.1. Oxygen Saturation (Ox%)

Analysis indicated no main effect of treatment, as shown in Table 4.3., however a significant interaction effect between treatment and day was identified for oxygen saturation [$F(1, 1369) = 4.72$, $p = .03$]. Sidak corrected post-hoc comparisons indicated that this effect was limited to Day 1, where oxygen saturation was lower following resveratrol (mean change from

baseline -.51) in comparison to placebo (mean change from baseline -.064), *p* = .02; no significant differences between treatment were observed on Day 84 (*p* = .38). However, planned comparisons indicated no significant difference between treatment groups for each individual epoch within Day 84 (*p* = >.005).

Table 4.3. NIRS Oxygen Saturation by treatment group during post-dose assessment. Estimated marginal means and standard error (SE) of change from baseline scores are presented with F and *p* values of the main effects and interactions from the three-way ANOVA analysis.

			Post dose		Main Effects		
		n	Mean	SE		F	*p*
Ox %	Placebo	55	-0.43	.10	Treatment	0.82	.36
					Treatment*Day	**4.72**	**.03***
	Resveratrol		-.057	.10	Treatment*Epoch	0.25	.99
					Treatment*Day*Epoch	0.12	1.00

4.3.2.1.2. Total Haemoglobin (THC)

Analysis indicated a significant main effect of treatment, [$F(1, 1377) = 46.77$, $p < .001$], where THC was increased following resveratrol (mean change from baseline .62), in comparison with placebo (mean change from baseline -.42), as indicated in Table 4.4. and Figure 4.5. A significant interaction effect between treatment and day was identified [$F(1, 1377) = 20.26$, *p* = <.001]. Planned comparisons indicated that this effect was limited to Day 84 (*p* = <.001) where total haemoglobin was higher following resveratrol (mean change from baseline .81) in comparison to placebo (mean change from baseline -.925); the same effect was observed approaching trending towards significance on Day 1 (*p* = .08). Sidak corrected planned comparisons indicated that within Day 84, this effect was significant at all epochs (*p* = <.005) with the exception of epochs 11 (serial 3, rep 3, *p* = .11) and 13 (RVIP rep 3, *p* = .13), as detailed within Table 4.5 and Figure 4.6.

Table 4.4. NIRS Total Haemoglobin concentration by treatment group during post-dose assessment. Estimated marginal means and standard error (SE) of change from baseline scores are presented with F and *p* values of the main effects and interactions from the three-way ANOVA analysis.

			Post dose		Main Effects		
		n	Mean	SE		F	*p*
THC	Placebo	55	-0.42	.10	**Treatment**	**46.77**	**<.001***
					Treatment*Day	**20.26**	**<.001***
	Resveratrol		0.62	.11	Treatment*Epoch	.02	1.00
					Treatment*Day*Epoch	.07	1.00

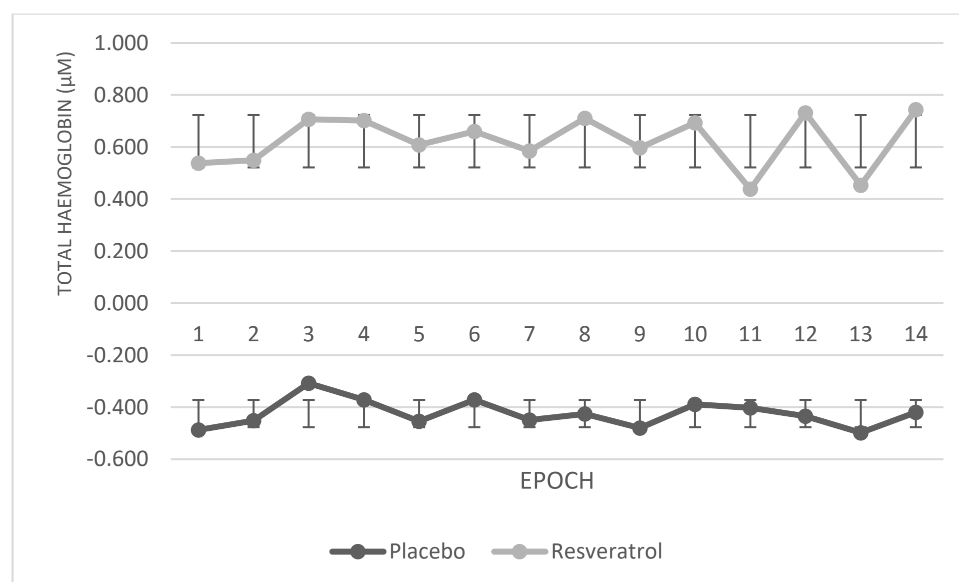

Figure 4.6. QNIRS Total Haemoglobin Concentration. Main effect of treatment on total haemoglobin concentration. Data presented is change from baseline scores by treatment group over 14 epochs during post-dose assessment (p = <.001).

Table 4.5. NIRS Total Haemoglobin concentration planned comparisons by day, epoch and treatment group during post-dose assessment. Estimated marginal means and standard error (SE) of change from baseline scores are presented with F and *p* value from the sidak corrected planned comparisons.

Day	Epoch		Mean	SE	Planned comparisons F	Planned comparisons *p*
1	1	Placebo	.08	.54	.01	.90
		Resveratrol	.17	.55		
	2	Placebo	.07	.54	.03	.84
		Resveratrol	.22	.55		
	3	Placebo	.17	.54	.12	.72
		Resveratrol	.45	.55		
	4	Placebo	.10	.54	.17	.67
		Resveratrol	.42	.55		
	5	Placebo	.02	.54	.18	.66
		Resveratrol	.35	.55		
	6	Placebo	.07	.54	.08	.77
		Resveratrol	.29	.55		
	7	Placebo	-.04	.54	.11	.74
		Resveratrol	.21	.55		
	8	Placebo	.04	.54	.41	.51
		Resveratrol	.54	.56		
	9	Placebo	.05	.54	.20	.64
		Resveratrol	.41	.56		
	10	Placebo	.14	.54	.29	.58
		Resveratrol	.57	.65		
	11	Placebo	.13	.54	.20	.65
		Resveratrol	.48	.56		
	12	Placebo	.03	.54	.47	.49
		Resveratrol	.57	.56		
	13	Placebo	.03	.54	.63	.42
		Resveratrol	.65	.56		
	14	Placebo	.12	.54	.46	.42
		Resveratrol	.66	.56		
84	1	Placebo	-1.05	.56	**5.50**	**.02***
		Resveratrol	.90	.61		
	2	Placebo	-.97	.56	**4.91**	**.03***
		Resveratrol	.87	.61		
	3	Placebo	-.78	.56	**4.39**	**.04***
		Resveratrol	.96	.61		
	4	Placebo	-.84	.56	**4.75**	**.03***
		Resveratrol	.97	.61		
	5	Placebo	-.92	.56	**4.56**	**.03***

	Resveratrol	.85	.61		
6	Placebo	-.82	.56	**4.84**	**.03***
	Resveratrol	1.02	.61		
7	Placebo	-.85	.56	**4.69**	**.03***
	Resveratrol	.95	.61		
8	Placebo	-.89	.56	**4.46**	**.03***
	Resveratrol	.87	.61		
9	Placebo	-1.01	.56	**4.61**	**.03***
	Resveratrol	.77	.61		
10	Placebo	-.92	.56	**4.32**	**.03***
	Resveratrol	.81	.61		
11	Placebo	-.93	.56	2.46	.11
	Resveratrol	.39	.63		
12	Placebo	-.90	.56	**4.59**	**.03***
	Resveratrol	.88	.61		
13	Placebo	-1.03	.56	2.27	.13
	Resveratrol	.24	.63		
14	Placebo	-.96	.56	**4.57**	**.03***
	Resveratrol	.82	.61		

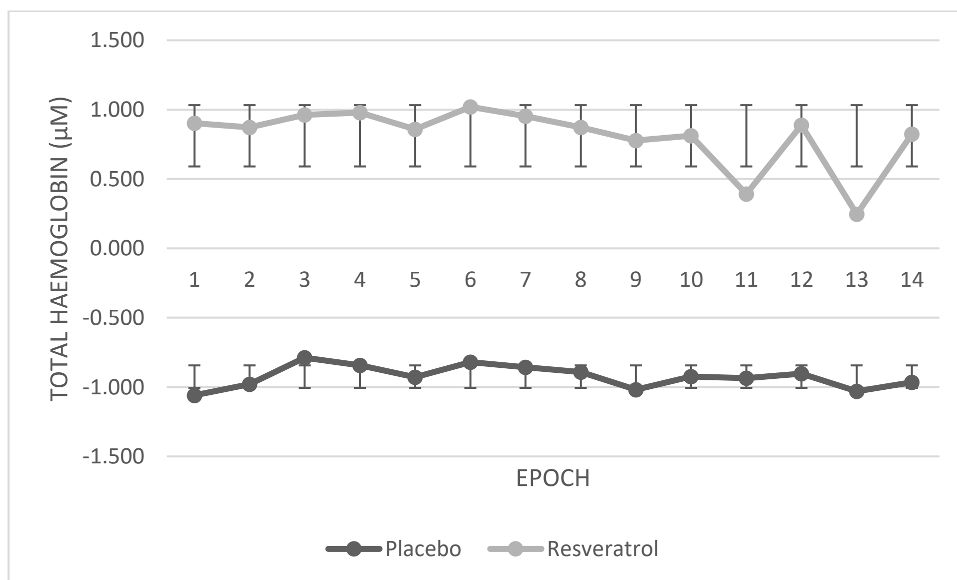

Figure 4.7. QNIRS Total Haemoglobin Concentration Day 84. Post hoc comparisons of effect of treatment on total haemoglobin concentration (change from baseline score) on Day 84. Presented by treatment group over 14 epochs during post-dose assessment. Epochs 1-10, 12, 14 significant to $p = <.05$.

4.3.2.1.3. Oxygenated Haemoglobin (HbO)

A significant main effect of treatment was observed, [$F(1, 1367) = 24.54$, $p < .001$], where HbO was increased following resveratrol (mean change from baseline .23) in comparison with placebo (mean change from baseline -.44) as indicated in Table 4.6. and Figure 4.7. A significant interaction effect between treatment and day was identified [$F(1, 1367) = 25.96$, $p = < .001$]. Post hoc planned comparisons indicated that this effect was limited to Day 84 ($p = .001$) where oxygenated haemoglobin was higher following resveratrol (mean change from baseline .44) in comparison to placebo (mean change from baseline .03). Sidak corrected planned comparisons indicated that within Day 84, the effect was significant at epochs 1, 2 and 13 (rest and during RVIP rep 3, $p = < .05$); and trending towards significant during epochs 4, 7, 10, 11 and 14 (serial 7s rep 1, serial 3s rep 2 and 3, RVIP rep 2 and 3, $p = .06$), as shown in Table 4.7 and Figure 4.8.

Table 4.6. NIRS Oxygenated Haemoglobin concentration by treatment group during post-dose assessment. Estimated marginal means and standard error (SE) are presented with F and *p* values of the main effects and interactions from the three-way ANOVA analysis.

			Post dose		Main Effects		
		n	Mean	SE		F	*p*
HbO	Placebo	55	-0.44	.09	**Treatment**	**24.54**	**<.001***
	Resveratrol		0.23	.10	**Treatment*Day**	**25.96**	**<.001***
					Treatment*Epoch	.05	1.00
					Treatment*Day*Epoch	.05	1.00

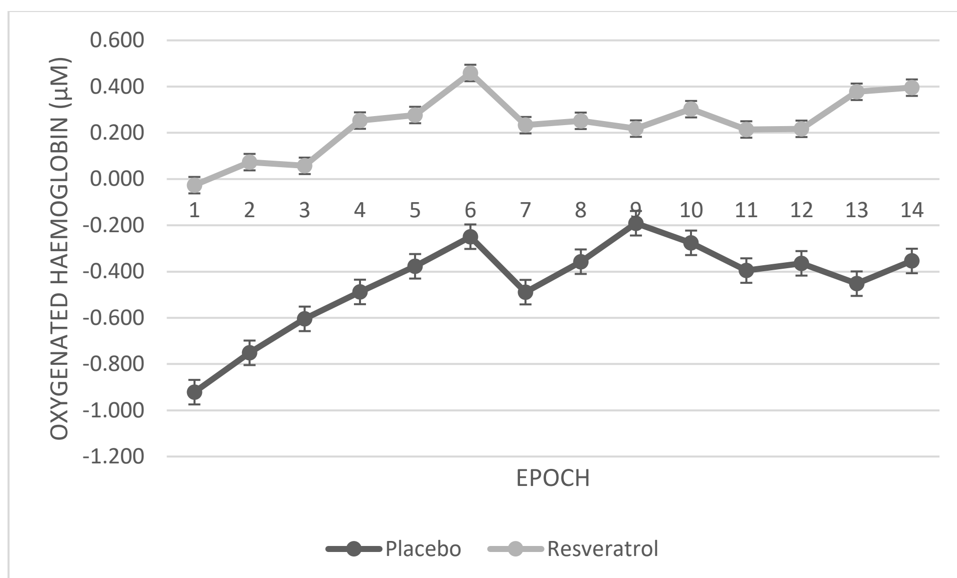

Figure 4.8. QNIRS Oxygenated Haemoglobin Concentration on day 84. Main effect of treatment on oxygenated haemoglobin concentration on day 84. Data presented is change from baseline data by treatment group over 14 epochs during post-dose assessment, p = <.001).

Table 4.7. NIRS Oxygenated Haemoglobin concentration planned comparisons by day, epoch and treatment group during post-dose assessment. Estimated marginal means and standard error (SE) of change from baseline scores are presented with F and p value from the sidak corrected planned comparisons.

					Planned comparisons	
Day	Epoch		Mean	SE	F	p
1	1	Placebo	-.34	.48	.003	.95
		Resveratrol	-.30	.49		
	2	Placebo	-.20	.48	.002	.96
		Resveratrol	-.17	.49		
	3	Placebo	-.09	.48	.000	.99
		Resveratrol	-.09	.49		
	4	Placebo	.04	.48	.01	.90
		Resveratrol	.12	.49		
	5	Placebo	-.07	.48	.10	.74
		Resveratrol	.15	.49		
	6	Placebo	.05	.48	.03	.84
		Resveratrol	.18	.49		
	7	Placebo	-.01	.48	.000	.99
		Resveratrol	-.01	.49		
	8	Placebo	.16	.48	.01	.90
		Resveratrol	.07	.49		
	9	Placebo	.25	.48	.20	.65
		Resveratrol	-.06	.49		

	10	Placebo	.31	.48	.16	.68
		Resveratrol	.03	.49		
	11	Placebo	.18	.48	.12	.72
		Resveratrol	-.06	.49		
	12	Placebo	.15	.48	.000	.99
		Resveratrol	.15	.49		
	13	Placebo	.11	.48	.01	.90
		Resveratrol	.19	.49		
	14	Placebo	.18	.48	.004	.94
		Resveratrol	.23	.49		
84	1	Placebo	-1.49	.50	**5.31**	**.02***
		Resveratrol	.25	.56		
	2	Placebo	-1.29	.50	**4.54**	**.03***
		Resveratrol	.32	.56		
	3	Placebo	-1.11	.50	3.01	.08
		Resveratrol	.20	.56		
	4	Placebo	-1.02	.50	**1.19**	**.06^{T}**
		Resveratrol	.37	.56		
	5	Placebo	-.68	.51	1.99	.15
		Resveratrol	.40	.56		
	6	Placebo	-.55	.51	2.80	.09
		Resveratrol	.73	.56		
	7	Placebo	-.96	.50	**3.58**	**.06^{T}**
		Resveratrol	.47	.56		
	8	Placebo	-.87	.50	2.93	.08
		Resveratrol	.42	.56		
	9	Placebo	-.63	.51	2.19	.13
		Resveratrol	.49	.56		
	10	Placebo	-.86	.50	**3.57**	**.06^{T}**
		Resveratrol	.56	.56		
	11	Placebo	-.97	.50	**3.73**	**.05^{T}**
		Resveratrol	.49	.56		
	12	Placebo	-.88	.50	2.24	.13
		Resveratrol	.28	.59		
	13	Placebo	-1.01	.50	**4.31**	**.03***
		Resveratrol	.56	.56		
	14	Placebo	-.89	.50	**3.67**	**.05^{T}**
		Resveratrol	.55	.56		

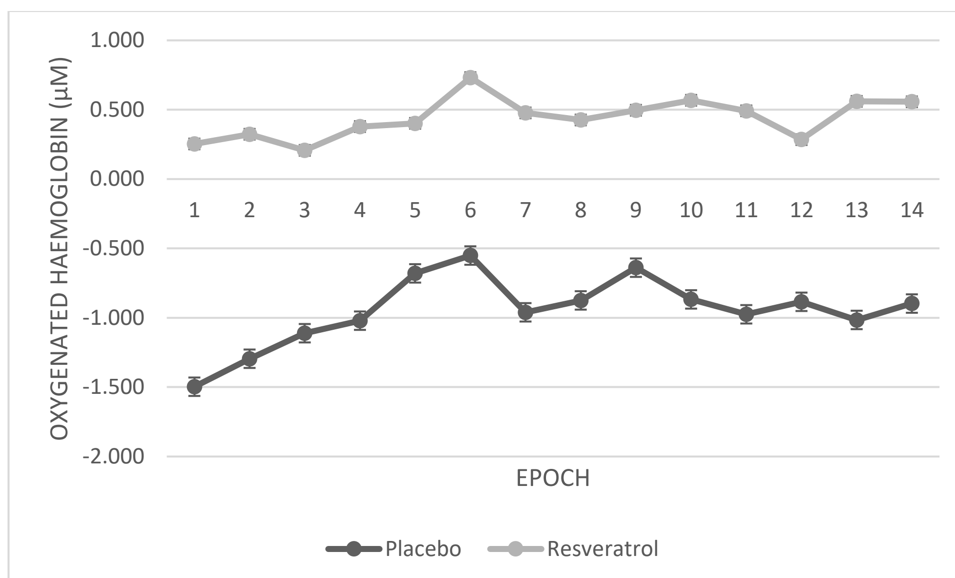

Figure 4.9. QNIRS Oxygenated Haemoglobin Concentration Day 84. Post hoc comparisons of effect of treatment on oxygenated haemoglobin concentration (change from baseline score) on Day 84. Presented by treatment group over 14 epochs during post-dose assessment. Epochs 1, 2 and 14 significant to p = <.05; epochs 4, 7, 10, 11 and 14 trending towards significance p = .06.

4.3.2.1.4. Deoxygenated Haemoglobin (Hb)

A significant main effect of treatment was observed, [F (1, 1349) = 19.48, p <.001], where Hb was increased following resveratrol (mean change from baseline .34) in comparison with placebo (mean change from baseline .07), as indicated in Table 4.8. and Figure 4.9. However, planned comparisons indicated no significant difference between treatment groups for each individual epoch within Day 1 and 84 (p = >.005), as shown in Table 4.9.

Table 4.8. NIRS Deoxygenated Haemoglobin concentration by treatment group during post-dose assessment. Estimated marginal means and standard error (SE) are presented with F and p values of the main effects and interactions from the three-way ANOVA analysis.

			Post dose		Main Effects		
		n	Mean	SE		F	*p*
Hb	Placebo	55	.07	.04	**Treatment**	**19.48**	**<.001***
					Treatment*Day	2.19	.13
	Resveratrol		.34	.04	Treatment*Epoch	.18	.99
					Treatment*Day*Epoch	.02	1.00

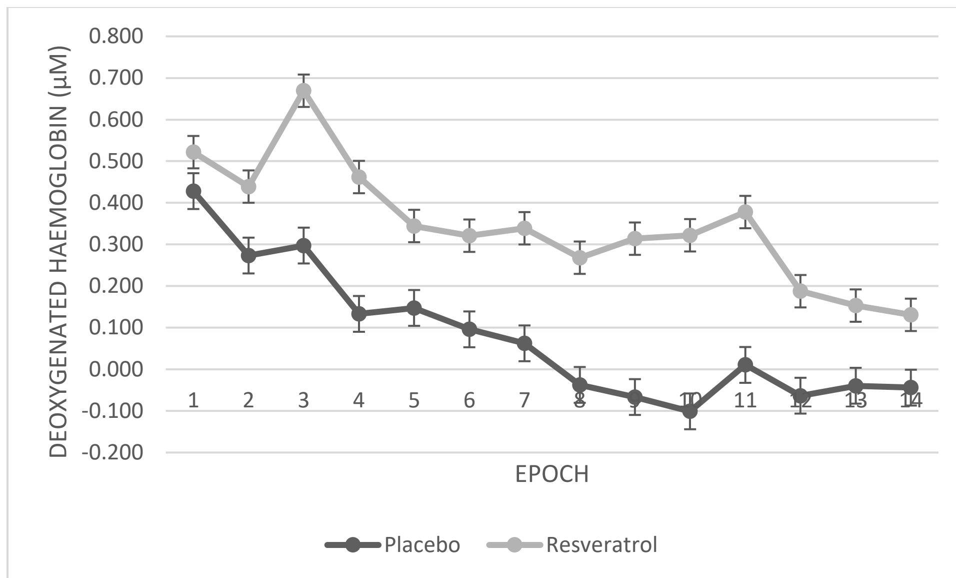

Figure 4.10. QNIRS Deoxygenated Haemoglobin Concentration on day 84. Main effect of treatment on total haemoglobin concentration on day 84. Presented by treatment group over 14 epochs during post-dose assessment, $p = <.001$.

Table 4.9. NIRS Deoxygenated Haemoglobin concentration planned comparisons by day, epoch and treatment group during post-dose assessment. Estimated marginal means and standard error (SE) of change from baseline scores are presented with F and *p* value from the sidak corrected planned comparisons.

					Planned comparisons	
Day	Epoch		Mean	SE	*F*	*p*
1	1	Placebo	.43	.21	.02	.88
		Resveratrol	.48	.21		
	2	Placebo	.28	.21	.14	.70
		Resveratrol	.40	.21		
	3	Placebo	.28	.21	.72	.39
		Resveratrol	.54	.21		
	4	Placebo	.07	.21	.71	.39
		Resveratrol	.33	.22		
	5	Placebo	.11	.21	.09	.75
		Resveratrol	.20	.21		
	6	Placebo	.03	.21	.06	.80
		Resveratrol	.11	.21		
	7	Placebo	-.00	.21	.55	.45
		Resveratrol	.22	.21		
	8	Placebo	-.09	.21	.49	.48
		Resveratrol	.12	.21		
	9	Placebo	-.18	.21	.86	.35
		Resveratrol	.09	.21		

	10	Placebo	-.16	.21	1.04	.30
		Resveratrol	.14	.21		
	11	Placebo	-.03	.21	.48	.48
		Resveratrol	.18	.21		
	12	Placebo	-.13	.21	.37	.54
		Resveratrol	.05	.21		
	13	Placebo	-.08	.21	.17	.67
		Resveratrol	.04	.21		
	14	Placebo	-.04	.21	.03	.85
		Resveratrol	.00	.21		
84	1	Placebo	.42	.22	.18	.66
		Resveratrol	.56	.24		
	2	Placebo	.26	.22	.40	.52
		Resveratrol	.47	.24		
	3	Placebo	.31	.22	2.01	.15
		Resveratrol	.79	.25		
	4	Placebo	.19	.22	1.42	.23
		Resveratrol	.59	.24		
	5	Placebo	.18	.22	.80	.36
		Resveratrol	.48	.24		
	6	Placebo	.15	.22	1.24	.26
		Resveratrol	.52	.24		
	7	Placebo	.12	.22	.95	.33
		Resveratrol	.45	.24		
	8	Placebo	.01	.22	1.41	.23
		Resveratrol	.41	.24		
	9	Placebo	.05	.22	2.00	.15
		Resveratrol	.53	.24		
	10	Placebo	-.03	.22	2.51	.11
		Resveratrol	.49	.24		
	11	Placebo	.05	.22	2.40	.12
		Resveratrol	.57	.24		
	12	Placebo	.00	.22	.89	.34
		Resveratrol	.32	.24		
	13	Placebo	.00	.22	.59	.43
		Resveratrol	.25	.24		
	14	Placebo	-.04	.22	.79	.37
		Resveratrol	.25	.24		

4.3.2.2. NIRS Cognitive Task Performance

4.3.2.2.1. Serial 3 subtractions

A significant effect of treatment was observed on total subtractions on Day 84, repetition 2, [F (1, 44) = 7.82, p = .008, d = .84], where participants performed more subtractions following placebo (42.50), in comparison to resveratrol (33.90). The same effect was also observed on Day 84, repetition 3, [F (1, 45) = 4.81, p = .03, d = .65], where participants performed more subtractions following placebo (41.81), in comparison to resveratrol (34.05).

A significant effect of treatment was observed on number of correct subtractions on Day 84, repetition 2, [F (1, 44) = 8.91, p = .005, d = .89], where participants performed more correct subtractions following placebo (40.58), in comparison to resveratrol (31.45). The same effect was also observed on Day 84, repetition 3, [F (1, 45) = 5.34, p = .02, d = .68], where participants performed more correct subtractions following placebo (39.85), in comparison to resveratrol (31.57).

No effects observed on Day 1 or on any other outcome at Day 84, as shown in Tables 4.10 and 4.11.

Table 4.10. Serial threes subtraction on Day 1 during NIRS assessment. Comparisons by treatment group. Serial subtraction of threes task during NIRS post-dose assessment on Day 1. Estimated marginal means and standard deviations (SD) are presented with F and *p* values.

		Post-dose			Main Effects		
		n	Mean	SD	df	F	p
			Rep 1				
SS3 Total	Placebo	28	38.86	11.94	1	2.52	.11
	Resveratrol	27	33.74	11.90			
SS3 Correct	Placebo	28	36.96	12.09	1	2.62	.11
	Resveratrol	27	31.59	12.49			
SS3 Errors	Placebo	28	1.89	1.83	1	.21	.64
	Resveratrol	27	2.15	2.28			
			Rep 2				
SS3 Total	Placebo	28	39.36	13.70	1	1.44	.23
	Resveratrol	27	35.00	13.17			
SS3 Correct	Placebo	28	37.14	13.49	1	1.16	.28
	Resveratrol	27	33.19	13.75			
SS3 Errors	Placebo	28	2.21	2.06	1	.62	.43
	Resveratrol	27	1.81	1.64			
			Rep 3				
SS3 Total	Placebo	28	38.29	11.50	1	1.02	.31
	Resveratrol	27	35.04	12.29			
SS3 Correct	Placebo	28	36.21	11.72	1	1.18	.28
	Resveratrol	27	32.63	12.66			
SS3 Errors	Placebo	28	2.07	2.43	1	.23	.62
	Resveratrol	27	2.41	2.67			

Table 4.11. Serial threes subtraction on Day 84 during NIRS assessment. Comparisons by treatment group. Serial subtraction of threes task during NIRS post-dose assessment on Day 84. Estimated marginal means and standard deviations (SD) are presented with F and *p* values.

		Post-dose			Main Effects		
		n	Mean	SD	df	F	p
			Rep 1				
SS3	Placebo	26	40.42	13.08	1	.80	.37
Total	Resveratrol	21	36.95	13.34			
SS3	Placebo	26	38.46	13.28	1	.62	.43
Correct	Resveratrol	21	35.33	13.78			
SS3	Placebo	26	1.96	1.75	1	.45	.50
Errors	Resveratrol	21	1.62	1.68			
			Rep 2				
SS3	**Placebo**	**26**	**42.50**	**11.49**	**1**	**7.82**	**.008***
Total	**Resveratrol**	**20**	**33.90**	**8.58**			
SS3	**Placebo**	**26**	**40.58**	**11.22**	**1**	**8.91**	**.005***
Correct	**Resveratrol**	**20**	**31.45**	**8.87**			
SS3	Placebo	26	1.92	2.29	1	.64	.42
Errors	Resveratrol	20	2.45	2.08			
			Rep 3				
SS3	**Placebo**	**26**	**41.81**	**12.28**	**1**	**4.81**	**.03***
Total	**Resveratrol**	**21**	**34.05**	**11.83**			
SS3	**Placebo**	**26**	**39.85**	**12.27**	**1**	**5.34**	**.02***
Correct	**Resveratrol**	**21**	**31.57**	**12.10**			
SS3	Placebo	26	1.96	2.42	1	.67	.41
Errors	Resveratrol	21	2.48	1.69			

4.3.2.2.2. Serial 7 subtractions

No significant effects were observed on any outcome at any time period, as shown in Tables 4.12 and 4.13.

Table 4.12. Serial sevens subtractions on Day 1 during NIRS assessment. Comparisons by treatment group. Serial subtraction of sevens task during NIRS post-dose assessment on Day 1. Estimated marginal means and standard deviations (SD) are presented with F and *p* values.

		Post-dose			Main Effects		
		n	Mean	SD	df	F	p
			Rep 1				
SS7	Placebo	28	26.68	10.12	1	1.57	.21
Total	Resveratrol	27	23.26	10.04			
SS7	Placebo	28	23.93	10.00	1	1.88	.17
Correct	Resveratrol	27	20.11	10.64			
SS7	Placebo	28	2.75	2.15	1	.33	.56
Errors	Resveratrol	27	3.15	2.91			
			Rep 2				
SS7	Placebo	28	26.11	10.67	1	1.34	.25
Total	Resveratrol	27	22.85	10.16			
SS7	Placebo	28	23.00	10.92	1	1.18	.28
Correct	Resveratrol	27	19.81	10.78			
	Placebo	28	3.11	1.83	1	.01	.90

SS7 Errors	Resveratrol	27	3.04	2.37			
			Rep 3				
SS7 Total	Placebo	28	26.25	11.01	1	2.05	.15
	Resveratrol	27	22.22	9.74			
SS7 Correct	Placebo	28	23.43	10.91	1	2.12	.15
	Resveratrol	27	19.30	10.06			
SS7 Errors	Placebo	28	2.82	2.59	1	.02	.87
	Resveratrol	27	2.93	2.16			

Table 4.13. Serial sevens subtractions on Day 84 during NIRS assessment. Comparisons by treatment group. Serial subtraction of sevens task during NIRS post-dose assessment on Day 84. Estimated marginal means and standard deviations (SD) are presented with F and *p* values.

		Post-dose			Main Effects		
		n	Mean	SD	df	F	p
			Rep 1				
SS7 Total	Placebo	25	27.08	9.47	1	2.94	.09
	Resveratrol	21	22.52	8.31			
SS7 Correct	Placebo	25	24.84	9.91	1	3.12	.08
	Resveratrol	21	20.05	8.17			
SS7 Errors	Placebo	25	2.24	1.83	1	.13	.71
	Resveratrol	21	2.48	2.52			
			Rep 2				
SS7 Total	Placebo	26	27.69	10.32	1	1.41	.24
	Resveratrol	22	24.41	8.45			
SS7 Correct	Placebo	26	25.77	10.55	1	2.36	.13
	Resveratrol	22	21.41	8.79			
SS7 Errors	Placebo	26	1.92	1.78	1	2.67	.10
	Resveratrol	22	3.00	2.74			
			Rep 3				
SS7 Total	Placebo	26	27.58	9.28	1	2.21	.14
	Resveratrol	22	23.59	9.20			
SS7 Correct	Placebo	26	24.69	9.54	1	2.73	.10
	Resveratrol	22	20.27	8.83			
SS7 Errors	Placebo	26	2.88	2.38	1	.45	.50
	Resveratrol	22	3.32	2.00			

4.3.2.2.3. Rapid Visual Information Processing

A significant acute effect of treatment was observed for RVIP accuracy on Day 1, repetition 1, [F (1, 50) = 6.71, p = .01, d = .73], where participants performed better following placebo (66.82) in comparison with resveratrol (50.00). The same effect was observed on Day 1, repetition 2, [F (1, 50) = 4.31, p = .04, d = .58] where participants performed better following placebo (62.31) in comparison with resveratrol (49.60). And at Day 1 repetition 3, [F (1, 52) = 4.91, p = .03, d = .61], where participants performed better following placebo (59.90) in comparison with resveratrol (45.83).

A significant acute effect of treatment was observed for RVIP accuracy on Day 84, repetition 2, [F (1, 43) = 5.04, p = .03, d = .68], where participants performed better following placebo

(65.62) in comparison with resveratrol (50.23). The same effect was observed on Day 84, repetition 3, [F (1, 43) = 5.30, p = .02, d = .70] where participants performed better following placebo (63.26) in comparison with resveratrol (47.18).

A significant acute effect of treatment was observed for RVIP false alarms on Day 1, repetition 1, [F (1, 50) = 9.32, p = .004, d = .86], where participants had less false alarms following placebo (1.08), in comparison with resveratrol (4.08). The same effect was observed on Day 1, repetition 3, [F (1, 52) = 6.48, p = .01, d = .70], where participants had less false alarms following placebo (1.78), in comparison with resveratrol (5.00).

A significant acute effect of treatment was observed for RVIP false alarms on Day 84, repetition 2, [F (1, 43) = 5.62, p = .02, d = .72], where participants had less false alarms following placebo (1.46), in comparison with resveratrol (4.24). The same effect was observed on Day 84, repetition 3, [F (1, 43) = 17.77, p<.001, d = 1.28], where participants had less false alarms following placebo (1.30), in comparison with resveratrol (5.82).

Table 4.14. RVIP performance on Day 1 during NIRS assessment. Comparisons by treatment group. Rapid visual information processing task during NIRS post-dose assessment on Day 1. Estimated marginal means and standard deviations (SD) are presented with F and p values.

		Post-dose			Main Effects	
		n	Mean	SD	F	p
		Rep 1				
RVIP Accuracy	**Placebo**	**26**	**66.82**	**23.59**	**6.71**	**.01***
	Resveratrol	**26**	**50.00**	**23.21**		
RVIP Correct RT	Placebo	26	505.01	53.16	.24	.62
	Resveratrol	26	497.47	56.56		
RVIP False alarms	**Placebo**	**26**	**1.08**	**1.69**	**9.32**	**.004***
	Resveratrol	**26**	**4.08**	**4.69**		
		Rep 2				
RVIP Accuracy	**Placebo**	**27**	**62.31**	**23.13**	**4.31**	**.04***
	Resveratrol	**25**	**49.60**	**20.85**		
RVIP Correct RT	Placebo	27	503.29	56.20	.00	.95
	Resveratrol	25	504.26	59.01		
RVIP False alarms	Placebo	27	1.89	2.19	.95	.33
	Resveratrol	25	2.68	3.52		
		Rep 3				
RVIP Accuracy	**Placebo**	**27**	**59.90**	**24.79**	**4.91**	**.03***
	Resveratrol	**27**	**45.83**	**21.73**		
RVIP Correct RT	Placebo	27	512.83	57.05	.23	.63
	Resveratrol	27	505.31	57.08		
RVIP False alarms	**Placebo**	**27**	**1.78**	**2.19**	**6.48**	**.01***
	Resveratrol	**27**	**5.00**	**6.20**		

Table 4.15. RVIP performance on Day 84 during NIRS assessment. Comparisons by treatment group. Rapid visual information processing task during NIRS post-dose assessment on Day 84. Estimated marginal means and standard deviations (SD) are presented with F and *p* values.

		Post-dose			Main Effects	
		n	Mean	SD	F	p
		Rep 1				
RVIP Accuracy	Placebo	25	67.80	23.16	2.86	.09
	Resveratrol	21	56.78	20.51		
RVIP Correct RT	Placebo	25	492.06	50.45	.12	.72
	Resveratrol	21	498.40	69.25		
RVIP False alarms	Placebo	25	1.92	2.79	1.84	.18
	Resveratrol	21	3.43	4.65		
		Rep 2				
RVIP Accuracy	**Placebo**	**24**	**65.62**	**23.62**	**5.04**	**.03***
	Resveratrol	**21**	**50.23**	**22.07**		
RVIP Correct RT	Placebo	24	495.97	47.85	.13	.71
	Resveratrol	21	501.19	45.42		
RVIP False alarms	**Placebo**	**24**	**1.46**	**1.93**	**5.69**	**.02***
	Resveratrol	**21**	**4.24**	**5.32**		
		Rep 3				
RVIP Accuracy	**Placebo**	**23**	**63.26**	**22.74**	**5.30**	**.02***
	Resveratrol	**22**	**47.18**	**24.08**		
RVIP Correct RT	Placebo	23	509.24	48.21	.08	.77
	Resveratrol	22	504.99	49.14		
RVIP False alarms	**Placebo**	**23**	**1.30**	**1.22**	**17.77**	**<.001***
	Resveratrol	**22**	**5.82**	**4.98**		

4.3.2.2.4. VAS

No significant effect was observed at any time period, as presented in Tables 4.16 and 4.17.

Table 4.16. VAS response on Day 1 during NIRS assessment. Comparisons by treatment group. VAS responses during NIRS post-dose assessment on Day 1. Estimated marginal means and standard deviations (SD) are presented with F and *p* values.

		Post-dose			Main Effects	
		n	Mean	SD	F	p
		Rep 1				
Task Difficulty	Placebo	27	69.00	15.72	1.56	.21
	Resveratrol	27	74.15	14.48		
Mental Fatigue	Placebo	27	71.07	15.92	.37	.54
	Resveratrol	27	73.56	13.61		
		Rep 2				
Task Difficulty	Placebo	26	79.92	10.42	.11	.74
	Resveratrol	27	78.81	13.65		
Mental Fatigue	Placebo	26	82.65	8.10	1.70	.19
	Resveratrol	27	78.67	13.36		
		Rep 3				
Task Difficulty	Placebo	27	79.85	14.53	.03	.85
	Resveratrol	27	80.59	14.07		
Mental Fatigue	Placebo	27	83.85	9.17	.45	.50
	Resveratrol	27	81.70	13.78		

Table 4.17. VAS response on Day 84 during NIRS assessment. Comparisons by treatment group. VAS responses during NIRS post-dose assessment on Day 84. Estimated marginal means and standard deviations (SD) are presented with F and *p* values.

		Post-dose			Main Effects	
		n	Mean	SD	F	p
		Rep 1				
Task Difficulty	Placebo	26	68.50	18.94	.14	.70
	Resveratrol	22	70.45	15.85		
Mental Fatigue	Placebo	26	68.15	17.15	.72	.39
	Resveratrol	22	72.18	15.18		
		Rep 2				
Task Difficulty	Placebo	26	72.54	17.47	1.94	.17
	Resveratrol	22	79.09	14.63		
Mental Fatigue	Placebo	26	73.77	16.42	1.89	.17
	Resveratrol	22	80.32	16.38		
		Rep 3				
Task Difficulty	Placebo	26	75.50	18.02	1.83	.18
	Resveratrol	22	82.00	14.64		
Mental Fatigue	Placebo	26	76.58	19.05	1.20	.27
	Resveratrol	22	82.23	16.05		

4.3.2.3. Cognitive Assessments

4.3.2.3.1. Numeric Working Memory

Analysis of the data indicated a significant effect of treatment on Day 84 assessment 3, for overall reaction time, [F (1, 72) = 4.82, p = .031, d = .52], where participants performed quicker following placebo (801.42 msecs) when compared with resveratrol (855.70 msecs). The same effect was observed as a trend towards a significant effect during Day 84 assessment 2, [F (1, 72) = 3.60, p = .062, d = .45], where participants performed quicker following placebo (806.84 msecs) when compared with resveratrol (853.71 msecs).

Likewise, during Day 84, assessment 3, a significant effect of treatment was observed for Correct reaction time, [F (1, 72) = 4.62, p = .035, d = .51], where participants performed quicker following placebo (798.18 msecs) when compared with resveratrol (849.42 msecs). And during Day 84, assessment 3, a significant effect of treatment was observed for 'Yes' reaction time, [F (1, 72) = 6.29, p = .014, d = .60], where participants performed quicker following placebo (745.21 msecs) when compared with resveratrol (802.86 msecs).

Analysis of the pure chronic effects on Day 84 indicated a significant effect of treatment for Overall accuracy at Day 84 baseline assessment, [F (1, 69) = 9.55, p = .003, d = .75], where participants performed better following resveratrol (97.22) compared with placebo (94.38). Additionally, the same treatment effect was observed for 'Yes' accuracy, [F (1, 69) = 6.92, p =

.01, $d = .63$] and 'No' accuracy [$F(1, 69) = 7.38$, $p = .008$, $d = .65$], with resveratrol performing more accurately on all outcomes.

No additional significant effects were observed for any task outcome, as presented in Tables 4.18, 4.19 and 4.20.

Table 4.18. Numeric Working Memory performance on Day 1. Comparisons by treatment group. Numeric working memory performance on Day 1. Baseline and post-dose estimated marginal means and standard errors (SE) are presented with F and *p* values, for Assessments 2 and 3, for all task outcome measures, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
% Correct	Assessment 2	Placebo	42	94.44	.73	95.66	.55	.00	.94
		Resveratrol	50	95.38	.64	95.61	.51		
	Assessment 3	Placebo	42	94.44	.73	95.90	.63	.11	.74
		Resveratrol	50	95.38	.64	95.62	.57		
Overall RT	Assessment 2	Placebo	42	920.76	35.49	871.62	22.85	.21	.64
		Resveratrol	50	938.77	29.49	886.16	20.94		
	Assessment 3	Placebo	42	920.76	35.49	847.12	23.11	1.65	.20
		Resveratrol	50	938.77	29.49	827.50	21.18		
Correct RT	Assessment 2	Placebo	42	915.88	35.50	862.00	22.21	.47	.49
		Resveratrol	50	928.20	28.85	882.69	20.35		
	Assessment 3	Placebo	42	915.88	35.50	840.87	22.62	1.66	.20
		Resveratrol	50	928.20	28.85	880.45	20.73		
Correct Yes %	Assessment 2	Placebo	42	93.45	.81	93.85	.87	.10	.75
		Resveratrol	50	94.50	.76	93.47	.80		
	Assessment 3	Placebo	42	93.45	.81	94.60	.83	.06	.79
		Resveratrol	50	94.50	.76	94.89	.76		
Correct No %	Assessment 2	Placebo	42	95.43	.84	97.51	.46	.11	.73
		Resveratrol	50	96.26	.80	97.73	.42		
	Assessment 3	Placebo	42	95.43	.84	97.20	.64	.95	.33
		Resveratrol	50	96.26	.80	96.35	.58		
Yes RT	Assessment 2	Placebo	42	866.85	32.08	817.24	23.00	.08	.77
		Resveratrol	50	871.89	27.57	826.31	21.08		
	Assessment 3	Placebo	42	866.85	32.08	796.25	21.63	1.37	.24
		Resveratrol	50	871.89	27.57	830.66	19.82		
No RT	Assessment 2	Placebo	42	974.66	41.37	923.26	28.32	.42	.51
		Resveratrol	50	1005.66	33.61	948.31	25.94		
	Assessment 3	Placebo	42	974.66	41.37	896.46	29.38	1.51	.22
		Resveratrol	50	1005.66	33.61	945.60	26.92		

Table 4.19. Numeric Working Memory performance on Day 84. Comparisons by treatment group. Numeric working memory performance on Day 84. Baseline and post-dose estimated marginal means and standard errors (SE) are presented with F and *p* values for Assessments 2 and 3, for all task outcome measures from ANCOVA analysis.

				Baseline		Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
% Correct	Assessment 2	Placebo	38	93.52	1.00	96.64	.60	.22	.63
		Resveratrol	35	96.93	.55	96.21	.63		
	Assessment 3	Placebo	38	93.52	1.00	96.75	.43	1.15	.28
		Resveratrol	35	96.93	.55	96.06	.45		
Overall RT	**Assessment 2**	**Placebo**	**38**	**949.97**	**38.71**	**806.84**	**17.06**	**3.60**	**.06[t]**
		Resveratrol	**35**	**914.42**	**37.04**	**853.71**	**17.78**		
	Assessment 3	**Placebo**	**38**	**949.97**	**38.71**	**801.42**	**17.09**	**4.82**	**.03***
		Resveratrol	**35**	**914.42**	**37.04**	**855.70**	**17.81**		
Correct RT	Assessment 2	Placebo	38	939.83	38.15	804.89	15.91	2.37	.12
		Resveratrol	35	907.27	36.70	840.35	16.59		
	Assessment 3	**Placebo**	**38**	**939.83**	**38.15**	**798.18**	**16.46**	**4.62**	**.03***
		Resveratrol	**35**	**907.27**	**36.70**	**849.42**	**17.15**		
Correct Yes %	Assessment 2	Placebo	38	91.83	1.18	95.40	.83	.06	.79
		Resveratrol	35	95.82	.70	95.08	.87		
	Assessment 3	Placebo	38	91.83	1.18	95.53	.59	.05	.81
		Resveratrol	35	95.82	.70	95.32	.61		
Correct No %	Assessment 2	Placebo	38	95.21	.99	97.67	.52	.01	.89
		Resveratrol	35	98.04	.58	97.57	.55		
	Assessment 3	Placebo	38	95.21	.99	97.83	.54	1.15	.28
		Resveratrol	35	98.04	.58	96.96	.57		
Yes RT	Assessment 2	Placebo	38	901.67	39.86	755.22	17.79	2.77	.10
		Resveratrol	35	845.35	31.64	796.67	17.91		
	Assessment 3	**Placebo**	**38**	**901.67**	**39.86**	**745.21**	**15.89**	**6.29**	**.01***
		Resveratrol	**35**	**845.35**	**31.64**	**802.86**	**16.56**		
No RT	Assessment 2	Placebo	38	998.26	40.22	857.42	21.35	3.11	.08
		Resveratrol	35	983.49	46.82	911.89	22.25		
	Assessment 3	Placebo	38	998.26	40.22	856.64	23.95	2.33	.13
		Resveratrol	35	983.49	46.82	909.62	24.96		

Table 4.20. Numeric Working Memory Pure Chronic Analysis. Comparisons by treatment group. Numeric working memory performance pure chronic analysis. Baseline (Day 1 assessment 1) and post-dose (Day 84, all assessments) estimated marginal means and standard errors (SE) are presented with F and *p* values, from ANCOVA analysis.

			Baseline (Day 1 A1)			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
% Correct	**Assessment 1**	**Placebo**	**38**	**94.44**	**.73**	**94.38**	**.63**	**9.55**	**.003***
		Resveratrol	**34**	**95.38**	**.64**	**97.22**	**.66**		
	Assessment 2	Placebo	38	94.44	.73	96.12	.61	.45	.50
		Resveratrol	34	95.38	.64	96.72	.64		
	Assessment 3	Placebo	38	94.44	.73	96.38	.41	.03	.85
		Resveratrol	34	95.38	.64	96.49	.44		
Overall RT	Assessment 1	Placebo	38	920.76	35.49	900.14	21.04	.57	.44
		Resveratrol	34	938.77	29.49	876.66	22.26		
	Assessment 2	Placebo	38	920.76	35.49	817.90	20.08	.87	.35
		Resveratrol	34	938.77	29.49	845.50	21.25		
	Assessment 3	Placebo	38	920.76	35.49	813.35	20.35	1.20	.27
		Resveratrol	34	938.77	29.49	846.15	21.53		
Correct RT	Assessment 1	Placebo	38	915.88	35.50	886.05	20.50	.23	.62
		Resveratrol	34	928.20	28.85	871.37	21.69		
	Assessment 2	Placebo	38	915.88	35.50	812.37	19.32	.68	.41
		Resveratrol	34	928.20	28.85	835.77	20.44		
	Assessment 3	Placebo	38	915.88	35.50	806.13	20.26	1.64	.20
		Resveratrol	34	928.20	28.85	844.15	21.44		
Correct Yes %	**Assessment 1**	**Placebo**	**38**	**93.45**	**.81**	**92.50**	**.93**	**6.92**	**.01***
		Resveratrol	**34**	**94.50**	**.76**	**96.09**	**.99**		
	Assessment 2	Placebo	38	93.45	.81	94.79	.83	.54	.46
		Resveratrol	34	94.50	.76	95.69	.88		
	Assessment 3	Placebo	38	93.45	.81	95.11	.60	.49	.48
		Resveratrol	34	94.50	.76	95.72	.63		
Correct No %	**Assessment 1**	**Placebo**	**38**	**95.43**	**.84**	**96.25**	**.53**	**7.38**	**.008***
		Resveratrol	**34**	**96.26**	**.80**	**98.37**	**.56**		
	Assessment 2	Placebo	38	95.43	.84	97.35	.54	.41	.51
		Resveratrol	34	96.26	.80	97.86	.57		
	Assessment 3	Placebo	38	95.43	.84	97.64	.51	.24	.62
		Resveratrol	34	96.26	.80	97.27	.54		
Yes RT	Assessment 1	Placebo	38	866.85	32.08	839.49	19.37	.67	.41
		Resveratrol	34	871.89	27.57	816.08	20.50		

	Assessment 2	Placebo	38	866.85	32.08	765.77	19.35	.69	.40
		Resveratrol	34	871.89	27.57	789.51	20.47		
	Assessment 3	Placebo	38	866.85	32.08	755.88	19.82	1.71	.19
		Resveratrol	34	871.89	27.57	793.99	20.97		
No RT	Assessment 1	Placebo	38	974.66	41.37	957.81	27.88	.17	.67
		Resveratrol	34	1005.66	33.61	940.57	29.50		
	Assessment 2	Placebo	38	974.66	41.37	867.74	24.37	1.03	.31
		Resveratrol	34	1005.66	33.61	904.07	25.78		
	Assessment 3	Placebo	38	974.66	41.37	868.55	25.75	.73	.39
		Resveratrol	34	1005.66	33.61	900.85	27.24		

4.3.2.3.2. Choice Reaction Time

Analysis indicated a trend towards a significant pure chronic effect of treatment during the Day 84 assessment 2, [F (1, 82) = 3.80, p = .055, d = .42], with participants performing more accurately following placebo (98.61), in comparison to resveratrol (97.86).

No additional significant or interaction effects were observed for this task, as presented in Table 4.21.

Table 4.21. Choice Reaction Time. Comparisons by treatment group. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Day 1						
% Correct	Assessment 2	Placebo	54	98.04	.29	98.56	.21	.04	.83
		Resveratrol	51	98.56	.25	98.50	.21		
	Assessment 3	Placebo	54	98.04	.29	98.20	.23	.01	.88
		Resveratrol	51	98.56	.25	98.25	.24		
Overall RT	Assessment 2	Placebo	54	483.17	10.25	485.20	7.02	.00	.96
		Resveratrol	51	475.66	10.16	485.68	7.22		
	Assessment 3	Placebo	54	483.17	10.25	475.82	6.33	2.05	.15
		Resveratrol	51	475.66	10.16	488.86	6.51		
	Assessment 2	Placebo	54	484.98	10.28	486.48	7.06	.00	.94

Correct RT		Resveratrol	51	476.79	10.11	487.23	7.27		
	Assessment 3	Placebo	54	484.98	10.28	477.29	6.38	2.05	.15
		Resveratrol	51	476.79	10.11	490.45	6.56		
				Day 84					
% Correct	Assessment 2	Placebo	47	98.63	.28	98.52	.27	1.77	.18
		Resveratrol	38	98.44	.29	97.98	.30		
	Assessment 3	Placebo	47	98.63	.28	98.25	.30	.79	.37
		Resveratrol	38	98.44	.29	97.84	.34		
Overall RT	Assessment 2	Placebo	47	484.07	10.76	490.98	6.55	.29	.58
		Resveratrol	38	498.98	14.41	496.34	7.29		
	Assessment 3	Placebo	47	484.07	10.76	486.06	7.06	1.72	.19
		Resveratrol	38	498.98	14.41	499.96	7.85		
Correct RT	Assessment 2	Placebo	47	485.63	10.75	491.93	6.65	.44	.50
		Resveratrol	38	500.53	14.53	498.57	7.39		
	Assessment 3	Placebo	47	485.63	10.75	487.65	7.12	1.82	.18
		Resveratrol	38	500.53	14.53	502.03	7.91		

			Pure Chronic						
			Baseline (Day 1 A1)			Post dose		Main Effects	
			n	Mean	SE	Mean	SE	F	p
% Correct	Assessment 1	Placebo	47	98.04	.29	98.69	.27	.65	.42
		Resveratrol	38	98.56	.25	98.35	.31		
	Assessment 2	**Placebo**	**47**	**98.04**	**.29**	**98.61**	**.25**	**3.80**	**.05[t]**
		Resveratrol	**38**	**98.56**	**.25**	**97.86**	**.28**		
	Assessment 3	Placebo	47	98.04	.29	98.26	.30	.89	.34
		Resveratrol	38	98.56	.25	97.83	.34		
Overall RT	Assessment 1	Placebo	47	483.17	10.25	477.70	8.01	.01	.90
		Resveratrol	38	475.66	10.16	479.11	8.91		
	Assessment 2	Placebo	47	483.17	10.25	489.60	8.51	.43	.51
		Resveratrol	38	475.66	10.16	498.03	9.46		
	Assessment 3	Placebo	47	483.17	10.25	484.75	8.27	1.84	.17
		Resveratrol	38	475.66	10.16	501.58	9.20		
Correct RT	Assessment 1	Placebo	47	484.98	10.28	478.90	7.99	.02	.88
		Resveratrol	38	476.79	10.11	480.69	8.90		
	Assessment 2	Placebo	47	484.98	10.28	490.26	8.58	.64	.42
		Resveratrol	38	476.79	10.11	500.63	9.55		
	Assessment 3	Placebo	47	484.98	10.28	486.16	8.37	1.99	.16
		Resveratrol	38	476.79	10.11	503.88	9.31		

4.3.2.3.3. Corsi Blocks

No acute effects or pure chronic effects were observed, as presented in Table 4.22.

Table 4.22. Corsi Blocks. Comparisons by treatment group. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Day 1						
Corsi block span	Assessment 2	Placebo	54	5.90	.11	5.73	.09	.73	.39
		Resveratrol	54	5.76	.11	5.85	.09		
	Assessment 3	Placebo	54	5.90	.11	5.86	.11	.45	.50
		Resveratrol	54	5.76	.11	5.74	.11		
			Day 84						
Corsi block span	Assessment 2	Placebo	48	5.87	.15	5.99	.10	.02	.87
		Resveratrol	45	5.66	.13	5.97	.10		
	Assessment 3	Placebo	48	5.87	.15	5.74	.12	.00	.94
		Resveratrol	45	5.66	.13	5.73	.13		
			Pure Chronic						
			Baseline (Day 1 A1)			Post dose		Main Effects	
			n	Mean	SE	Mean	SE	F	p
Corsi block span	Assessment 1	Placebo	48	5.90	.11	5.91	.11	1.34	.25
		Resveratrol	45	5.76	.11	5.72	.12		
	Assessment 2	Placebo	48	5.90	.11	5.99	.10	.05	.80
		Resveratrol	45	5.76	.11	5.96	.10		
	Assessment 3	Placebo	48	5.90	.11	5.74	.12	.01	.90
		Resveratrol	45	5.76	.11	5.72	.12		

4.3.2.3.4. Serial 3 subtractions

Analysis of pure chronic data indicated a significant effect of treatment for Total number of subtractions at the Day 84, assessment 3, repetition 2 time-point [F (1, 86) = 4.89, p = .03, d = .48] and repetition 3 [F (1, 87) = 4.63, p = .03, d = .46]; with participants performing more subtractions following placebo (38.20, 37.12) in comparison to resveratrol (34.90, 34.16). In

addition, a trend towards a significant effect was observed during assessment 2, repetition 3, [F (1, 87) = 3.89, p = .052, d = .42]; again with placebo completing more subtractions (39.00) in comparison to resveratrol (36.60). A further trend of a pure chronic effect was observed at the Day 84 baseline assessment during repetition 3, [F (1, 86) = 3.33, p = .07, d = .39]

Analysis of pure chronic effects on correct subtractions indicated that a significant effect of treatment was observed on Day 84, assessment 3, repetition 2, [F (1, 86) = 4.64, p = .03, d = .46], Day 84, assessment 1, repetition 3 [F (1, 87) = 4.55, p = .03, d = .45], Day 84, assessment 2, repetition 3 [F (1, 87) = 6.02, p = .01, d = .53] and Day 84, assessment 3, repetition 3 [F (1, 87) = 7.55, p = .007, d = .59]. In each of these cases, participants who had consumed placebo (36.19, 35.76, 36.92, 35.13, respectively) completed more correct subtractions than resveratrol (32.77, 35.35, 33.50, 30.62, respectively).

In addition, an acute significant effect of treatment on Correct subtractions was observed on Day 1, Assessment 2, during repetition 3, [F (1, 103) = 4.35, p = .03, d = .41], where placebo participants performed more correct responses (34.35) in comparison to resveratrol (31.85).

In terms of pure chronic effects on number of Errors on this task, a significant effect of treatment was observed on the Day 84 baseline assessment, repetition 1, [F (1, 86) = 5.49, p = .02, d = .51], with participants performing less errors following placebo (1.34) in comparison with resveratrol (2.40).

And an acute significant effect of treatment was observed on Day 1, assessment 2, at repetition 3, [F (1, 103) = 5.76, p = .01, d = .47], where participants made fewer errors following placebo (1.66) in comparison with resveratrol (2.69).

No other significant effects were observed, as presented in Tables 4.23, 4.24 and 4.25.

Table 4.23. Serial three subtractions on Day 1. Comparisons by treatment group. Serial three's task performance on Day 1. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values, for Assessments 2 and 3, for all task outcome measures, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Rep 1						
Total	Assessment 2	Placebo	53	33.41	1.89	35.74	.67	.98	.32
		Resveratrol	52	29.44	1.56	34.79	.67		
	Assessment 3	Placebo	53	33.41	1.89	34.89	.70	.26	.60
		Resveratrol	52	29.44	1.56	34.37	.71		
Correct	Assessment 2	Placebo	53	30.89	1.93	33.51	.78	2.00	.16
		Resveratrol	52	27.33	1.59	31.93	.79		
	Assessment 3	Placebo	53	30.89	1.93	32.71	.70	.28	.59
		Resveratrol	52	27.33	1.59	32.17	.70		
Error	Assessment 2	Placebo	53	2.52	.34	2.45	.31	.17	.67
		Resveratrol	52	2.02	.21	2.63	.31		
	Assessment 3	Placebo	53	2.52	.34	2.26	.26	.17	.70
		Resveratrol	52	2.02	.21	2.11	.26		
			Rep 2						
Total	Assessment 2	Placebo	54	36.26	1.83	35.82	.66	.12	.72
		Resveratrol	52	31.96	1.65	35.48	.68		
	Assessment 3	Placebo	54	36.26	1.83	36.02	.65	.61	.43
		Resveratrol	52	31.96	1.65	35.28	.66		
Correct	Assessment 2	Placebo	54	34.11	1.88	33.26	.73	.05	.81
		Resveratrol	52	29.62	1.64	33.51	.74		
	Assessment 3	Placebo	54	34.11	1.88	33.47	.75	.15	.69
		Resveratrol	52	29.62	1.64	33.04	.76		
Error	Assessment 2	Placebo	54	2.15	.26	2.48	.28	1.05	.30
		Resveratrol	52	2.35	.26	2.06	.29		
	Assessment 3	Placebo	54	2.15	.26	2.44	.29	.04	.82
		Resveratrol	52	2.35	.26	2.34	.30		
			Rep 3						
Total	Assessment 2	Placebo	53	36.20	1.81	35.96	.74	1.65	.20
		Resveratrol	53	31.76	1.65	34.60	.74		
	Assessment 3	Placebo	53	36.20	1.81	35.02	.71	.01	.90
		Resveratrol	53	31.76	1.65	34.90	.71		
Correct	**Assessment 2**	**Placebo**	**53**	**33.98**	**1.90**	**34.35**	**.84**	**4.35**	**.03***
		Resveratrol	**53**	**29.78**	**1.67**	**31.85**	**.84**		
	Assessment 3	Placebo	53	33.98	1.90	32.69	.91	.03	.86

		Resveratrol	53	29.78	1.67	32.46	.91		
Error	**Assessment 2**	**Placebo**	**53**	**2.22**	**.31**	**1.66**	**.30**	**5.76**	**.01***
		Resveratrol	**53**	**1.98**	**.25**	**2.69**	**.30**		
	Assessment 3	Placebo	53	2.22	.31	2.43	.33	.03	.85
		Resveratrol	53	1.98	.25	2.34	.33		

Table 4.24. Serial three subtractions on Day 84. Comparisons by treatment group. Serial three's task performance on Day 84. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values, for Assessments 2 and 3, for all task outcome measures, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Rep 1						
Total	Assessment 2	Placebo	45	34.11	1.86	37.45	.86	.47	.49
		Resveratrol	44	30.34	1.80	36.60	.87		
	Assessment 3	Placebo	45	34.11	1.86	38.16	.92	1.87	.17
		Resveratrol	44	30.34	1.80	36.35	.93		
Correct	Assessment 2	Placebo	45	32.72	1.90	34.27	1.03	.04	.82
		Resveratrol	44	28.00	1.76	33.94	1.04		
	Assessment 3	Placebo	45	32.72	1.90	35.35	.99	.34	.55
		Resveratrol	44	28.00	1.76	34.52	1.00		
Error	Assessment 2	Placebo	45	1.38	.26	3.02	.38	.12	.72
		Resveratrol	44	2.34	.35	2.82	.39		
	Assessment 3	Placebo	45	1.38	.26	2.51	.33	.62	.43
		Resveratrol	44	2.34	.35	2.13	.34		
			Rep 2						
Total	Assessment 2	Placebo	46	38.24	1.91	37.25	.66	.22	.63
		Resveratrol	44	32.11	1.96	36.79	.68		
	Assessment 3	Placebo	46	38.24	1.91	37.25	.86	2.11	.14
		Resveratrol	44	32.11	1.96	35.43	.88		
Correct	Assessment 2	Placebo	46	36.22	1.95	34.91	.76	.00	.94
		Resveratrol	44	29.61	2.04	34.99	.78		
	Assessment 3	Placebo	46	36.22	1.95	34.95	.94	1.23	.26
		Resveratrol	44	29.61	2.04	33.43	.95		
Error	Assessment 2	Placebo	46	2.02	.31	2.30	.33	.94	.33
		Resveratrol	44	2.50	.34	1.83	.34		
	Assessment 3	Placebo	46	2.02	.31	2.15	.27	.00	.98
		Resveratrol	44	2.50	.34	2.15	.27		
			Rep 3						
Total	Assessment 2	Placebo	48	39.08	1.87	38.03	.72	.12	.72

		Resveratrol	44	33.98	1.71	37.66	.75		
	Assessment 3	Placebo	48	39.08	1.87	36.26	.73	1.27	.26
		Resveratrol	44	33.98	1.71	35.05	.76		
Correct	Assessment 2	Placebo	48	36.78	2.00	35.64	.81	.45	.50
		Resveratrol	44	31.57	1.77	34.84	.84		
	Assessment 3	Placebo	48	36.78	2.00	34.03	.96	3.00	.08
		Resveratrol	44	31.57	1.77	31.59	1.00		
Error	Assessment 2	Placebo	48	2.31	.40	2.32	.32	1.43	.23
		Resveratrol	44	2.41	.33	2.89	.33		
	Assessment 3	Placebo	48	2.31	.40	2.32	.42	2.81	.09
		Resveratrol	44	2.41	.33	3.35	.44		

Table 4.25. Serial three subtractions Pure Chronic analysis. Comparisons by treatment group. Serial three's task performance pure chronic analysis. Baseline (Day 1 assessment 1) and post-dose (Day 84, all assessments) estimated marginal means and standard errors (SE) are presented with F and *p* values, from ANCOVA analysis.

			Baseline (Day 1 A1)			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Rep 1						
Total	Assessment 1	Placebo	45	33.41	1.89	32.28	.89	.12	.72
		Resveratrol	44	29.44	1.56	31.82	.90		
	Assessment 2	Placebo	45	33.41	1.89	37.53	.98	.52	.47
		Resveratrol	44	29.44	1.56	36.51	.99		
	Assessment 3	Placebo	45	33.41	1.89	38.22	1.00	1.81	.18
		Resveratrol	44	29.44	1.56	36.29	1.01		
Correct	Assessment 1	Placebo	45	30.89	1.93	31.00	.92	1.63	.20
		Resveratrol	44	27.33	1.59	29.31	.93		
	Assessment 2	Placebo	45	30.89	1.93	34.93	1.12	1.07	.30
		Resveratrol	44	27.33	1.59	33.27	1.13		
	Assessment 3	Placebo	45	30.89	1.93	36.03	1.10	1.97	.16
		Resveratrol	44	27.33	1.59	33.82	1.11		
Error	**Assessment 1**	**Placebo**	**45**	**2.52**	**.34**	**1.38**	**.30**	**5.49**	**.02***
		Resveratrol	**44**	**2.02**	**.21**	**2.40**	**.30**		
	Assessment 2	Placebo	45	2.52	.34	2.66	.42	.76	.38
		Resveratrol	44	2.02	.21	3.18	.42		
	Assessment 3	Placebo	45	2.52	.34	2.26	.36	.06	.80
		Resveratrol	44	2.02	.21	2.38	.36		
			Rep 2						
Total	Assessment 1	Placebo	45	36.26	1.83	36.38	.85	1.90	.17

		Resveratrol	44	31.96	1.65	34.67	.86		
	Assessment 2	Placebo	45	36.26	1.83	38.06	.88	2.00	.16
		Resveratrol	44	31.96	1.65	36.27	.89		
	Assessment 3	**Placebo**	**45**	**36.26**	**1.83**	**38.20**	**1.03**	**4.89**	**.03***
		Resveratrol	**44**	**31.96**	**1.65**	**34.90**	**1.04**		
Correct	Assessment 1	Placebo	45	34.11	1.88	34.37	.90	2.55	.11
		Resveratrol	44	29.62	1.64	32.29	.91		
	Assessment 2	Placebo	45	34.11	1.88	35.83	.91	1.15	.28
		Resveratrol	44	29.62	1.64	34.41	.93		
	Assessment 3	**Placebo**	**45**	**34.11**	**1.88**	**36.19**	**1.10**	**4.64**	**.03***
		Resveratrol	**44**	**29.62**	**1.64**	**32.77**	**1.11**		
Error	Assessment 1	Placebo	45	2.15	.26	1.93	.29	1.59	.21
		Resveratrol	44	2.35	.26	2.45	.29		
	Assessment 2	Placebo	45	2.15	.26	2.14	.36	.15	.69
		Resveratrol	44	2.35	.26	1.94	.36		
	Assessment 3	Placebo	45	2.15	.26	1.87	.27	.92	.33
		Resveratrol	44	2.35	.26	2.26	.28		
				Rep 3					
Total	**Assessment 1**	**Placebo**	**47**	**36.20**	**1.81**	**37.73**	**.69**	**3.33**	**.07^{t}**
		Resveratrol	**43**	**31.76**	**1.65**	**35.89**	**.72**		
	Assessment 2	**Placebo**	**47**	**36.20**	**1.81**	**39.00**	**.83**	**3.86**	**.05^{t}**
		Resveratrol	**43**	**31.76**	**1.65**	**36.60**	**.87**		
	Assessment 3	**Placebo**	**47**	**36.20**	**1.81**	**37.12**	**.94**	**4.63**	**.03***
		Resveratrol	**43**	**31.76**	**1.65**	**34.16**	**.98**		
Correct	**Assessment 1**	**Placebo**	**47**	**33.98**	**1.90**	**35.76**	**.77**	**4.55**	**.03***
		Resveratrol	**43**	**29.78**	**1.67**	**33.35**	**.81**		
	Assessment 2	**Placebo**	**47**	**33.98**	**1.90**	**36.92**	**.95**	**6.02**	**.01***
		Resveratrol	**43**	**29.78**	**1.67**	**33.50**	**.99**		
	Assessment 3	**Placebo**	**47**	**33.98**	**1.90**	**35.13**	**1.12**	**7.55**	**.007***
		Resveratrol	**43**	**29.78**	**1.67**	**30.62**	**1.17**		
Error	Assessment 1	Placebo	47	2.22	.31	2.08	.32	.52	.47
		Resveratrol	43	1.98	.25	2.42	.33		
	Assessment 2	Placebo	47	2.22	.31	2.20	.34	2.32	.13
		Resveratrol	43	1.98	.25	2.95	.35		
	Assessment 3	**Placebo**	**47**	**2.22**	**.31**	**2.20**	**.42**	**3.20**	**.07^{t}**
		Resveratrol	**43**	**1.98**	**.25**	**3.31**	**.44**		

4.3.2.3.5. Serial 7 subtractions

Analysis indicated a significant pure chronic effect of treatment for Total subtractions observed on Day 84 during assessment 2, repetition 2, [F (1, 87) = 5.13, p = .02, d = .49], where participants completed more subtractions following placebo (25.53) in comparison to resveratrol (23.49). In addition, a significant treatment effect was observed during Day 84, assessment 1, repetition 3, [F (1, 87) = 4.25, p = .04, d = .44], with the placebo condition completing more subtractions (24.36) in comparison to resveratrol (22.61). Likewise, at Day 84, assessment 3, repetition 3, [F (1, 87) = 6.42, p = .01, d = .54], the placebo condition completed more subtractions (24.94) in comparison to resveratrol (22.65).

In terms of acute effects within Day 84, a significant treatment effect was observed on Day 84 during assessment 2, repetition 2, [F (1, 88) = 9.60, p = .003, d = .65], with participants completing more subtractions following placebo (25.51) in comparison with resveratrol (23.13). And again, at Day 84, assessment 3, repetition 2, [F (1, 88) = 9.36, p = .003, d = .65], where participants completed more subtractions following placebo (25.19), in comparison with resveratrol (23.15).

When considering treatment effects on the number of correct subtractions completed, analysis indicated a significant pure chronic treatment effect at Day 84 during assessment 2, repetition 2, [F (1, 87) = 4.42, p = .03, d = .45], with participants completing more correct subtractions following placebo (22.73) when compared with resveratrol (20.49). Additionally, the same pure-chronic effect was observed on Day 84, assessment 2, repetition 3, [F (1, 87) = 4.58, p = .03, d = .45], with placebo (22.36) performing greater than resveratrol (19.98). Again, placebo (22.06) performed more correct responses than resveratrol (19.24) at Day 84, assessment 3, repetition 3, [F (1, 87) = 7.43, p = .008, d = .58.].

In terms of acute changes within testing visits, on Day 84 analysis observed two significant treatment effects on number of correct subtractions. The first at assessment 2, repetition 2, [F (1, 88) = 7.33, p = .008, d = .57], with participants completing more correct subtractions following placebo (22.28) in comparison to resveratrol (20.19). In addition, at assessment 3, repetition 2, [F (1, 88) = 5.16, p = .026, d = .48], with participants completing more correct subtractions following placebo (22.66), in comparison to resveratrol (20.19).

No significant effects were observed on number of errors at any time point, as presented in Tables 4.26, 4.27 and 4.28.

Table 4.26. Serial seven subtractions on Day 1. Comparisons by treatment group. Serial sevens task performance on Day 1. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values for Assessments 2 and 3, for all task outcome measures, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Rep 1						
Total	Assessment 2	Placebo	54	22.80	1.27	23.09	.50	.00	.97
		Resveratrol	53	19.46	1.23	23.11	.50		
	Assessment 3	Placebo	54	22.80	1.27	22.71	.52	1.41	.23
		Resveratrol	53	19.46	1.23	23.61	.53		
Correct	Assessment 2	Placebo	54	20.11	1.34	20.24	.60	.00	.92
		Resveratrol	53	16.85	1.27	20.16	.61		
	Assessment 3	Placebo	54	20.11	1.34	19.74	.66	.80	.37
		Resveratrol	53	16.85	1.27	20.59	.66		
Error	Assessment 2	Placebo	54	2.69	.25	2.87	.30	.01	.91
		Resveratrol	53	2.61	.25	2.92	.30		
	Assessment 3	Placebo	54	2.69	.25	3.00	.32	.00	.94
		Resveratrol	53	2.61	.25	2.97	.32		
			Rep 2						
Total	Assessment 2	Placebo	54	24.43	1.43	23.67	.53	.00	.93
		Resveratrol	54	20.27	1.24	23.73	.53		
	Assessment 3	Placebo	54	24.43	1.43	23.88	.58	1.91	.16
		Resveratrol	54	20.27	1.24	22.74	.58		
Correct	Assessment 2	Placebo	54	21.19	1.42	20.53	.67	.01	.90
		Resveratrol	54	17.11	1.32	20.65	.67		
	Assessment 3	Placebo	54	21.19	1.42	20.30	.75	.75	.38
		Resveratrol	54	17.11	1.32	19.36	.75		
Error	Assessment 2	Placebo	54	3.24	.34	3.11	.34	.00	.97
		Resveratrol	54	3.16	.32	3.10	.34		
	Assessment 3	Placebo	54	3.24	.34	3.66	3.78	.49	.48
		Resveratrol	54	3.16	.32	3.29	.37		
			Rep 3						
Total	Assessment 2	Placebo	54	23.65	1.37	23.97	.55	.26	.60
		Resveratrol	53	21.02	1.27	23.57	.55		
	Assessment 3	Placebo	54	23.65	1.37	23.44	.50	1.42	.23
		Resveratrol	53	21.02	1.27	22.58	.51		
Correct	Assessment 2	Placebo	54	20.38	1.37	20.49	.63	.31	.57
		Resveratrol	53	17.89	1.37	19.98	.63		
	Assessment 3	Placebo	54	20.38	1.37	20.38	.59	2.17	.14

		Resveratrol	53	17.89	1.37	19.13	.59		
Error	Assessment 2	Placebo	54	3.27	.34	3.54	.34	.00	.96
		Resveratrol	53	3.13	.30	3.52	.35		
	Assessment 3	Placebo	54	3.27	.34	3.14	.35	.19	.66
		Resveratrol	53	3.13	.30	3.36	.36		

Table 4.27. Serial seven subtractions on Day 84. Comparisons by treatment group. Serial sevens task performance on Day 84. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values for Assessments 2 and 3, for all task outcome measures, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Rep 1						
Total	Assessment 2	Placebo	49	23.39	1.33	24.56	.57	.91	.34
		Resveratrol	42	19.93	1.52	23.75	.61		
	Assessment 3	Placebo	49	23.39	1.33	23.94	.51	.21	.64
		Resveratrol	42	19.93	1.52	24.29	.55		
Correct	Assessment 2	Placebo	49	21.12	1.41	21.46	.63	.41	.52
		Resveratrol	42	17.55	1.57	20.85	.68		
	Assessment 3	Placebo	49	21.12	1.41	21.13	.61	1.48	.22
		Resveratrol	42	17.55	1.57	22.24	.66		
Error	Assessment 2	Placebo	49	2.27	.31	3.11	.32	.28	.59
		Resveratrol	42	2.39	.28	2.86	.35		
	Assessment 3	Placebo	49	2.27	.31	2.74	.28	2.16	.14
		Resveratrol	42	2.39	.28	2.13	.30		
			Rep 2						
Total	Assessment 2	**Placebo**	**49**	**24.61**	**1.41**	**25.51**	**.51**	**9.60**	**.003***
		Resveratrol	**42**	**21.79**	**1.52**	**23.13**	**.56**		
	Assessment 3	**Placebo**	**49**	**24.61**	**1.41**	**25.19**	**.45**	**9.36**	**.003***
		Resveratrol	**42**	**21.79**	**1.52**	**23.15**	**.48**		
Correct	Assessment 2	**Placebo**	**49**	**22.04**	**1.52**	**22.66**	**.61**	**7.33**	**.008***
		Resveratrol	**42**	**19.14**	**1.51**	**20.19**	**.66**		
	Assessment 3	**Placebo**	**49**	**22.04**	**1.52**	**22.28**	**.62**	**5.16**	**.02***
		Resveratrol	**42**	**19.14**	**1.51**	**20.19**	**.67**		
Error	Assessment 2	Placebo	49	2.57	.38	2.86	.29	.01	.91
		Resveratrol	42	2.65	.36	2.91	.31		
	Assessment 3	Placebo	49	2.57	.38	2.90	.33	.02	.88
		Resveratrol	42	2.65	.36	2.97	.36		

			n	Mean	SE	Mean	SE	F	p
			Rep 3						
Total	Assessment 2	Placebo	48	25.57	1.33	24.02	.48	.03	.85
		Resveratrol	42	22.05	1.48	24.15	.52		
	Assessment 3	Placebo	48	25.57	1.33	24.31	.54	1.33	.25
		Resveratrol	42	22.05	1.48	23.38	.57		
Correct	Assessment 2	Placebo	48	22.98	1.40	21.60	.56	.78	.37
		Resveratrol	42	19.37	1.56	20.86	.60		
	Assessment 3	Placebo	48	22.98	1.40	21.42	.65	2.22	.14
		Resveratrol	42	19.37	1.56	19.97	.70		
Error	Assessment 2	Placebo	48	2.59	.30	2.47	.32	2.67	.10
		Resveratrol	42	2.67	.38	3.24	.34		
	Assessment 3	Placebo	48	2.59	.30	2.92	.34	.81	.36
		Resveratrol	42	2.67	.38	3.37	.36		

Table 4.28. Serial seven subtractions Pure Chronic analysis. Comparisons by treatment group. Serial sevens task performance pure chronic analysis. Baseline (Day 1 assessment 1) and post-dose (Day 84, all assessments) estimated marginal means and standard errors (SE) are presented with F and *p* values, from ANCOVA analysis.

			Baseline (Day 1 A1)			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Rep 1						
Total	Assessment 1	Placebo	49	22.80	1.27	22.32	.60	.24	.62
		Resveratrol	41	19.46	1.23	21.88	.65		
	Assessment 2	Placebo	49	22.80	1.27	24.80	.60	1.56	.21
		Resveratrol	41	19.46	1.23	23.67	.66		
	Assessment 3	Placebo	49	22.80	1.27	24.41	.55	.06	.79
		Resveratrol	41	19.46	1.23	24.42	.61		
Correct	Assessment 1	Placebo	49	20.11	1.34	20.12	.66	.68	.41
		Resveratrol	41	16.85	1.27	19.31	.72		
	Assessment 2	Placebo	49	20.11	1.34	21.84	.68	1.42	.23
		Resveratrol	41	16.85	1.27	20.63	.74		
	Assessment 3	Placebo	49	20.11	1.34	21.54	.61	.65	.42
		Resveratrol	41	16.85	1.27	22.27	.67		
Error	Assessment 1	Placebo	49	2.69	.25	2.24	.29	.35	.55
		Resveratrol	41	2.61	.25	2.50	.32		
	Assessment 2	Placebo	49	2.69	.25	3.05	.33	.06	.80
		Resveratrol	41	2.61	.25	2.93	.36		
	Assessment 3	Placebo	49	2.69	.25	2.67	.26	1.96	.16
		Resveratrol	41	2.61	.25	2.13	.28		

Rep 2									
Total	Assessment 1	Placebo	48	24.43	1.43	23.53	.62	.09	.75
		Resveratrol	42	20.27	1.24	23.81	.66		
	Assessment 2	**Placebo**	**48**	**24.43**	**1.43**	**25.53**	**.61**	**5.13**	**.02***
		Resveratrol	**42**	**20.27**	**1.24**	**23.49**	**.66**		
	Assessment 3	Placebo	48	24.43	1.43	25.07	.62	3.02	.08
		Resveratrol	42	20.27	1.24	23.46	.66		
Correct	Assessment 1	Placebo	48	21.19	1.42	20.98	.74	.01	.91
		Resveratrol	42	17.11	1.32	21.09	.79		
	Assessment 2	**Placebo**	**48**	**21.19**	**1.42**	**22.73**	**.72**	**4.42**	**.03***
		Resveratrol	**42**	**17.11**	**1.32**	**20.49**	**.77**		
	Assessment 3	Placebo	48	21.19	1.42	22.10	.74	2.15	.14
		Resveratrol	42	17.11	1.32	20.49	.79		
Error	Assessment 1	Placebo	48	3.24	.34	2.56	.36	.07	.79
		Resveratrol	42	3.16	.32	2.70	.38		
	Assessment 2	Placebo	48	3.24	.34	2.83	.33	.04	.82
		Resveratrol	42	3.16	.32	2.94	.35		
	Assessment 3	Placebo	48	3.24	.34	2.94	.35	.00	.92
		Resveratrol	42	3.16	.32	2.99	.37		
Rep 3									
Total	**Assessment 1**	**Placebo**	**48**	**23.65**	**1.37**	**24.36**	**.57**	**4.25**	**.04***
		Resveratrol	**42**	**21.02**	**1.27**	**22.61**	**.61**		
	Assessment 2	Placebo	48	23.65	1.37	24.75	.63	2.39	.12
		Resveratrol	42	21.02	1.27	23.32	.67		
	Assessment 3	**Placebo**	**48**	**23.65**	**1.37**	**24.94**	**.61**	**6.42**	**.01***
		Resveratrol	**42**	**21.02**	**1.27**	**22.65**	**.65**		
Correct	**Assessment 1**	**Placebo**	**48**	**20.38**	**1.37**	**21.75**	**.63**	**3.92**	**.05[t]**
		Resveratrol	**42**	**17.89**	**1.37**	**19.90**	**.68**		
	Assessment 2	**Placebo**	**48**	**20.38**	**1.37**	**22.36**	**.75**	**4.58**	**.03***
		Resveratrol	**42**	**17.89**	**1.37**	**19.98**	**.81**		
	Assessment 3	**Placebo**	**48**	**20.38**	**1.37**	**22.06**	**.70**	**7.43**	**.008***
		Resveratrol	**42**	**17.89**	**1.37**	**19.24**	**.75**		
Error	Assessment 1	Placebo	48	3.27	.34	2.60	.31	.04	.82
		Resveratrol	42	3.13	.30	2.71	.33		
	Assessment 2	Placebo	48	3.27	.34	2.44	.38	2.21	.14
		Resveratrol	42	3.13	.30	3.28	.41		
	Assessment 3	Placebo	48	3.27	.34	2.90	.35	.92	.33
		Resveratrol	42	3.13	.30	3.39	.37		

4.3.2.3.6. Rapid Visual Information Processing

A significant pure-chronic treatment effect was observed during Day 84, assessment 3, repetition 3, [F (1, 71) = 4.05, p = .04, d = .48], where participants completed fewer false alarms following placebo (1.39) in comparison to resveratrol (2.82). Additionally, a trend towards a significant pure-chronic treatment effect was observed during Day 84, assessment 1, repetition 3, [F(1, 71) = 3.83, p = .054, d = .46], with the placebo condition completing fewer false alarms (1.44) in comparison with resveratrol (2.44).

No other effects were observed on the false alarms outcome at any time point. No effects were observed on Accuracy or Reaction time outcomes at any time point, as presented in Tables 4.29, 4.30 and 4.31.

Table 4.29. Rapid Visual Information Processing on Day 1. Comparisons by treatment group. RVIP performance on Day 1. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values for Assessments 2 and 3, for all task outcome measures, from ANCOVA analysis.

				Baseline		Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Rep 1						
% Correct	Assessment 2	Placebo	50	58.43	3.04	54.51	1.70	.80	.37
		Resveratrol	45	52.88	2.68	56.75	1.79		
	Assessment 3	Placebo	50	58.43	3.04	52.88	1.97	.64	.42
		Resveratrol	45	52.88	2.68	55.18	2.07		
Correct RT	Assessment 2	Placebo	50	502.10	7.98	504.10	6.04	.37	.54
		Resveratrol	45	499.46	8.00	498.755	6.37		
	Assessment 3	Placebo	50	502.10	7.98	500.62	5.07	.09	.76
		Resveratrol	45	499.46	8.00	502.83	5.34		
False alarms	Assessment 2	Placebo	50	3.53	.52	2.63	.41	.10	.74
		Resveratrol	45	4.20	.62	2.83	.44		
	Assessment 3	Placebo	50	3.53	.52	2.66	.40	.02	.87
		Resveratrol	45	4.20	.62	2.75	.42		
			Rep 2						
% Correct	Assessment 2	Placebo	49	57.30	3.03	51.13	1.65	2.35	.12
		Resveratrol	49	52.19	2.86	47.53	1.65		
	Assessment 3	Placebo	49	57.30	3.03	54.44	1.51	1.86	.17
		Resveratrol	49	52.19	2.86	51.52	1.51		
Correct RT	Assessment 2	Placebo	49	515.39	7.94	507.43	6.40	.15	.69
		Resveratrol	49	503.22	6.67	511.02	6.40		

	Assessment 3	Placebo	49	515.39	7.94	512.95	4.72	.31	.57
		Resveratrol	49	503.22	6.67	509.16	4.72		
False alarms	Assessment 2	Placebo	49	2.78	.35	3.22	.41	.33	.56
		Resveratrol	49	3.82	.61	3.56	.41		
	Assessment 3	Placebo	49	2.78	.35	2.93	.41	1.11	.29
		Resveratrol	49	3.82	.61	3.55	.41		
			Rep 3						
% Correct	Assessment 2	Placebo	49	57.50	3.31	50.46	1.70	.48	.48
		Resveratrol	44	51.25	2.80	52.50	1.80		
	Assessment 3	Placebo	49	57.50	3.31	52.91	1.48	.02	.86
		Resveratrol	44	51.25	2.80	53.28	1.56		
Correct RT	Assessment 2	Placebo	49	517.02	8.37	506.10	6.55	.03	.84
		Resveratrol	44	501.84	7.14	507.99	6.91		
	Assessment 3	Placebo	49	517.02	8.37	504.75	4.39	1.60	.20
		Resveratrol	44	501.84	7.14	512.87	4.63		
False alarms	Assessment 2	Placebo	49	2.86	.39	2.64	.31	.01	.91
		Resveratrol	44	3.86	.61	2.69	.33		
	Assessment 3	Placebo	49	2.86	.39	2.55	.29	.79	.37
		Resveratrol	44	3.86	.61	2.93	.31		

Table 4.30. Rapid Visual Information Processing on Day 84. Comparisons by treatment group. RVIP performance on Day 84. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values for Assessments 2 and 3, for all task outcome measures, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Rep 1						
% Correct	Assessment 2	Placebo	42	60.85	3.37	59.17	1.86	.39	.53
		Resveratrol	34	56.73	3.35	57.41	2.07		
	Assessment 3	Placebo	42	60.85	3.37	55.94	1.89	.02	.87
		Resveratrol	34	56.73	3.35	56.40	2.11		
Correct RT	Assessment 2	Placebo	42	502.52	9.19	500.10	4.99	.86	.35
		Resveratrol	34	494.43	10.66	507.05	5.55		
	Assessment 3	Placebo	42	502.52	9.19	498.11	4.97	.83	.36
		Resveratrol	34	494.43	10.66	504.89	5.23		
False alarms	Assessment 2	Placebo	42	2.60	.39	2.11	.31	.08	.77
		Resveratrol	34	3.31	.76	1.97	.35		
	Assessment 3	Placebo	42	2.60	.39	2.24	.30	.38	.53
		Resveratrol	34	3.31	.76	1.96	.34		

			Rep 2						
% Correct	Assessment 2	Placebo	44	60.81	3.45	57.29	2.13	.03	.85
		Resveratrol	33	52.30	3.47	56.70	2.44		
	Assessment 3	Placebo	44	60.81	3.45	50.99	2.16	1.41	.23
		Resveratrol	33	52.30	3.47	54.92	2.47		
Correct RT	Assessment 2	Placebo	44	517.46	8.36	504.80	5.42	.71	.39
		Resveratrol	33	505.37	9.85	511.79	6.19		
	Assessment 3	Placebo	44	517.46	8.36	506.83	5.04	.00	.99
		Resveratrol	33	505.37	9.85	506.77	5.76		
False alarms	Assessment 2	Placebo	44	2.09	.30	2.49	.36	.00	.98
		Resveratrol	33	4.77	1.06	2.48	.41		
	Assessment 3	Placebo	44	2.09	.30	2.38	.42	.37	.54
		Resveratrol	33	4.77	1.06	1.98	.48		
			Rep 3						
% Correct	Assessment 2	Placebo	44	59.42	3.30	53.80	1.69	.26	.60
		Resveratrol	33	50.83	3.22	55.15	1.96		
	Assessment 3	Placebo	44	59.42	3.30	51.08	1.69	.04	.83
		Resveratrol	33	50.83	3.22	50.52	1.96		
Correct RT	Assessment 2	Placebo	44	519.64	8.20	511.69	4.66	2.83	.09
		Resveratrol	33	510.08	9.59	523.70	5.39		
	Assessment 3	Placebo	44	519.64	8.20	510.94	5.46	.00	.95
		Resveratrol	33	510.08	9.59	511.39	6.31		
False alarms	Assessment 2	Placebo	44	2.25	.45	2.33	.32	.01	.90
		Resveratrol	33	4.49	1.05	2.27	.37		
	Assessment 3	Placebo	44	2.25	.45	1.74	.33	1.16	.28
		Resveratrol	33	4.49	1.05	2.30	.39		

Table 4.31. Rapid Visual Information Processing Pure Chronic analysis. Comparisons by treatment group. RVIP performance pure chronic analysis. Baseline (Day 1 assessment 1) and post-dose (Day 84, all assessments) estimated marginal means and standard errors (SE) are presented with F and *p* values, from ANCOVA analysis.

			Baseline (Day 1 A1)			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Rep 1						
% Correct	Assessment 1	Placebo	40	58.43	3.04	62.76	1.71	.53	.46
		Resveratrol	31	52.88	2.68	60.86	1.94		
	Assessment 2	Placebo	40	58.43	3.04	60.06	2.13	1.41	.23
		Resveratrol	31	52.88	2.68	56.21	2.42		
	Assessment 3	Placebo	40	58.43	3.04	56.73	2.09	.01	.91
		Resveratrol	31	52.88	2.68	56.39	2.38		

Correct RT	Assessment 1	Placebo	40	502.10	7.98	496.98	6.15	.52	.47
		Resveratrol	31	499.46	8.00	490.22	6.99		
	Assessment 2	Placebo	40	502.10	7.98	500.42	6.72	.01	.89
		Resveratrol	31	499.46	8.00	501.79	7.63		
	Assessment 3	Placebo	40	502.10	7.98	499.24	6.35	.24	.62
		Resveratrol	31	499.46	8.00	494.52	7.22		
False alarms	Assessment 1	Placebo	40	3.53	.52	1.89	.40	2.32	.13
		Resveratrol	31	4.20	.62	2.82	.45		
	Assessment 2	Placebo	40	3.53	.52	1.71	.50	.83	.36
		Resveratrol	31	4.20	.62	2.40	.57		
	Assessment 3	Placebo	40	3.53	.52	1.68	.37	.89	.34
		Resveratrol	31	4.20	.62	2.21	.42		
Rep 2									
% Correct	Assessment 1	Placebo	42	57.30	3.03	58.63	2.10	.84	.36
		Resveratrol	32	52.19	2.86	55.69	2.40		
	Assessment 2	Placebo	42	57.30	3.03	57.54	2.02	.83	.36
		Resveratrol	32	52.19	2.86	54.71	2.32		
	Assessment 3	Placebo	42	57.30	3.03	51.75	2.29	.08	.77
		Resveratrol	32	52.19	2.86	52.77	2.63		
Correct RT	Assessment 1	Placebo	42	515.39	7.94	515.23	6.62	.00	.95
		Resveratrol	32	503.22	6.67	514.66	7.59		
	Assessment 2	Placebo	42	515.39	7.94	504.15	6.39	.83	.36
		Resveratrol	32	503.22	6.67	513.04	7.32		
	Assessment 3	Placebo	42	515.39	7.94	506.25	6.48	.00	.95
		Resveratrol	32	503.22	6.67	505.73	7.43		
False alarms	Assessment 1	Placebo	42	2.78	.35	2.05	.38	2.19	.14
		Resveratrol	32	3.82	.61	2.92	.43		
	Assessment 2	Placebo	42	2.78	.35	2.23	.44	.67	.41
		Resveratrol	32	3.82	.61	2.78	.50		
	Assessment 3	Placebo	42	2.78	.35	1.97	.54	.33	.56
		Resveratrol	32	3.82	.61	2.46	.62		
Rep 3									
% Correct	Assessment 1	Placebo	42	57.50	3.31	58.43	2.06	1.22	.27
		Resveratrol	32	51.25	2.80	54.94	2.36		
	Assessment 2	Placebo	42	57.50	3.31	55.58	2.22	.12	.72
		Resveratrol	32	51.25	2.80	54.38	2.55		
	Assessment 3	Placebo	42	57.50	3.31	52.49	2.15	.68	.41
		Resveratrol	32	51.25	2.80	49.77	2.46		
Correct RT	Assessment 1	Placebo	42	517.02	8.37	516.47	6.72	.01	.89
		Resveratrol	32	501.84	7.14	517.86	7.71		

	Assessment 2	Placebo	42	517.02	8.37	507.57	6.59	2.65	.10
		Resveratrol	32	501.84	7.14	523.97	7.56		
	Assessment 3	Placebo	42	517.02	8.37	505.01	6.63	.18	.66
		Resveratrol	32	501.84	7.14	509.39	7.60		
False alarms	**Assessment 1**	**Placebo**	**42**	**2.86**	**.39**	**1.44**	**.32**	**3.83**	**.05[t]**
		Resveratrol	**32**	**3.86**	**.61**	**2.42**	**.37**		
	Assessment 2	Placebo	42	2.86	.39	1.96	.39	1.26	.26
		Resveratrol	32	3.86	.61	2.64	.45		
	Assessment 3	**Placebo**	**42**	**2.86**	**.39**	**1.39**	**.46**	**4.05**	**.04***
		Resveratrol	**32**	**3.86**	**.61**	**2.82**	**.53**		

4.3.2.3.7. Peg and Ball

Analysis indicated two trends towards significant pure-chronic treatment effects on ‘thinking reaction time’. On Day 84, assessment 1, [F (1, 82) = 3.45, p = .06, d = .41], participants performed quicker following placebo (2803.45), in comparison with resveratrol (3108.11). Again, at Day 84, assessment 2, [F (1, 82) = 3.42, p = .06, d = .41], participants performed quicker following placebo (2543.10), in comparison with resveratrol (2802.78).

In addition, analysis of acute effects within testing visits indicated a trend towards a significant effect of treatment on Day 1 during assessment 2, [F (1, 103) = 3.69, p = .057, d = .38], where participants performed fewer errors following placebo (3.48), in comparison with resveratrol (4.91).

No effects were observed on ‘Completion reaction time’ outcomes at any time point. No other significant effects were observed on any outcome at any time point, as presented in Table 4.32.

Table 4.32. Peg and Ball. Comparisons by treatment group. Peg and Ball performance on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
Average Thinking RT	Assessment 2	Placebo	54	3245.89	168.00	2605.37	91.98	2.39	.12
		Resveratrol	52	2891.07	183.63	2809.72	93.75		
	Assessment 3	Placebo	54	3245.89	168.00	2778.49	70.48	.12	.73
		Resveratrol	52	2891.07	183.63	2743.46	71.83		
Average Complete RT	Assessment 2	Placebo	54	9676.58	269.17	8774.40	163.78	.04	.83
		Resveratrol	52	9610.91	323.60	8724.52	166.91		
	Assessment 3	Placebo	54	9676.58	269.17	8826.78	135.95	.91	.34
		Resveratrol	52	9610.91	323.60	9012.91	138.54		
Total Errors	Assessment 2	Placebo	54	3.44	.38	3.99	.43	.08	.76
		Resveratrol	52	4.70	.47	4.17	.44		
	Assessment 3	**Placebo**	**54**	**3.44**	**.38**	**3.48**	**.51**	**3.69**	**.05[t]**
		Resveratrol	**52**	**4.70**	**.47**	**4.91**	**.52**		
Day 84									
Average Thinking RT	Assessment 2	Placebo	45	2922.94	143.91	2666.49	81.59	.00	.98
		Resveratrol	40	3149.45	204.76	2663.96	86.55		
	Assessment 3	Placebo	45	2922.94	143.91	2581.11	103.19	.12	.72
		Resveratrol	40	3149.45	204.76	2634.94	109.46		
Average Complete RT	Assessment 2	Placebo	45	9272.04	308.12	8635.08	139.76	2.61	.11
		Resveratrol	40	9499.18	370.88	8694.58	148.23		
	Assessment 3	Placebo	45	9272.04	308.12	8349.40	207.79	.54	.46
		Resveratrol	40	9499.18	370.88	8573.09	220.40		
Total Errors	Assessment 2	Placebo	45	3.94	.52	3.57	.55	2.17	.14
		Resveratrol	40	3.50	.44	4.75	.58		
	Assessment 3	Placebo	45	3.94	.52	4.02	.51	.64	.42
		Resveratrol	40	3.50	.44	3.42	.54		
Pure Chronic									
			Day 1 A1			Post dose (Day 84)		Main Effects	
			n	Mean	SE	Mean	SE	F	p
Average Thinking RT	**Assessment 1**	**Placebo**	**45**	**3245.89**	**168.00**	**2803.45**	**112.02**	**3.45**	**.06[t]**
		Resveratrol	**40**	**2891.07**	**183.63**	**3108.11**	**118.85**		
	Assessment 2	**Placebo**	**45**	**3245.89**	**168.00**	**2543.10**	**95.98**	**3.42**	**.06[t]**
		Resveratrol	**40**	**2891.07**	**183.63**	**2802.78**	**101.83**		

	Assessment 3	Placebo	45	3245.89	168.00	2472.56	114.75	2.87	.09
		Resveratrol	40	2891.07	183.63	2757.06	121.76		
Average Complete RT	Assessment 1	Placebo	45	9676.58	269.17	9210.56	190.53	.00	.96
		Resveratrol	40	9610.91	323.60	9223.16	202.08		
	Assessment 2	Placebo	45	9676.58	269.17	8360.43	164.79	1.99	.16
		Resveratrol	40	9610.91	323.60	8699.80	174.79		
	Assessment 3	Placebo	45	9676.58	269.17	8345.71	220.16	.52	.47
		Resveratrol	40	9610.91	323.60	8577.24	233.51		
Total Errors	Assessment 1	Placebo	45	3.44	.38	3.91	.47	.52	.47
		Resveratrol	40	4.70	.47	3.40	.50		
	Assessment 2	Placebo	45	3.44	.38	3.82	.55	.62	.42
		Resveratrol	40	4.70	.47	4.47	.58		
	Assessment 3	Placebo	45	3.44	.38	4.18	.54	1.33	.25
		Resveratrol	40	4.70	.47	3.24	.57		

4.3.2.3.8. Name-to-face recall

In terms of acute effects within testing visits, analysis indicated a significant treatment effect was observed for Overall accuracy on Day 1, during assessment 3, [F (1, 98) = 4.61, p = .03, d = .43], where participants performed more accurately following placebo (59.00) in comparison with resveratrol (52.64). Additionally, a trend towards a significant treatment effect was observed on Day 1 during assessment 2, [F (1, 98) = 3.51, p = .06, d = .38], where participants performed better following placebo (55.94) in comparison to resveratrol (51.10).

A trend towards a significant treatment effect on 'Correct Forename %' was observed on Day 1 during assessment 3, [F (1, 98) = 3.58, p = .06, d = .38], where participants performed better following placebo (61.46) in comparison with resveratrol (54.61).

Additionally, a significant treatment effect was observed for 'Correct Surname %' on Day 1 during assessment 3, [F (1, 98) = 4.32, p = .04, d = .42], where participants performed better following placebo (56.27) in comparison with resveratrol (49.76). This effect was also observed on Day 84 during assessment 2, [F (1, 87) = 5.73, p = .01, d = .51], where participants performed better following placebo (60.79) in comparison with resveratrol (51.78).

For 'Forename Correct RT' analysis indicated a trend towards a significant pure chronic effect, at the Day 84 baseline assessment, [F (1, 87) = 3.79, p = .055, d = .42], where participants performed quicker following placebo (4584.95) in comparison to resveratrol (5188.13).

Additionally, a trend towards a significant pure chronic effect at the Day 84 baseline assessment was observed for 'Surname Correct RT', [$F(1, 87) = 3.22$, $p = .07$, $d = .39$], where participants were quicker following placebo (6062.27) in comparison to resveratrol (6723.05).

Additionally, an acute significant treatment effect was observed for 'Overall Correct RT' on Day 1 during assessment 3, [$F(1, 98) = 4.70$, $p = .03$, $d = .44$], where participants performed quicker following placebo (9850.80) in comparison with resveratrol (11552.31).

No other significant effects were observed on any outcome at any time point, as shown in Tables 4.33, 4.34. and 4.35.

Table 4.33. Name-to-face recall on Day 1. Comparisons by treatment group. Name to face recall performance on Day 1. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values for Assessments 2 and 3, for all task outcome measures, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
% Correct	**Assessment 2**	**Placebo**	**51**	**59.24**	**2.48**	**55.94**	**1.81**	**3.51**	**.06[t]**
		Resveratrol	**50**	**62.34**	**2.37**	**51.10**	**1.83**		
	Assessment 3	**Placebo**	**51**	**59.24**	**2.48**	**59.00**	**2.08**	**4.61**	**.03***
		Resveratrol	**50**	**62.34**	**2.37**	**52.64**	**2.10**		
Overall RT	Assessment 2	Placebo	51	12322.811	484.78	11952.41	354.24	.13	.71
		Resveratrol	50	13557.01	567.71	11769.18	357.83		
	Assessment 3	Placebo	51	12322.811	484.78	12295.34	344.72	.00	.96
		Resveratrol	50	13557.01	567.71	12318.29	348.21		
Correct RT	**Assessment 2**	**Placebo**	**51**	**9784.28**	**493.72**	**9850.80**	**537.67**	**4.70**	**.03***
		Resveratrol	**50**	**12382.22**	**509.52**	**11552.31**	**543.30**		
	Assessment 3	Placebo	51	9784.28	493.72	10467.43	521.06	1.11	.29
		Resveratrol	50	12382.22	509.52	9664.17	526.51		
Overall forename RT	Assessment 2	Placebo	51	5370.06	236.49	5210.36	173.68	.12	.72
		Resveratrol	50	5974.11	287.46	5122.91	175.44		
	Assessment 3	Placebo	51	5370.06	236.49	5403.97	169.10	.22	.63
		Resveratrol	50	5974.11	287.46	5287.91	170.81		
Correct Forename RT	Assessment 2	Placebo	51	4770.61	182.41	4587.52	196.19	1.20	.27
		Resveratrol	50	5398.69	240.99	4898.27	198.20		
	Assessment 3	Placebo	51	4770.61	182.41	4826.07	214.77	.06	.80
		Resveratrol	50	5398.69	240.99	4748.62	216.97		
Overall Surname RT	Assessment 2	Placebo	51	6952.74	261.49	6730.29	192.48	.06	.79
		Resveratrol	50	7582.89	292.38	6658.25	194.42		
	Assessment 3	Placebo	51	6952.74	261.49	6877.49	187.54	.38	.53
		Resveratrol	50	7582.89	292.38	7044.53	189.44		

Correct Surname RT	Assessment 2	Placebo	51	6438.65	247.00	6206.58	223.30	2.32	.13
		Resveratrol	50	7253.21	28382	6695.76	225.57		
	Assessment 3	Placebo	51	6438.65	247.00	6410.95	248.63	.07	.78
		Resveratrol	50	7253.21	28382	6313.45	251.15		
Correct Forename %	Assessment 2	Placebo	51	60.30	2.74	55.63	2.43	.85	.35
		Resveratrol	50	62.19	2.63	52.42	2.46		
	Assessment 3	**Placebo**	**51**	**60.30**	**2.74**	**61.64**	**2.61**	**3.58**	**.06[t]**
		Resveratrol	**50**	**62.19**	**2.63**	**54.61**	**2.63**		
Correct Surname %	**Assessment 2**	**Placebo**	**51**	**58.18**	**2.72**	**56.27**	**2.19**	**4.32**	**.04***
		Resveratrol	**50**	**62.49**	**2.66**	**49.76**	**2.21**		
	Assessment 3	Placebo	51	58.18	2.72	56.36	2.56	2.43	.12
		Resveratrol	50	62.49	2.66	50.67	2.58		

Table 4.34. Name-to-face recall on Day 84. Comparisons by treatment group. Name to face recall performance on Day 84. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values for Assessments 2 and 3, for all task outcome measures, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
% Correct	Assessment 2	Placebo	46	61.05	2.57	59.76	2.07	1.77	.18
		Resveratrol	44	62.97	2.46	55.79	2.12		
	Assessment 3	Placebo	46	61.05	2.57	58.94	2.00	2.32	.13
		Resveratrol	44	62.97	2.46	54.56	2.05		
Overall RT	Assessment 2	Placebo	46	11385.05	481.52	12305.35	357.06	1.97	.16
		Resveratrol	44	12762.44	452.85	11578.66	365.28		
	Assessment 3	Placebo	46	11385.05	481.52	11727.43	322.86	.65	.42
		Resveratrol	44	12762.44	452.85	11350.38	330.29		
Correct RT	Assessment 2	Placebo	46	10077.31	525.28	10240.29	532.35	1.53	.21
		Resveratrol	44	11710.72	563.91	9282.20	544.65		
	Assessment 3	Placebo	46	10077.31	525.28	10316.64	465.96	.00	.99
		Resveratrol	44	11710.72	563.91	10313.62	476.73		
Overall forename RT	Assessment 2	Placebo	46	4957.50	234.57	5414.99	188.08	1.92	.16
		Resveratrol	44	5666.32	246.87	5037.82	192.41		
	Assessment 3	Placebo	46	4957.50	234.57	5154.03	174.23	.57	.45
		Resveratrol	44	5666.32	246.87	4963.74	178.24		
Correct Forename RT	Assessment 2	Placebo	46	4444.61	217.03	4743.17	188.97	.30	.58
		Resveratrol	44	5327.26	281.57	4592.76	193.36		
	Assessment 3	Placebo	46	4444.61	217.03	4512.81	187.52	.40	.52
		Resveratrol	44	5327.26	281.57	4686.97	191.87		
Overall Surname RT	Assessment 2	Placebo	46	6427.55	261.81	6880.45	182.74	1.55	.21
		Resveratrol	44	7096.12	227.32	6551.21	186.93		
	Assessment 3	Placebo	46	6427.55	261.81	6568.48	163.43	.55	.45
		Resveratrol	44	7096.12	227.32	6391.78	167.18		

Correct Surname RT	Assessment 2	Placebo	46	6045.19	278.79	6346.65	220.33	.05	.81
		Resveratrol	44	6847.28	266.20	6271.22	225.44		
	Assessment 3	Placebo	46	6045.19	278.79	6267.29	203.87	.01	.91
		Resveratrol	44	6847.28	266.20	6297.35	208.59		
Correct Forename %	Assessment 2	Placebo	46	64.79	2.78	58.85	2.71	.04	.83
		Resveratrol	44	61.74	2.75	59.68	2.78		
	Assessment 3	Placebo	46	64.79	2.78	59.61	2.74	.44	.50
		Resveratrol	44	61.74	2.75	56.99	2.80		
Correct Surname %	**Assessment 2**	**Placebo**	**46**	**57.31**	**3.03**	**60.79**	**2.61**	**5.73**	**.01***
		Resveratrol	**44**	**64.20**	**2.84**	**51.78**	**2.67**		
	Assessment 3	Placebo	46	57.31	3.03	58.07	2.38	2.77	.09
		Resveratrol	44	64.20	2.84	52.35	2.44		

Table 4.35. Name-to-face recall Pure Chronic analysis. Comparisons by treatment group. Name to face recall performance pure chronic analysis. Baseline (Day 1 assessment 1) and post-dose (Day 84, all assessments) estimated marginal means and standard errors (SE) are presented with F and *p* values, from ANCOVA analysis.

			Baseline (Day 1 A1)			Post Dose		Main Effects	
			n	Mean	SE	Mean	SE	F	p
% Correct	Assessment 1	Placebo	46	59.24	2.48	61.52	2.08	.29	.58
		Resveratrol	44	62.34	2.37	63.14	2.12		
	Assessment 2	Placebo	46	59.24	2.48	59.27	2.11	.96	.32
		Resveratrol	44	62.34	2.37	56.31	2.15		
	Assessment 3	Placebo	46	59.24	2.48	58.49	2.21	1.19	.27
		Resveratrol	44	62.34	2.37	55.04	2.26		
Overall RT	**Assessment 1**	**Placebo**	**46**	**12322.811**	**484.78**	**11528.94**	**394.53**	**3.36**	**.07[t]**
		Resveratrol	**44**	**13557.01**	**567.71**	**12567.18**	**403.45**		
	Assessment 2	Placebo	46	12322.811	484.78	12027.70	394.92	.07	.78
		Resveratrol	44	13557.01	567.71	11868.94	403.85		
	Assessment 3	Placebo	46	12322.811	484.78	11462.18	365.66	.10	.75
		Resveratrol	44	13557.01	567.71	11627.69	373.92		
Correct RT	Assessment 1	Placebo	46	9784.28	493.72	10383.80	504.04	1.37	.24
		Resveratrol	44	12382.22	509.52	11246.46	515.80		
	Assessment 2	Placebo	46	9784.28	493.72	10239.42	537.72	1.48	.22
		Resveratrol	44	12382.22	509.52	9283.11	550.26		
	Assessment 3	Placebo	46	9784.28	493.72	10295.34	476.92	.00	.95
		Resveratrol	44	12382.22	509.52	10335.89	488.04		
Overall forename RT	Assessment 1	Placebo	46	5370.06	236.49	5045.68	211.45	2.96	.08
		Resveratrol	44	5974.11	287.46	5567.73	216.23		
	Assessment 2	Placebo	46	5370.06	236.49	5292.10	204.56	.18	.66
		Resveratrol	44	5974.11	287.46	5166.29	209.19		

	Assessment 3	Placebo	46	5370.06	236.49	5025.38	199.70	.06	.80
		Resveratrol	44	5974.11	287.46	5098.24	204.22		
Correct Forename RT	**Assessment 1**	**Placebo**	**46**	**4770.61**	**182.41**	**4584.95**	**215.20**	**3.79**	**.05**[t]
		Resveratrol	**44**	**5398.69**	**240.99**	**5188.13**	**220.09**		
	Assessment 2	Placebo	46	4770.61	182.41	4612.36	206.95	.15	.69
		Resveratrol	44	5398.69	240.99	4729.52	211.66		
	Assessment 3	Placebo	46	4770.61	182.41	4446.72	186.11	1.33	.25
		Resveratrol	44	5398.69	240.99	4756.06	190.34		
Overall Surname RT	**Assessment 1**	**Placebo**	**46**	**6952.74**	**261.49**	**6476.31**	**206.11**	**3.22**	**.07**[t]
		Resveratrol	**44**	**7582.89**	**292.38**	**7006.71**	**210.76**		
	Assessment 2	Placebo	46	6952.74	261.49	6732.96	204.96	.00	.92
		Resveratrol	44	7582.89	292.38	6705.40	209.58		
	Assessment 3	Placebo	46	6952.74	261.49	6431.39	184.93	.15	.69
		Resveratrol	44	7582.89	292.38	6535.10	189.11		
Correct Surname RT	**Assessment 1**	**Placebo**	**46**	**6438.65**	**247.00**	**6062.27**	**247.86**	**3.42**	**.06**[t]
		Resveratrol	**44**	**7253.21**	**28382**	**6723.05**	**253.51**		
	Assessment 2	Placebo	46	6438.65	247.00	6288.81	215.34	.01	.89
		Resveratrol	44	7253.21	28382	6331.69	220.25		
	Assessment 3	Placebo	46	6438.65	247.00	6220.91	200.73	.18	.66
		Resveratrol	44	7253.21	28382	6345.83	205.30		
Correct Forename %	Assessment 1	Placebo	46	60.30	2.74	64.28	2.55	.33	.56
		Resveratrol	44	62.19	2.63	62.15	2.61		
	Assessment 2	Placebo	46	60.30	2.74	59.13	2.79	.00	.94
		Resveratrol	44	62.19	2.63	59.39	2.85		
	Assessment 3	Placebo	46	60.30	2.74	60.02	2.93	.67	.41
		Resveratrol	44	62.19	2.63	56.56	3.00		
Correct Surname %	Assessment 1	Placebo	46	58.18	2.72	58.89	2.41	2.17	.14
		Resveratrol	44	62.49	2.66	63.99	2.47		
	Assessment 2	Placebo	46	58.18	2.72	59.58	2.69	2.87	.09
		Resveratrol	44	62.49	2.66	53.05	2.75		
	Assessment 3	Placebo	46	58.18	2.72	57.01	2.48	.99	.32
		Resveratrol	44	62.49	2.66	53.46	2.54		

4.3.2.3.9. Picture recognition

Analysis indicated a significant pure chronic effect of treatment for 'Correct Yes' on Day 84, assessment 3, [F (1, 81) = 6.69, p = .01, d = .57], where participants performed more accurately following resveratrol (94.54) in comparison to placebo (89.43).

In addition, a trend towards a significant acute effect of treatment was observed for 'Overall accuracy' on Day 1 during assessment 3, [F(1, 93) = 3.54, p = .06, d = .39], where participants performed better following placebo (94.61), in comparison with resveratrol (92.70).

A significant acute effect of treatment was observed for 'Correct No%' on Day 84 during assessment 3, [F(1, 83) = 4.42, p = .03, d = .46], where participants performed better following placebo (95.89), in comparison with resveratrol (92.81).

A significant acute effect of treatment was observed for 'Correct Yes %' on Day 84 during assessment 3, [F(1, 83) = 6.33, p = .01, d = .55], where participants performed better following resveratrol (94.40), in comparison with placebo (89.75).

In terms of reaction time, a significant pure chronic effect of treatment was observed for 'Yes RT' on Day 84 during assessment 1, [F (1, 81) = 6.35, p = .01, d = .56], where participants performed quicker following resveratrol (812.70), in comparison with placebo (897.33).

A significant acute effect of treatment was observed for 'Yes RT' on Day 1 during assessment 3, [F(1, 93) = 6.02, p = .01, d = .51], where participants performed quicker following resveratrol (825.02), in comparison with placebo (885.07).

No other significant effects were observed for any outcome at any time point, as presented in Tables 4.36, 4.37 and 4.38.

Table 4.36. Picture Recognition on Day 1. Comparisons by treatment group. Picture recognition performance on Day 1. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values for Assessments 2 and 3, for all task outcome measures, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
% Correct	Assessment 2	Placebo	49	94.40	.74	91.91	.96	.58	.44
		Resveratrol	47	95.25	.55	92.97	.98		
	Assessment 3	**Placebo**	**49**	**94.40**	**.74**	**94.61**	**.70**	**3.54**	**.06[t]**
		Resveratrol	**47**	**95.25**	**.55**	**92.70**	**.72**		
Overall RT	Assessment 2	Placebo	49	933.74	19.69	934.08	17.06	2.61	.10
		Resveratrol	47	902.62	17.76	894.54	17.42		
	Assessment 3	Placebo	49	933.74	19.69	922.42	15.72	.00	.99
		Resveratrol	47	902.62	17.76	922.59	16.05		

Correct RT	Assessment 2	Placebo	49	918.04	18.64	912.46	16.39	2.67	.10
		Resveratrol	47	889.06	18.16	873.99	16.74		
	Assessment 3	Placebo	49	918.04	18.64	905.11	15.49	.23	.63
		Resveratrol	47	889.06	18.16	894.41	15.82		
Correct Yes %	Assessment 2	Placebo	49	91.57	1.39	89.27	1.59	.80	.37
		Resveratrol	47	93.46	1.05	91.32	1.62		
	Assessment 3	Placebo	49	91.57	1.39	93.02	1.15	2.80	.09
		Resveratrol	47	93.46	1.05	90.25	1.17		
Correct No %	Assessment 2	Placebo	49	97.23	.68	94.44	.82	.05	.81
		Resveratrol	47	97.05	.59	94.72	.84		
	Assessment 3	Placebo	49	97.23	.68	96.22	.74	1.03	.31
		Resveratrol	47	97.05	.59	95.14	.75		
Yes RT	**Assessment 2**	**Placebo**	**49**	**872.22**	**19.69**	**885.07**	**17.09**	**6.02**	**.01***
		Resveratrol	**47**	**840.96**	**18.37**	**825.02**	**17.45**		
	Assessment 3	Placebo	49	872.22	19.69	880.60	17.87	.17	.67
		Resveratrol	47	840.96	18.37	869.87	18.25		
No RT	Assessment 2	Placebo	49	995.26	28.34	987.20	25.71	.55	.45
		Resveratrol	47	964.29	26.72	959.76	26.54		
	Assessment 3	Placebo	49	995.26	28.34	969.44	24.52	.00	.98
		Resveratrol	47	964.29	26.72	969.91	25.04		

Table 4.37. Picture Recognition on Day 84. Comparisons by treatment group. Picture recognition performance on Day 84. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values for Assessments 2 and 3, for all task outcome measures, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
% Correct	Assessment 2	Placebo	47	95.88	.81	93.51	.77	.56	.45
		Resveratrol	39	97.13	.52	94.39	.85		
	Assessment 3	Placebo	47	95.88	.81	92.89	.78	.27	.60
		Resveratrol	39	97.13	.52	93.51	.86		
Overall RT	Assessment 2	Placebo	47	945.07	23.67	905.88	15.95	1.49	.22
		Resveratrol	39	908.24	24.03	935.06	17.53		
	Assessment 3	Placebo	47	945.07	23.67	910.76	14.88	.12	.72
		Resveratrol	39	908.24	24.03	918.55	16.36		
Correct RT	Assessment 2	Placebo	47	932.52	21.86	889.58	15.43	.78	.37
		Resveratrol	39	896.56	22.74	910.03	16.95		
	Assessment 3	Placebo	47	932.52	21.86	896.18	13.98	.01	.89
		Resveratrol	39	896.56	22.74	899.08	15.36		
	Assessment 2	Placebo	47	94.46	1.25	92.07	1.34	.19	.66

Correct Yes %		Resveratrol	39	96.43	.86	92.96	1.47		
	Assessment 3	**Placebo**	**47**	**94.46**	**1.25**	**89.75**	**1.23**	**6.33**	**.01***
		Resveratrol	**39**	**96.43**	**.86**	**94.40**	**1.36**		
Correct No %	Assessment 2	Placebo	47	97.30	.82	94.89	.96	.49	.48
		Resveratrol	39	97.82	.61	95.90	1.05		
	Assessment 3	**Placebo**	**47**	**97.30**	**.82**	**95.89**	**.98**	**4.42**	**.03***
		Resveratrol	**39**	**97.82**	**.61**	**92.81**	**1.07**		
Yes RT	Assessment 2	Placebo	47	903.01	27.57	854.03	20.76	2.25	.13
		Resveratrol	39	822.50	24.02	901.29	22.87		
	Assessment 3	Placebo	47	903.01	27.57	874.44	21.12	.32	.57
		Resveratrol	39	822.50	24.02	856.24	23.27		
No RT	Assessment 2	Placebo	47	987.13	26.26	960.51	24.14	.01	.89
		Resveratrol	39	993.98	32.29	965.49	26.50		
	Assessment 3	Placebo	47	987.13	26.26	951.08	20.98	.64	.42
		Resveratrol	39	993.98	32.29	976.05	23.03		

Table 4.38. Picture recognition Pure Chronic analysis. Comparisons by treatment group. Picture recognition performance pure chronic analysis. Baseline (Day 1 assessment 1) and post-dose (Day 84, all assessments) estimated marginal means and standard errors (SE) are presented with F and *p* values, from ANCOVA analysis.

			Baseline (Day 1 A1)			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
% Correct	Assessment 1	Placebo	46	94.40	.74	95.99	.65	1.16	.28
		Resveratrol	38	95.25	.55	97.04	.71		
	Assessment 2	Placebo	46	94.40	.74	93.07	.85	1.45	.23
		Resveratrol	38	95.25	.55	94.61	.94		
	Assessment 3	Placebo	46	94.40	.74	92.49	.88	1.01	.31
		Resveratrol	38	95.25	.55	93.82	.97		
Overall RT	Assessment 1	Placebo	46	933.74	19.69	932.28	19.14	.62	.43
		Resveratrol	38	902.62	17.76	909.68	21.09		
	Assessment 2	Placebo	46	933.74	19.69	907.58	17.38	1.14	.28
		Resveratrol	38	902.62	17.76	935.48	19.15		
	Assessment 3	Placebo	46	933.74	19.69	910.88	14.98	.00	.96
		Resveratrol	38	902.62	17.76	911.91	16.50		
Correct RT	Assessment 1	Placebo	46	918.04	18.64	921.08	17.90	.64	.42
		Resveratrol	38	889.06	18.16	899.58	19.72		
	Assessment 2	Placebo	46	918.04	18.64	889.67	15.87	.82	.36
		Resveratrol	38	889.06	18.16	911.30	17.48		

	Assessment 3	Placebo	46	918.04	18.64	895.50	13.93	.01	.90
		Resveratrol	38	889.06	18.16	897.88	15.34		
Correct Yes %	Assessment 1	Placebo	46	91.57	1.39	94.57	1.04	.87	.35
		Resveratrol	38	93.46	1.05	96.03	1.15		
	Assessment 2	Placebo	46	91.57	1.39	91.65	1.42	.45	.50
		Resveratrol	38	93.46	1.05	93.08	1.56		
	Assessment 3	**Placebo**	**46**	**91.57**	**1.39**	**89.43**	**1.32**	**6.69**	**.01***
		Resveratrol	**38**	**93.46**	**1.05**	**94.54**	**1.45**		
Correct No %	Assessment 1	Placebo	46	97.23	.68	97.35	.62	.67	.41
		Resveratrol	38	97.05	.59	98.11	.68		
	Assessment 2	Placebo	46	97.23	.68	94.44	1.01	1.35	.24
		Resveratrol	38	97.05	.59	96.20	1.12		
	Assessment 3	Placebo	46	97.23	.68	95.46	1.07	1.99	.16
		Resveratrol	38	97.05	.59	93.21	1.17		
Yes RT	**Assessment 1**	**Placebo**	**46**	**872.22**	**19.69**	**897.33**	**22.51**	**6.35**	**.01***
		Resveratrol	**38**	**840.96**	**18.37**	**812.70**	**24.78**		
	Assessment 2	Placebo	46	872.22	19.69	868.86	22.20	.16	.68
		Resveratrol	38	840.96	18.37	882.22	24.44		
	Assessment 3	Placebo	46	872.22	19.69	880.72	20.04	1.27	.26
		Resveratrol	38	840.96	18.37	847.01	22.06		
No RT	Assessment 1	Placebo	46	995.26	28.34	972.40	24.09	.60	.43
		Resveratrol	38	964.29	26.72	1000.38	26.53		
	Assessment 2	Placebo	46	995.26	28.34	951.21	26.10	.65	.42
		Resveratrol	38	964.29	26.72	982.78	28.75		
	Assessment 3	Placebo	46	995.26	28.34	946.86	21.38	.51	.47
		Resveratrol	38	964.29	26.72	969.76	23.55		

4.3.2.3.10. Word recognition

Analysis indicated a significant pure chronic effect on treatment on ‘Overall %’ at Day 84, assessment 2, [F(1, 85) = 6.68, p = .01, d = .56], where participants performed better following placebo (76.55), in comparison with resveratrol (71.51). Additionally, a significant pure chronic effect of treatment was observed on ‘Correct No %’ at Day 84, assessment 2, [F (1, 85) = 6.48, p = .01, d = .55], where participants performed better following placebo (79.14), in comparison with resveratrol (71.55).

In terms of acute effects within testing visits. Analysis indicated a significant acute effect of treatment for ‘Overall %’ on Day 84 during assessment 2, [F(1, 85) = 5.39, p = .02, d = .50],

where participants performed better following placebo (76.37), in comparison with resveratrol (71.71).

A significant acute effect of treatment was observed for 'Correct No%' on Day 1 during assessment 3, [F(1, 99) = 5.12, p = .02, d = .45], where participants performed better following placebo (75.51), in comparison with resveratrol (69.71). Similarly, a significant acute effect of treatment was observed for 'Correct No%' on Day 84 during assessment 2, [F(1, 85) = 5.10, p = .02, d = .49], where participants performed better following placebo (78.46), in comparison with resveratrol (72.32).

In addition, a trend towards a significant acute effect of treatment was observed for 'Overall %' on Day 1 during assessment 2, [F (1, 99) = 3.59, p = .06, d = .38], where participants performed better following placebo (71.76), in comparison with resveratrol (68.36).

No other significant effects were observed on any outcome at any time point, as presented in Tables 4.39, 4.40 and 4.41.

Table 4.39. Word Recognition on Day 1. Comparisons by treatment group. Word recognition performance on Day 1. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and p values for Assessments 2 and 3, for all task outcome measures, from ANCOVA analysis..

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	P
% Correct	**Assessment 2**	**Placebo**	**51**	**72.57**	**1.64**	**71.76**	**1.26**	**3.59**	**.06[t]**
		Resveratrol	**51**	**73.45**	**1.14**	**68.36**	**1.26**		
	Assessment 3	Placebo	51	72.57	1.64	72.87	1.35	1.51	.22
		Resveratrol	51	73.45	1.14	70.52	1.35		
Overall RT	Assessment 2	Placebo	51	999.01	22.32	998.19	20.08	1.57	.21
		Resveratrol	51	1033.80	30.90	962.55	20.08		
	Assessment 3	Placebo	51	999.01	22.32	999.79	16.61	.13	.71
		Resveratrol	51	1033.80	30.90	991.14	16.61		
Correct RT	Assessment 2	Placebo	51	963.66	21.07	947.43	18.61	.00	.93
		Resveratrol	51	970.64	26.25	949.52	18.61		
	Assessment 3	Placebo	51	963.66	21.07	958.39	17.80	.01	.89
		Resveratrol	51	970.64	26.25	955.07	17.80		
Correct Yes %	Assessment 2	Placebo	51	64.40	2.43	67.51	1.88	.34	.55
		Resveratrol	51	66.91	1.84	65.94	1.88		
		Placebo	51	64.40	2.43	67.83	2.06	.04	.82

	Assessment 3	Resveratrol	51	66.91	1.84	67.19	2.06		
Correct No %	Assessment 2	Placebo	51	80.75	2.56	78.13	1.75	1.38	.24
		Resveratrol	51	79.99	1.76	75.19	1.75		
	Assessment 3	**Placebo**	**51**	**80.75**	**2.56**	**75.51**	**1.81**	**5.12**	**.02***
		Resveratrol	**51**	**79.99**	**1.76**	**69.71**	**1.81**		
Yes RT	Assessment 2	Placebo	51	986.00	23.37	995.33	22.23	.87	.35
		Resveratrol	51	1016.76	32.02	965.87	22.23		
	Assessment 3	Placebo	51	986.00	23.37	982.97	22.85	2.26	.13
		Resveratrol	51	1016.76	32.02	934.37	22.85		
No RT	Assessment 2	Placebo	51	1012.01	27.88	1010.87	26.15	.22	.63
		Resveratrol	51	1050.84	34.26	993.27	26.15		
	Assessment 3	Placebo	51	1012.01	27.88	1002.82	24.13	.19	.66
		Resveratrol	51	1050.84	34.26	1017.83	24.13		

Table 4.40. Word Recognition on Day 84. Comparisons by treatment group. Word recognition performance on Day 84. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values for Assessments 2 and 3, for all task outcome measures, from ANCOVA analysis..

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
% Correct	**Assessment 2**	**Placebo**	**47**	**76.45**	**1.47**	**76.37**	**1.36**	**5.39**	**.02***
		Resveratrol	**41**	**77.06**	**1.35**	**71.71**	**1.46**		
	Assessment 3	Placebo	47	76.45	1.47	72.02	1.34	.12	.72
		Resveratrol	41	77.06	1.35	71.33	1.43		
Overall RT	Assessment 2	Placebo	47	991.68	24.07	972.35	18.02	.00	.94
		Resveratrol	41	1002.66	28.23	970.40	19.30		
	Assessment 3	Placebo	47	991.68	24.07	957.92	18.99	.39	.53
		Resveratrol	41	1002.66	28.23	975.49	20.33		
Correct RT	Assessment 2	Placebo	47	964.23	24.28	935.08	19.34	.16	.69
		Resveratrol	41	978.94	29.83	946.45	20.71		
	Assessment 3	Placebo	47	964.23	24.28	926.54	18.64	.05	.82
		Resveratrol	41	978.94	29.83	932.79	19.96		
Correct Yes %	Assessment 2	Placebo	47	66.94	2.46	73.79	2.02	51	.47
		Resveratrol	41	70.79	2.41	71.66	2.16		
	Assessment 3	Placebo	47	66.94	2.46	71.04	1.97	.82	.36
		Resveratrol	41	70.79	2.41	73.67	2.11		
Correct No %	**Assessment 2**	**Placebo**	**47**	**85.97**	**1.96**	**78.46**	**1.84**	**5.10**	**.02***
		Resveratrol	**41**	**83.33**	**1.56**	**72.32**	**1.97**		

	Assessment 3	Placebo	47	85.97	1.96	72.80	2.14	1.29	.25
		Resveratrol	41	83.33	1.56	69.22	2.29		
Yes RT	Assessment 2	Placebo	47	993.25	30.06	949.11	22.50	.35	.55
		Resveratrol	41	973.31	30.41	929.42	24.09		
	Assessment 3	Placebo	47	993.25	30.06	919.94	21.24	.16	.68
		Resveratrol	41	973.31	30.41	932.44	22.74		
No RT	Assessment 2	Placebo	47	990.11	29.90	995.77	25.08	.17	.67
		Resveratrol	41	1032.01	33.40	1011.16	26.86		
	Assessment 3	Placebo	47	990.11	29.90	995.24	27.74	.34	.55
		Resveratrol	41	1032.01	33.40	1019.28	29.71		

Table 4.41. Word Recognition Pure Chronic analysis. Comparisons by treatment group. Word recognition performance pure chronic analysis. Baseline (Day 1 assessment 1) and post-dose (Day 84, all assessments) estimated marginal means and standard errors (SE) are presented with F and *p* values, from ANCOVA analysis.

			Baseline (Day 1 A1)			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
% Correct	Assessment 1	Placebo	47	72.57	1.64	76.85	1.31	.00	.96
		Resveratrol	41	73.45	1.14	76.77	1.41		
	Assessment 2	**Placebo**	**47**	**72.57**	**1.64**	**76.55**	**1.33**	**6.68**	**.01***
		Resveratrol	**41**	**73.45**	**1.14**	**71.51**	**1.42**		
	Assessment 3	Placebo	47	72.57	1.64	72.17	1.29	.28	.59
		Resveratrol	41	73.45	1.14	71.16	1.38		
Overall RT	Assessment 1	Placebo	47	999.01	22.32	994.68	18.76	.04	.83
		Resveratrol	41	1033.80	30.90	1000.34	20.09		
	Assessment 2	Placebo	47	999.01	22.32	971.15	19.27	.00	.93
		Resveratrol	41	1033.80	30.90	971.77	20.63		
	Assessment 3	Placebo	47	999.01	22.32	956.67	20.44	.45	.50
		Resveratrol	41	1033.80	30.90	976.91	21.89		
Correct RT	Assessment 1	Placebo	47	963.66	21.07	966.64	20.05	.33	.56
		Resveratrol	41	970.64	26.25	983.63	21.47		
	Assessment 2	Placebo	47	963.66	21.07	929.81	19.09	.65	.42
		Resveratrol	41	970.64	26.25	952.49	20.44		
	Assessment 3	Placebo	47	963.66	21.07	921.72	19.30	.34	.55
		Resveratrol	41	970.64	26.25	938.31	20.67		
Correct Yes %	Assessment 1	Placebo	47	64.40	2.43	68.10	2.15	.21	.64
		Resveratrol	41	66.91	1.84	69.57	2.31		
	Assessment 2	Placebo	47	64.40	2.43	73.93	2.01	.67	.41
		Resveratrol	41	66.91	1.84	71.50	2.16		

	Assessment 3	Placebo	47	64.40	2.43	70.91	2.13	.86	.35
		Resveratrol	41	66.91	1.84	73.83	2.28		
Correct No %	Assessment 1	Placebo	47	80.75	2.56	85.83	1.50	.92	.33
		Resveratrol	41	79.99	1.76	83.71	1.61		
	Assessment 2	**Placebo**	**47**	**80.75**	**2.56**	**79.14**	**2.03**	**6.48**	**.01***
		Resveratrol	**41**	**79.99**	**1.76**	**71.55**	**2.17**		
	Assessment 3	Placebo	47	80.75	2.56	23.26	2.04	2.32	.13
		Resveratrol	41	79.99	1.76	68.69	2.18		
Yes RT	Assessment 1	Placebo	47	986.00	23.37	989.15	25.92	.14	.70
		Resveratrol	41	1016.76	32.02	974.61	27.75		
	Assessment 2	Placebo	47	986.00	23.37	953.07	23.71	.65	.41
		Resveratrol	41	1016.76	32.02	924.88	25.38		
	Assessment 3	Placebo	47	986.00	23.37	923.50	23.94	.01	.89
		Resveratrol	41	1016.76	32.02	928.36	25.63		
No RT	Assessment 1	Placebo	47	1012.01	27.88	999.94	22.97	.61	.43
		Resveratrol	41	1050.84	34.26	1026.38	24.60		
	Assessment 2	Placebo	47	1012.01	27.88	988.95	25.19	.66	.41
		Resveratrol	41	1050.84	34.26	1018.98	26.97		
	Assessment 3	Placebo	47	1012.01	27.88	989.22	27.18	.86	.35
		Resveratrol	41	1050.84	34.26	1026.17	29.11		

4.3.2.3.11. Immediate word recall

Analysis indicated a significant acute effect of treatment of correct responses on Day 84, during assessment 2, [F(1, 88) = 3.89, p = .05, d = .42], where participants performed better following placebo (5.98), in comparison with resveratrol (5.39).

A significant pure chronic effect of treatment on number of errors was observed at Day 84, assessment 1, [F(1, 88) = 5.58, p = .02, d = .51], where participants had fewer errors following placebo (.51), in comparison with resveratrol (.93).

No other significant effects were observed on any outcome at any time point, as shown in Table 4.42.

Table 4.42. Immediate Word Recall. Comparisons by treatment group. Immediate Word Recall performance on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Day 1						
Correct	Assessment 2	Placebo	54	5.44	.27	5.38	.20	1.12	.29
		Resveratrol	54	5.50	.28	5.07	.20		
	Assessment 3	Placebo	54	5.44	.27	4.98	.22	.00	.92
		Resveratrol	54	5.50	.28	5.01	.22		
Incorrect	Assessment 2	Placebo	54	.65	.13	.65	.11	.72	.39
		Resveratrol	54	.80	.13	.79	.11		
	Assessment 3	Placebo	54	.65	.13	.79	.12	.14	.70
		Resveratrol	54	.80	.13	.85	.12		
			Day 84						
Correct	**Assessment 2**	**Placebo**	**47**	**5.77**	**.30**	**5.98**	**.21**	**3.83**	**.05[t]**
		Resveratrol	**44**	**6.08**	**.32**	**5.39**	**.21**		
	Assessment 3	Placebo	47	5.77	.30	5.24	.25	1.31	.25
		Resveratrol	44	6.08	.32	5.66	.26		
Incorrect	Assessment 2	Placebo	47	.57	.14	.57	.14	.03	.86
		Resveratrol	44	.98	.14	.61	.14		
	Assessment 3	Placebo	47	.57	.14	.85	.13	.86	.35
		Resveratrol	44	.98	.14	.66	.14		
			Pure Chronic						
			Baseline (Day 1 A1)			Post dose		Main Effects	
			n	Mean	SE	Mean	SE	F	p
Correct	Assessment 1	Placebo	47	5.44	.27	5.79	.27	.64	.42
		Resveratrol	44	5.50	.28	6.11	.28		
	Assessment 2	Placebo	47	5.44	.27	5.91	.24	1.69	.19
		Resveratrol	44	5.50	.28	5.46	.24		
	Assessment 3	Placebo	47	5.44	.27	5.19	.26	2.01	.16
		Resveratrol	44	5.50	.28	5.72	.27		
Incorrect	**Assessment 1**	**Placebo**	**47**	**.65**	**.13**	**.51**	**.12**	**5.58**	**.02***
		Resveratrol	**44**	**.80**	**.13**	**.93**	**.12**		
	Assessment 2	Placebo	47	.65	.13	.53	.13	.40	.52
		Resveratrol	44	.80	.13	.65	.14		

	Assessment 3	Placebo	47	.65	.13	.80	.13	.25	.61
		Resveratrol	44	.80	.13	.70	.14		

4.3.2.3.12. Delayed word recall

Analysis indicated a significant acute effect of treatment of correct responses on Day 1, during assessment 3, [*F* (1, 103) = 5.70, *p* = .01, *d* = .47], where participants performed better following resveratrol (1.82), in comparison with placebo (1.13).

No other significant effects were observed on any outcome at any time point, as shown in Table 4.43.

Table 4.43. Delayed Word Recall. Comparisons by treatment group. Delayed Word Recall performance on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Day 1						
Correct	Assessment 2	Placebo	53	3.42	.29	2.27	.21	.95	.33
		Resveratrol	53	3.41	.25	1.98	.21		
	Assessment 3	**Placebo**	**53**	**3.42**	**.29**	**1.13**	**.20**	**5.70**	**.01***
		Resveratrol	**53**	**3.41**	**.25**	**1.82**	**.20**		
Incorrect	Assessment 2	Placebo	53	.82	.14	1.45	.20	2.76	.09
		Resveratrol	53	1.22	.19	1.94	.20		
	Assessment 3	Placebo	53	.82	.14	2.04	.26	.04	.83
		Resveratrol	53	1.22	.19	2.12	.26		
			Day 84						
Correct	Assessment 2	Placebo	48	3.77	.33	2.69	.25	.06	.79
		Resveratrol	43	4.06	.36	2.59	.26		
	Assessment 3	Placebo	48	3.77	.33	2.17	.24	.43	.50
		Resveratrol	43	4.06	.36	1.93	.26		
Incorrect	Assessment 2	Placebo	48	.82	.14	1.34	.20	.21	.64
		Resveratrol	43	1.09	.16	1.47	.21		
		Placebo	48	.82	.14	1.53	.25	2.24	.13

	Assessment 3	Resveratrol	43	1.09	.16	2.08	.26		

			Pure Chronic						
			Baseline (Day 1 A1)			Post dose		Main Effects	
			n	Mean	SE	Mean	SE	F	p
Correct	Assessment 1	Placebo	48	3.42	.29	3.83	.29	.21	.64
		Resveratrol	43	3.41	.25	4.02	.30		
	Assessment 2	Placebo	48	3.42	.29	2.65	.26	.00	.95
		Resveratrol	43	3.41	.25	2.63	.27		
	Assessment 3	Placebo	48	3.42	.29	2.15	.22	.38	.53
		Resveratrol	43	3.41	.25	1.95	.24		
Incorrect	Assessment 1	Placebo	48	.82	.14	.93	.13	.00	.98
		Resveratrol	43	1.22	.19	.93	.14		
	Assessment 2	Placebo	48	.82	.14	1.38	.20	.01	.89
		Resveratrol	43	1.22	.19	1.42	.21		
	Assessment 3	Placebo	48	.82	.14	1.58	.26	1.21	.27
		Resveratrol	43	1.22	.19	2.01	.28		

4.3.2.3.13. VAS – Task difficulty

Analysis indicated a significant pure chronic effect of treatment at Day 84, assessment 2, repetition 2, [F (1, 88) = 4.70, p = .03, d = .46], where participants found the tasks less difficult following placebo (67.45), in comparison with resveratrol (75.23). Additionally, a trend towards a significant pure chronic effect of treatment was observed at Day 84, assessment 3, repetition 2, [F (1, 88) = 3.43, p = .06, d = .39], where participants found the tasks less difficult following placebo (73.17), in comparison with resveratrol (79.93). A trend towards a significant pure chronic effect of treatment was observed at Day 84, assessment 1, repetition 3, [F (1, 86) = 3.24, p = .07, d = .38], where participants found the tasks less difficult following placebo (68.61), in comparison with resveratrol (73.87).

A trend towards a significant acute effect of treatment was observed at Day 84, assessment 2, repetition 1 [F (1, 90) = 3.26, p = .07, d = .38], where participants found the tasks less difficult following placebo (64.39), in comparison with resveratrol (69.62).

No other effects were observed at any time point, as presented in Table 4.44.

Table 4.44. Task Difficulty VAS. Comparisons by treatment group. Task difficulty VAS response on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Day 1						
Rep 1	Assessment 2	Placebo	55	61.53	2.79	68.40	2.02	.44	.50
		Resveratrol	54	66.65	2.14	70.31	2.03		
	Assessment 3	Placebo	55	61.53	2.79	67.26	1.72	1.32	.25
		Resveratrol	54	66.65	2.14	70.10	1.74		
Rep 2	Assessment 2	Placebo	54	68.16	2.52	74.89	1.83	.55	.45
		Resveratrol	53	71.11	2.25	76.84	1.85		
	Assessment 3	Placebo	55	68.16	2.52	72.42	1.60	.63	.42
		Resveratrol	54	71.11	2.25	74.24	1.61		
Rep 3	Assessment 2	Placebo	54	69.11	2.48	78.68	1.81	.01	.89
		Resveratrol	53	74.93	2.01	79.01	1.83		
	Assessment 3	Placebo	55	69.11	2.48	75.91	1.64	.00	.98
		Resveratrol	54	74.93	2.01	75.87	1.66		
			Day 84						
Rep 1	**Assessment 2**	**Placebo**	**48**	**63.37**	**2.49**	**64.39**	**2.00**	**3.26**	**.07**[t]
		Resveratrol	**45**	**66.44**	**2.87**	**69.62**	**2.07**		
	Assessment 3	Placebo	48	63.37	2.49	67.04	2.14	2.75	.10
		Resveratrol	45	66.44	2.87	72.17	2.21		
Rep 2	Assessment 2	Placebo	48	63.02	2.69	69.28	1.76	1.63	.20
		Resveratrol	45	68.33	3.06	73.09	1.91		
	Assessment 3	Placebo	48	63.02	2.69	74.37	2.18	2.09	.15
		Resveratrol	45	68.33	3.06	78.53	2.36		
Rep 3	Assessment 2	Placebo	48	66.33	2.56	74.55	1.61	.16	.68
		Resveratrol	45	74.11	2.69	75.50	1.67		
	Assessment 3	Placebo	48	66.33	2.56	79.94	2.07	.08	.77
		Resveratrol	45	74.11	2.69	80.83	2.14		
			Pure Chronic						
			Baseline (Day 1 A1)			Post dose		Main Effects	
			n	Mean	SE	Mean	SE	F	p
Rep 1	Assessment 1	Placebo	48	61.53	2.79	64.31	2.20	.12	.72
		Resveratrol	45	66.65	2.14	65.44	2.27		
		Placebo	48	61.53	2.79	64.30	2.26	2.75	.10

	Assessment 2	Resveratrol	45	66.65	2.14	69.72	2.34		
	Assessment 3	Placebo	48	61.53	2.79	66.78	2.59	2.30	.13
		Resveratrol	45	66.65	2.14	72.45	2.67		
Rep 2	Assessment 1	Placebo	48	68.16	2.52	64.26	2.16	2.43	.12
		Resveratrol	45	71.11	2.25	69.26	2.33		
	Assessment 2	**Placebo**	**48**	**68.16**	**2.52**	**67.45**	**2.42**	**4.70**	**.03***
		Resveratrol	**45**	**71.11**	**2.25**	**75.23**	**2.61**		
	Assessment 3	**Placebo**	**48**	**68.16**	**2.52**	**73.17**	**2.46**	**3.43**	**.06[t]**
		Resveratrol	**45**	**71.11**	**2.25**	**79.93**	**2.66**		
Rep 3	**Assessment 1**	**Placebo**	**48**	**69.11**	**2.48**	**68.61**	**2.01**	**3.24**	**.07[t]**
		Resveratrol	**45**	**74.93**	**2.01**	**73.87**	**2.08**		
	Assessment 2	Placebo	48	69.11	2.48	72.85	2.09	2.16	.14
		Resveratrol	45	74.93	2.01	77.32	2.16		
	Assessment 3	Placebo	48	69.11	2.48	78.92	2.17	.90	.34
		Resveratrol	45	74.93	2.01	81.91	2.24		

4.3.2.3.14. VAS - Mental fatigue

Analysis indicated a significant pure chronic effect of treatment at Day 84, assessment 2, repetition 1, [F (1, 91) = 4.63, p = .03, d = .45], where participants were less fatigued following placebo (61.67), in comparison with resveratrol (68.66).

In addition, a trend towards a significant acute effect of treatment was observed at Day 84, assessment 2, repetition 1, [F (1, 91) = 3.91, p = .05, d = .41], where participants were less fatigued following placebo (62.28), in comparison with resveratrol (68.00).

No other effects were observed at any time point, as shown in Table 4.45.

Table 4.45. Mental Fatigue VAS. Comparisons by treatment group. Mental Fatigue VAS response on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

				Baseline		Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Day 1						
Rep 1	Assessment 2	Placebo	54	58.15	2.27	69.54	2.01	1.45	.23
		Resveratrol	54	58.47	2.14	72.99	2.02		
	Assessment 3	Placebo	54	58.15	2.27	63.61	1.90	.63	.42
		Resveratrol	54	58.47	2.14	65.76	1.92		
Rep 2	Assessment 2	Placebo	54	65.22	2.42	77.19	1.90	.25	.61
		Resveratrol	54	67.49	2.20	78.56	1.90		
	Assessment 3	Placebo	54	65.22	2.42	69.63	1.79	1.68	.19
		Resveratrol	54	67.49	2.20	72.93	1.79		
Rep 3	Assessment 2	Placebo	54	72.39	2.03	82.45	1.59	.75	.38
		Resveratrol	54	75.91	1.90	80.49	1.59		
	Assessment 3	Placebo	54	72.39	2.03	77.62	1.54	.13	.71
		Resveratrol	54	75.91	1.90	78.41	1.54		
			Day 84						
Rep 1	**Assessment 2**	**Placebo**	**49**	**54.53**	**2.02**	**62.28**	**2.00**	**3.91**	**.05**[t]
		Resveratrol	**45**	**56.42**	**2.86**	**68.00**	**2.08**		
	Assessment 3	Placebo	49	54.53	2.02	69.79	2.25	2.01	.16
		Resveratrol	45	56.42	2.86	74.41	2.35		
Rep 2	Assessment 2	Placebo	49	58.90	2.72	71.08	2.02	.06	.80
		Resveratrol	45	65.29	2.90	71.81	2.13		
	Assessment 3	Placebo	49	58.90	2.72	78.07	1.95	.32	.56
		Resveratrol	45	65.29	2.90	79.71	2.06		
Rep 3	Assessment 2	Placebo	48	66.82	2.49	75.31	1.88	.24	.62
		Resveratrol	44	71.93	2.82	76.67	1.96		
	Assessment 3	Placebo	48	66.82	2.49	80.61	1.83	1.13	.29
		Resveratrol	44	71.93	2.82	83.46	1.91		
			Pure Chronic						
				Baseline (Day 1 A1)		Post dose		Main Effects	
			n	Mean	SE	Mean	SE	F	p
Rep 1		Placebo	49	58.15	2.27	54.42	2.11	.42	.51

	Assessment 1	Resveratrol	45	58.47	2.14	56.42	2.20		
	Assessment 2	**Placebo**	**49**	**58.15**	**2.27**	**61.67**	**2.24**	**4.63**	**.03***
		Resveratrol	**45**	**58.47**	**2.14**	**68.66**	**2.34**		
	Assessment 3	Placebo	49	58.15	2.27	69.18	2.49	2.68	.10
		Resveratrol	45	58.47	2.14	75.09	2.60		
Rep 2	Assessment 1	Placebo	49	65.22	2.42	59.85	2.28	3.06	.08
		Resveratrol	44	67.49	2.20	65.68	2.41		
	Assessment 2	Placebo	49	65.22	2.42	69.47	2.38	1.40	.23
		Resveratrol	44	67.49	2.20	73.60	2.52		
	Assessment 3	Placebo	49	65.22	2.42	77.10	1.99	1.60	.20
		Resveratrol	44	67.49	2.20	80.79	2.10		
Rep 3	Assessment 1	Placebo	47	72.39	2.03	68.99	2.12	1.21	.27
		Resveratrol	44	75.91	1.90	72.38	2.20		
	Assessment 2	Placebo	47	72.39	2.03	74.43	2.21	.97	.32
		Resveratrol	44	75.91	1.90	77.59	2.28		
	Assessment 3	Placebo	47	72.39	2.03	80.11	1.83	2.00	.16
		Resveratrol	44	75.91	1.90	83.87	1.90		

4.3.2.4. Global Cognitive Domains

4.3.2.4.1. Accuracy of Attention

No effects observed at any time point.

Table 4.46. Accuracy of Attention. Comparisons by treatment group. Data presented are Z composite scores, calculated by clustering relevant tasks. Accuracy of Attention cognitive domain on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
Day 1 Acute								
Assessment 2	Placebo	49	.12	.73	.06	.05	.19	.66
	Resveratrol	44	-.01	.72	.03	.05		
Assessment 3	Placebo	49	.12	.73	.03	.05	.32	.56
	Resveratrol	44	-.01	.72	.07	.05		
Day 84 Acute								
Assessment 2	Placebo	44	.14	.75	.09	.06	.12	.72
	Resveratrol	33	-.07	.71	.06	.07		
Assessment 3	Placebo	44	.14	.75	.07	.06	.00	.99
	Resveratrol	33	-.07	.71	.07	.07		
Pure Chronic								
		Baseline (Day 1 A1)			Post dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
Assessment 1	Placebo	43	.12	.73	.07	.06		
	Resveratrol	32	-.01	.72	.00	.07	.52	.47
Assessment 2	Placebo	43	.12	.73	.08	.06	.45	.50
	Resveratrol	32	-.01	.72	.02	.07		
Assessment 3	Placebo	43	.12	.73	.06	.07	.15	.69
	Resveratrol	32	-.01	.72	.02	.08		

4.3.2.4.2. Speed of Attention

A trend towards a significant pure-chronic effect of treatment was observed at Day 84, assessment 2, [F (1, 71) = 3.46, p = .06, d = .43], where participants performed quicker following placebo (-.14), in comparison with resveratrol (.98).

A trend towards a significant acute effect of treatment was observed at Day 84, assessment 2, [F (1, 75) = 4.19, p = .05, d = .47], where participants performed quicker following placebo (-.06), in comparison with resveratrol (.10).

No other effects were observed at any time point, as shown in Table 4.47.

Table 4.47. Speed of Attention. Comparisons by treatment group. Data presented are Z composite scores, calculated by clustering relevant tasks. Speed of Attention cognitive domain on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
		Day 1 Acute						
Assessment 2	Placebo	50	.07	.12	-.05	.05	2.44	.12
	Resveratrol	47	-.11	.10	.06	.05		
Assessment 3	Placebo	50	.07	.12	-.08	.07	1.26	.26
	Resveratrol	47	-.11	.10	.02	.07		
		Day 84 Acute						
Assessment 2	**Placebo**	**44**	**.04**	**.12**	**-.06**	**.05**	**4.19**	**.05[t]**
	Resveratrol	**34**	**-.08**	**.13**	**.10**	**.06**		
Assessment 3	Placebo	44	.04	.12	-.05	.05	1.37	.24
	Resveratrol	34	-.08	.13	.04	.06		
		Pure Chronic						
		Baseline (Day 1 A1)			Post dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
Assessment 1	Placebo	42	.07	.12	-.05	.07	.43	.51
	Resveratrol	32	-.11	.10	.02	.08		
Assessment 2	**Placebo**	**42**	**.07**	**.12**	**-.14**	**.08**	**3.46**	**.06[t]**
	Resveratrol	**32**	**-.11**	**.10**	**.09**	**.09**		
Assessment 3	Placebo	42	.07	.12	-.12	.07	1.25	.26
	Resveratrol	32	-.11	.10	.00	.08		

4.3.2.4.3. Working Memory

A significant pure-chronic effect of treatment was observed at Day 84, assessment 1, [F (1, 69) = 6.16, p = .01, d = .59], where participants performed better following resveratrol (.27), in comparison with placebo (-.04).

No other effects were observed at any time point, as shown in Table 4.48.

Table 4.48. Working Memory. Comparisons by treatment group. Data presented are Z composite scores, calculated by clustering relevant tasks. Working Memory cognitive domain on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
		Day 1 Acute						
Assessment 2	Placebo	42	.05	.09	.03	.08	.08	.77
	Resveratrol	50	-.05	.11	.07	.08		
Assessment 3	Placebo	42	.05	.09	.10	.10	.27	.60
	Resveratrol	50	-.05	.11	.03	.10		
		Day 84 Acute						
Assessment 2	Placebo	38	-.10	.13	.03	.12	.04	.82
	Resveratrol	35	.10	.09	.00	.12		
Assessment 3	Placebo	38	-.10	.13	.20	.07	1.99	.16
	Resveratrol	35	.10	.09	.04	.07		
		Pure Chronic						
		Baseline (Day 1 A1)			Post dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
Assessment 1	**Placebo**	**38**	**.05**	**.09**	**-.04**	**.08**	**6.16**	**.01***
	Resveratrol	**34**	**-.05**	**.11**	**.27**	**.09**		
Assessment 2	Placebo	38	.05	.09	-.06	.12	.94	.33
	Resveratrol	34	-.05	.11	.10	.12		
Assessment 3	Placebo	38	.05	.09	.12	.07	.00	.98
	Resveratrol	34	-.05	.11	.12	.07		

4.3.2.4.4. Speed of Memory

A trend towards a significant acute effect of treatment was observed at Day 84, assessment 2, [F (1, 86) = 3.94, p = .05, d = .42], where participants performed quicker following placebo (-.11), in comparison with resveratrol (.05). In addition, a trend towards a significant acute effect of treatment was observed at Day 84, assessment 3, [F (1, 86) = 3.60, p = .06, d = .41], where participants performed quicker following placebo (-.07), in comparison with resveratrol (.11).

No other effects observed at any time point, as shown in Table 4.49.

Table 4.49. Speed of Memory. Comparisons by treatment group. Data presented are Z composite scores, calculated by clustering relevant tasks. Speed of Memory cognitive domain on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
		Day 1 Acute						
Assessment 2	Placebo	53	.03	.10	.00	.06	.02	.87
	Resveratrol	52	.00	.10	.01	.06		
Assessment 3	Placebo	53	.03	.10	-.00	.06	.70	.40
	Resveratrol	52	.00	.10	-.07	.06		
		Day 84 Acute						
Assessment 2	**Placebo**	**48**	**.02**	**.10**	**-.11**	**.05**	**3.94**	**.05[t]**
	Resveratrol	**41**	**-.11**	**.10**	**.05**	**.06**		
Assessment 3	**Placebo**	**48**	**.02**	**.10**	**-.07**	**.06**	**3.60**	**.06[t]**
	Resveratrol	**41**	**-.11**	**.10**	**.11**	**.07**		
		Pure Chronic						
		Baseline (Day 1 A1)			Post dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
Assessment 1	Placebo	48	.03	.10	.01	.06	1.35	.24
	Resveratrol	41	.00	.10	-.09	.06		
Assessment 2	Placebo	48	.03	.10	-.07	.07	.58	.44
	Resveratrol	41	.00	.10	.00	.07		
Assessment 3	Placebo	48	.03	.10	-.03	.06	.87	.35
	Resveratrol	41	.00	.10	.06	.07		

4.3.2.4.5. Episodic Memory

A significant acute effect of treatment was observed at Day 1, assessment 2, [F (1, 105) = 10.32, p = .002, d = .62], where participants performed better following placebo (.12), in comparison with resveratrol (-.14). In addition, a significant acute effect of treatment was observed at Day 84, assessment 2, [F (1, 91) = 4.73, p = .03, d = .45], where participants performed better following placebo (.08), in comparison with resveratrol (-.09).

No other effects observed at any time point, as presented in Table 4.50.

Table 4.50. Episodic Memory. Comparisons by treatment group. Data presented are Z composite scores, calculated by clustering relevant tasks. Episodic Memory cognitive domain on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
		Day 1 Acute						
Assessment 2	**Placebo**	**55**	**-.05**	**.08**	**.12**	**.05**	**10.32**	**.002***
	Resveratrol	**53**	**.04**	**.05**	**-.14**	**.06**		
Assessment 3	Placebo	55	-.05	.08	.05	.06	1.69	.19
	Resveratrol	53	.04	.05	-.05	.06		
		Day 84 Acute						
Assessment 2	**Placebo**	**49**	**-.05**	**.09**	**.08**	**.05**	**4.73**	**.03***
	Resveratrol	**45**	**.05**	**.08**	**-.09**	**.05**		
Assessment 3	Placebo	49	-.05	.09	.03	.06	.75	.38
	Resveratrol	45	.05	.08	-.03	.06		
		Pure Chronic						
		Baseline (Day 1 A1)			Post dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
Assessment 1	Placebo	49	-.05	.08	-.03	.06	.37	.54
	Resveratrol	45	.04	.05	.02	.06		
Assessment 2	Placebo	49	-.05	.08	.07	.06	2.71	.10
	Resveratrol	45	.04	.05	-.08	.06		
Assessment 3	Placebo	49	-.05	.08	.02	.06	.23	.63
	Resveratrol	45	.04	.05	-.02	.07		

4.3.2.4.6. Overall Accuracy

A significant acute effect of treatment was observed at Day 1, assessment 2, [F (1, 106) = 4.49, p = .03, d = .41], where participants performed better following placebo (.09), in comparison with resveratrol (-.11). In addition, a significant acute effect of treatment was observed at Day 84, assessment 2, [F (1, 91) = 10.71, p = .002, d = .69], where participants performed better following placebo (.07), in comparison with resveratrol (-.10). And, a significant acute effect of treatment was observed at Day 84, assessment 3, [F (1, 91) = 6.08, p = .01, d = .52], where participants performed better following placebo (.06), in comparison with resveratrol (-.08).

No other effects observed at any time point, as presented in Table 4.51.

Table 4.51. Overall Accuracy. Comparisons by treatment group. Data presented are Z composite scores, calculated by clustering relevant tasks. Overall Accuracy cognitive domain on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
		Day 1 Acute						
Assessment 2	**Placebo**	**55**	**.00**	**.05**	**.09**	**.06**	**4.49**	**.03***
	Resveratrol	**54**	**-.03**	**.07**	**-.11**	**.07**		
Assessment 3	Placebo	55	.00	.05	.04	.06	1.20	.27
	Resveratrol	54	-.03	.07	-.06	.06		
		Day 84 Acute						
Assessment 2	**Placebo**	**49**	**-.00**	**.08**	**.07**	**.03**	**10.71**	**.002***
	Resveratrol	**45**	**-.02**	**.07**	**-.10**	**.03**		
Assessment 3	**Placebo**	**49**	**-.00**	**.08**	**.06**	**.04**	**6.08**	**.01***
	Resveratrol	**45**	**-.02**	**.07**	**-.08**	**.04**		
		Pure Chronic						
		Baseline (Day 1 A1)			Post dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
Assessment 1	Placebo	49	.00	.05	-.00	.07	.03	.84
	Resveratrol	45	-.03	.07	-.02	.08		
Assessment 2	Placebo	49	.00	.05	.07	.07	3.06	.08
	Resveratrol	45	-.03	.07	-.11	.07		
Assessment 3	Placebo	49	.00	.05	.07	.07	2.34	.12
	Resveratrol	45	-.03	.07	-.09	.07		

4.3.2.4.7. Overall Speed

No effects observed at any time point, as shown in Table 4.52.

Table 4.52. Overall Speed. Comparisons by treatment group. Data presented are Z composite scores, calculated by clustering relevant tasks. Overall Speed cognitive domain on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
Day 1 Acute								
Assessment 2	Placebo	55	-.01	.08	.02	.04	.09	.75
	Resveratrol	54	.04	.08	.00	.04		
Assessment 3	Placebo	55	-.01	.08	.00	.04	.00	.97
	Resveratrol	54	.04	.08	.00	.04		
Day 84 Acute								
Assessment 2	Placebo	49	-.03	.08	.00	.04	.11	.73
	Resveratrol	45	.05	.09	.02	.04		
Assessment 3	Placebo	49	-.03	.08	-.00	.04	.98	.32
	Resveratrol	45	.05	.09	.05	.04		
Pure Chronic								
		Baseline (Day 1 A1)			Post dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
Assessment 1	Placebo	49	-.01	.08	-.01	.04	.31	.57
	Resveratrol	45	.04	.08	.03	.05		
Assessment 2	Placebo	49	-.01	.08	-.00	.05	.45	.50
	Resveratrol	45	.04	.08	.04	.05		
Assessment 3	Placebo	49	-.01	.08	-.02	.05	1.57	.21
	Resveratrol	45	.04	.08	.07	.05		

4.3.2.5. Mood

4.3.2.5.1. Profile of Mood States (POMS)

A trend towards a significant pure chronic treatment effect was observed on the Day 84 afternoon assessment, [F (1, 90) = 3.20, p = .07, d = .37], where participants scored higher on Vigour-Activity following resveratrol (13.18) in comparison to placebo (11.54).

No other effects were observed for any POMs outcome at any time point, as presented in Tables 4.53 and 4.54.

Table 4.53. Profile of Mood States on Day 1 and Day 84. Comparisons by treatment group. POMs responses on Day 1 and Day 84. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values, from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SE	Mean	SE	F	p
		Day 1 Acute						
Tension-Anxiety	Placebo	54	4.52	.48	5.97	.47	1.11	.29
	Resveratrol	54	5.05	.47	6.69	.47		
Depression-Dejection	Placebo	50	.83	.23	1.26	.29	1.68	.19
	Resveratrol	49	1.20	.28	1.80	.29		
Anger-Hostility	Placebo	52	.72	.15	.59	.12	.21	.64
	Resveratrol	48	.62	.13	.68	.13		
Vigour-Activity	Placebo	54	18.26	.89	12.36	.67	.01	.90
	Resveratrol	54	17.76	.89	12.25	.67		
Fatigue-Inertia	Placebo	54	3.74	.48	11.65	.72	.13	.71
	Resveratrol	54	4.95	.50	11.27	.72		
Confusion-Bewilderment	Placebo	54	6.04	.51	12.11	.65	.47	.49
	Resveratrol	54	7.49	.60	11.47	.65		
Friendliness	Placebo	54	17.04	.51	14.54	.39	.62	.43
	Resveratrol	54	17.60	.41	14.10	.39		
Total Mood Disturbance	Placebo	54	-2.44	2.2	19.45	2.15	.22	.63
	Resveratrol	54	1.40	2.0	20.91	2.15		
		Day 84 Acute						
Tension-Anxiety	Placebo	49	3.49	.45	4.96	.51	1.10	.29
	Resveratrol	45	4.62	.55	5.75	.54		
Depression-Dejection	Placebo	45	.57	.25	1.35	.18	1.52	.22
	Resveratrol	41	1.35	.33	1.02	.19		
Anger-Hostility	Placebo	48	.49	.12	.53	.13	.01	.89
	Resveratrol	40	1.02	.24	.50	.14		
Vigour-Activity	Placebo	50	17.24	.93	11.69	.60	1.67	.19
	Resveratrol	44	18.16	.99	12.84	.64		
Fatigue-Inertia	Placebo	49	3.96	.48	10.33	.80	.21	.64
	Resveratrol	45	4.56	.46	10.87	.83		
Confusion-Bewilderment	Placebo	49	6.24	.56	9.98	.58	.39	.53
	Resveratrol	45	6.47	.45	10.51	.61		
Friendliness	Placebo	49	16.41	.60	14.87	.41	.79	.37
	Resveratrol	45	17.73	.57	14.34	.42		
Total Mood Disturbance	Placebo	49	-2.49	2.12	15.40	2.18	.06	.79
	Resveratrol	45	-.27	2.25	16.20	2.27		

Table 4.54. Profile of Mood States Pure Chronic analysis. Comparisons by treatment group. POMs responses pure chronic analysis. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values. Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

			Baseline (Day 1 A1)			Post Dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
Tension-Anxiety	V2 AM	Placebo	48	4.52	.48	3.71	.40	.92	.33
		Resveratrol	45	5.05	.47	4.28	.41		
	V2 PM	Placebo	48	4.52	.48	4.99	.49	.96	.32
		Resveratrol	45	5.05	.47	5.69	.51		
Depression-Dejection	V2 AM	Placebo	43	.83	.23	.49	.19	1.67	.20
		Resveratrol	37	1.20	.28	.86	.20		
	V2 PM	Placebo	43	.83	.23	.97	.22	.21	.64
		Resveratrol	37	1.20	.28	.81	.24		
Anger-Hostility	V2 AM	Placebo	46	.72	.15	.45	.12	2.88	.09
		Resveratrol	36	.62	.13	.78	.14		
	V2 PM	Placebo	46	.72	.15	.42	.12	.00	.94
		Resveratrol	36	.62	.13	.40	.13		
Vigour-Activity	V2 AM	Placebo	49	18.26	.89	17.29	.67	.46	.49
		Resveratrol	44	17.76	.89	17.96	.71		
	V2 PM	**Placebo**	**49**	**18.26**	**.89**	**11.54**	**.62**	**3.20**	**.07**[t]
		Resveratrol	**44**	**17.76**	**.89**	**13.18**	**.66**		
Fatigue-Inertia	V2 AM	Placebo	48	3.74	.48	4.15	.41	.01	.90
		Resveratrol	45	4.95	.50	4.21	.43		
	V2 PM	Placebo	48	3.74	.48	10.51	.86	.01	.91
		Resveratrol	45	4.95	.50	10.67	.89		
Confusion-Bewilderment	V2 AM	Placebo	48	6.04	.51	6.59	.39	1.50	.22
		Resveratrol	45	7.49	.60	5.89	.40		
	V2 PM	Placebo	48	6.04	.51	10.38	.63	.19	.66
		Resveratrol	45	7.49	.60	9.97	.65		
Friendliness	V2 AM	Placebo	48	17.04	.51	16.87	.35	1.06	.30
		Resveratrol	45	17.60	.41	17.40	.36		
	V2 PM	Placebo	48	17.04	.51	14.71	.46	.01	.90
		Resveratrol	45	17.60	.41	14.63	.48		
Total Mood Disturbance	V2 AM	Placebo	48	-2.44	2.2	-1.32	1.49	.15	.69
		Resveratrol	45	1.40	2.0	-2.18	1.54		
	V2 PM	Placebo	48	-2.44	2.2	15.93	2.34	.03	.86
		Resveratrol	45	1.40	2.0	15.33	2.42		

4.3.2.5.2. Bond Lader

No acute or pure chronic effects were observed on any outcomes, as presented in Table 4.55.

Table 4.55. Bond Lader. Comparisons by treatment group. Bond Lader responses on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Day 1						
Alert	Assessment 2	Placebo	55	66.76	1.93	63.44	1.26	.31	.57
		Resveratrol	54	64.92	1.85	62.43	1.27		
	Assessment 3	Placebo	55	66.76	1.93	62.16	1.57	1.00	.31
		Resveratrol	54	64.92	1.85	59.90	1.59		
Content	Assessment 2	Placebo	55	73.69	1.71	71.69	.97	.03	.85
		Resveratrol	54	73.77	1.66	71.95	.98		
	Assessment 3	Placebo	55	73.69	1.71	72.54	1.18	.58	.44
		Resveratrol	54	73.77	1.66	71.26	1.19		
Calm	Assessment 2	Placebo	54	66.60	2.04	63.52	1.21	.57	.44
		Resveratrol	54	62.88	1.94	64.83	1.21		
	Assessment 3	Placebo	54	66.60	2.04	65.48	1.35	.25	.61
		Resveratrol	54	62.88	1.94	64.51	1.35		
			Day 84						
Alert	Assessment 2	Placebo	49	67.57	1.79	64.33	1.54	1.40	.24
		Resveratrol	45	64.95	2.33	61.68	1.61		
	Assessment 3	Placebo	49	67.57	1.79	64.44	1.99	1.46	.22
		Resveratrol	45	64.95	2.33	60.93	2.08		
Content	Assessment 2	Placebo	48	76.95	1.60	73.68	.88	1.15	.28
		Resveratrol	43	75.19	2.10	75.06	.93		
	Assessment 3	Placebo	48	76.95	1.60	74.50	1.14	.17	.67
		Resveratrol	43	75.19	2.10	75.21	1.21		
Calm	Assessment 2	Placebo	49	67.54	2.02	68.07	1.66	.04	.82
		Resveratrol	44	66.82	2.28	67.55	1.75		
	Assessment 3	Placebo	49	67.54	2.02	69.72	1.67	.00	1.00
		Resveratrol	44	66.82	2.28	69.72	1.76		

			Pure Chronic						
			Baseline (Day 1 A1)			Post dose		Main Effects	
			n	Mean	SE	Mean	SE	F	p
Alert	Assessment 1	Placebo	49	66.76	1.93	66.73	1.44	.17	.68
		Resveratrol	45	64.92	1.85	65.86	1.51		
	Assessment 2	Placebo	49	66.76	1.93	64.42	1.61	1.47	.22
		Resveratrol	45	64.92	1.85	61.59	1.68		
	Assessment 3	Placebo	49	66.76	1.93	64.36	1.89	1.47	.22
		Resveratrol	45	64.92	1.85	61.02	1.98		
Content	Assessment 1	Placebo	48	73.69	1.71	77.22	1.36	.76	.38
		Resveratrol	43	73.77	1.66	75.49	1.43		
	Assessment 2	Placebo	48	73.69	1.71	74.40	1.29	.00	.93
		Resveratrol	43	73.77	1.66	74.26	1.36		
	Assessment 3	Placebo	48	73.69	1.71	75.22	1.52	.13	.71
		Resveratrol	43	73.77	1.66	74.41	1.60		
Calm	Assessment 1	Placebo	49	66.60	2.04	66.22	1.72	.36	.54
		Resveratrol	44	62.88	1.94	67.74	1.81		
	Assessment 2	Placebo	49	66.60	2.04	66.98	1.52	.63	.43
		Resveratrol	44	62.88	1.94	68.76	1.61		
	Assessment 3	Placebo	49	66.60	2.04	68.52	1.53	1.26	.26
		Resveratrol	44	62.88	1.94	71.05	1.62		

4.3.2.6. Blood pressure and heart rate

Analysis indicated a significant acute treatment effect on diastolic blood pressure on Day 1, assessment 3, [F (1, 102) = 7.25, p = .008, d = .53], where diastolic blood pressure was lower following placebo (80.44), in comparison to resveratrol (84.54).

In addition, a significant acute effect of treatment on heart rate was observed on Day 1, assessment 3, [F (1, 103) = 4.31, p = .04, d = .41], where heart rate was lower following placebo (70.03), in comparison to resveratrol (72.61).

No effect was observed on systolic blood pressure and no further effects were observed at any other time point, as presented in Table 4.56.

Table 4.56. Blood pressure and heart rate. Comparisons by treatment group. Blood pressure and heart rate measures on Day 1, Day 84 and pure chronic analysis. Baseline raw scores and post-dose estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects for acute changes within Day 1 and Day 84, from ANCOVA analysis. For pure chronic analysis, Day 1 baseline (assessment 1) raw scores and Day 84 (all assessments) estimated marginal means and standard errors (SE) are presented with F and p values of treatment effects, from ANCOVA analysis.

			Baseline			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
			Day 1						
Systolic	Assessment 2	Placebo	55	123.71	1.89	123.95	1.40	.79	.37
		Resveratrol	53	123.65	1.82	122.16	1.42		
	Assessment 3	Placebo	55	123.71	1.89	122.31	1.57	2.03	.15
		Resveratrol	53	123.65	1.82	125.52	1.60		
Diastolic	Assessment 2	Placebo	52	82.62	1.42	81.78	1.03	.31	.57
		Resveratrol	53	81.91	1.58	82.59	1.02		
	Assessment 3	**Placebo**	**52**	**82.62**	**1.42**	**80.44**	**1.08**	**7.25**	**.008***
		Resveratrol	**53**	**81.91**	**1.58**	**84.54**	**1.07**		
Heart Rate	Assessment 2	Placebo	54	67.78	1.40	62.25	.73	.96	.32
		Resveratrol	52	65.33	1.25	63.29	.75		
	Assessment 3	**Placebo**	**54**	**67.78**	**1.40**	**70.03**	**.86**	**4.31**	**.04***
		Resveratrol	**52**	**65.33**	**1.25**	**72.61**	**.88**		
			Day 84						
Systolic	Assessment 2	Placebo	49	122.06	2.12	122.33	1.17	.81	.36
		Resveratrol	44	122.60	2.12	123.87	1.23		
	Assessment 3	Placebo	49	122.06	2.12	121.21	1.40	1.27	.26
		Resveratrol	44	122.60	2.12	123.51	1.47		
Diastolic	Assessment 2	Placebo	46	81.49	1.38	84.56	.91	.02	.88
		Resveratrol	41	83.53	1.87	84.37	.97		
	Assessment 3	Placebo	46	81.49	1.38	81.05	1.00	.11	.73
		Resveratrol	41	83.53	1.87	81.54	1.06		
Heart Rate	Assessment 2	Placebo	49	65.04	1.20	61.66	.68	1.05	.30
		Resveratrol	44	65.20	1.46	60.64	.72		
	Assessment 3	Placebo	49	65.04	1.20	71.01	.97	.07	.78
		Resveratrol	44	65.20	1.46	71.41	1.03		
			Pure Chronic						
			Baseline (Day 1 A1)			Post dose		Main Effects	
			n	Mean	SE	Mean	SE	F	p
Systolic		Placebo	49	123.71	1.89	121.71	1.65	.27	.60

	Assessment 1	Resveratrol	44	123.65	1.82	122.97	1.74		
	Assessment 2	Placebo	49	123.71	1.89	121.81	1.56	1.34	.24
		Resveratrol	44	123.65	1.82	124.45	1.64		
	Assessment 3	Placebo	49	123.71	1.89	120.75	1.70	1.74	.19
		Resveratrol	44	123.65	1.82	124.02	1.79		
Diastolic	Assessment 1	Placebo	46	82.62	1.42	80.85	1.10	2.43	.12
		Resveratrol	41	81.91	1.58	83.36	1.16		
	Assessment 2	Placebo	46	82.62	1.42	83.47	1.16	1.53	.21
		Resveratrol	41	81.91	1.58	85.58	1.23		
	Assessment 3	Placebo	46	82.62	1.42	80.07	1.29	1.84	.17
		Resveratrol	41	81.91	1.58	82.64	1.37		
Heart Rate	Assessment 1	Placebo	49	67.78	1.40	64.19	.91	1.96	.16
		Resveratrol	44	65.33	1.25	66.07	.97		
	Assessment 2	Placebo	49	67.78	1.40	60.86	.85	.29	.58
		Resveratrol	44	65.33	1.25	61.54	.90		
	Assessment 3	Placebo	49	67.78	1.40	70.07	1.05	2.37	.12
		Resveratrol	44	65.33	1.25	72.46	1.11		

4.3.2.7. Body Mass Index (BMI)

No significant differences were observed for BMI change.

Table 4.57. Body Mass Index. Comparisons by treatment group. Body Mass Index and weight measurements as taken at training visit and on Day 84. Baseline and post-dose (estimated marginal means and standard errors (SE) are presented with F and *p* values.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SE	F	p
Weight	Placebo	52	86.60	2.17	84.01	.22	.42	.51
	Resveratrol	49	83.03	2.01	83.80	.23		
BMI	Placebo	53	30.59	.58	29.70	.56	.65	.42
	Resveratrol	49	30.22	.58	30.35	.58		

4.3.3. Biological Results

4.3.3.1. Urinary metabolite analysis

Following LC-MS analysis, positive analysis identified 4662 mass spectral features. The resulting peak table was sequentially filtered to only include reproducible and stable peaks, which resulted in a final 2827 features in positive ionisation mode. PLS-DA analysis identified 15 mass spectral features which significantly differed between treatment groups. Of these, six were matched to existing metabolite databases (KEGG) and therefore could be identified, as illustrated in Figure 4.11 and Table 4.58.

Following this, negative analysis identified 3000 mass spectral features. The resulting peak table was sequentially filtered to only include reproducible and stable peaks, which resulted in a final 1212 features in negative ionisation mode. PLS-DA analysis identified 11 mass spectral features which significantly differed between treatment groups. Of these, five were matched to existing metabolite databases and therefore could be identified, as illustrated in Figure 4.12 and Table 4.58.

Table 4.58. Identified mass spectral features. Identified from both negative and positive mode that are influenced following resveratrol intervention.

Feature ID	Mass (m/z)	Formula	Compound name
	Positive mode ionisation analysis		
M0915	180.11.417	C11 H16 O2	Trienoic acid
M0023	340.06.226	C15 H16 O7S	Sulfonic acid
M0011	420.01.781	C18 H12 O10S	Dihydroxy-oxo-sulfanylium
M0049	310.05.164	C14 H14 O6S	Dihydroresveratrol 4'-sulfate
M0015	387.99121	C14 H12 09 S2	Trans-Resveratrol 3,4'-disulfate
M0164	516.09.431	C21 H24 O13S	Oxidanesulfonic acid
	Negative mode ionisation analysis		
M0164	516.09.431	C21 H24 O13S	Oxidanesulfonic acid
M0049	310.05.164	C14 H14 O6S	Dihydroresveratrol 4'-sulfate
M0011	420.01.781	C18 H12 O10S	Dihydroxy-oxo-sulfanylium
M0023	340.06.226	C15 H16 O7S	Sulfonic acid
M0015	387.99121	C14 H12 09 S2	Trans-Resveratrol 3,4'-disulfate

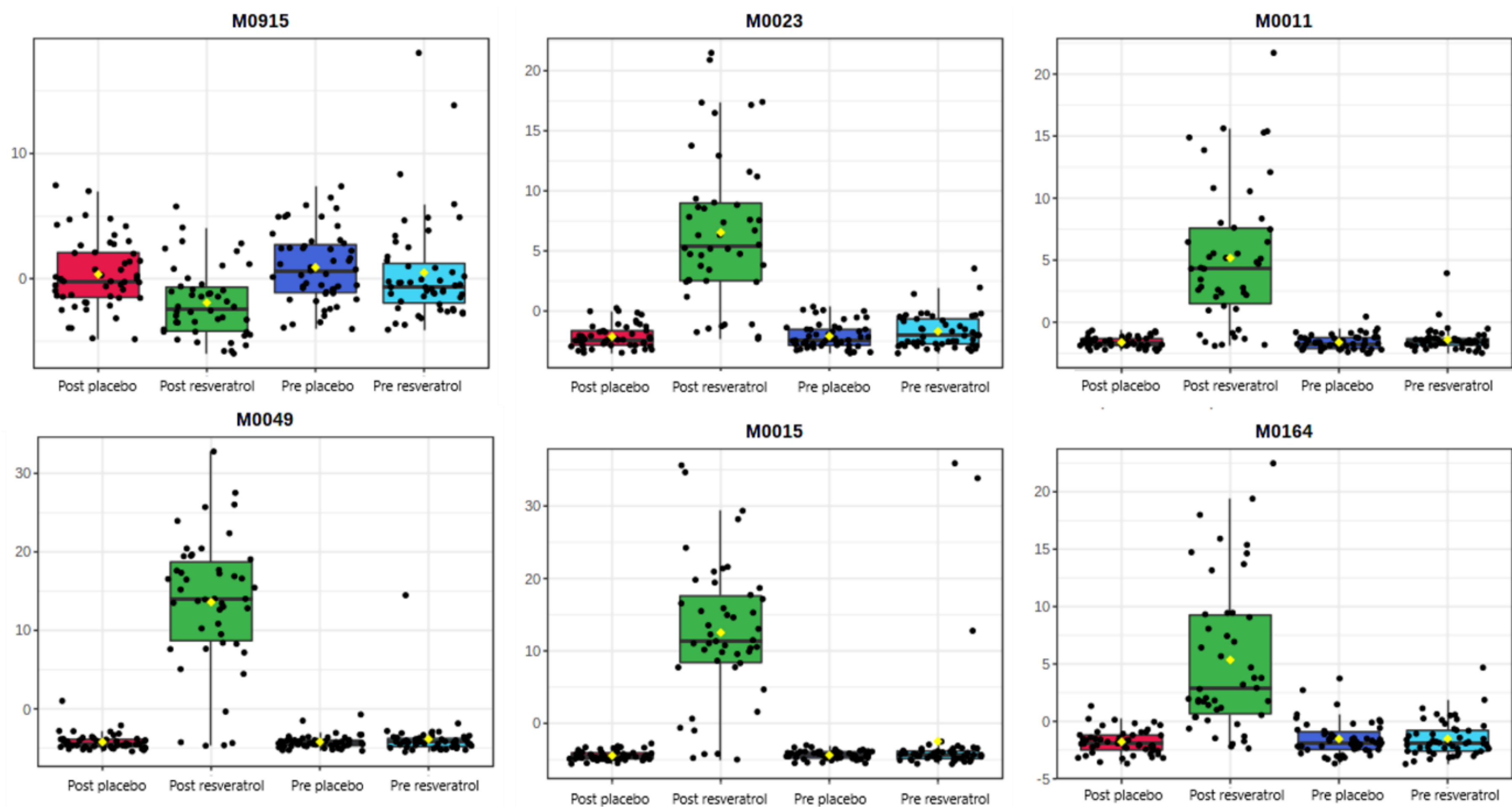

Figure 4.11. Intensity plots of significant features from HILIC Positive analysis. As identified by existing databases, detailed within Table 4.58.

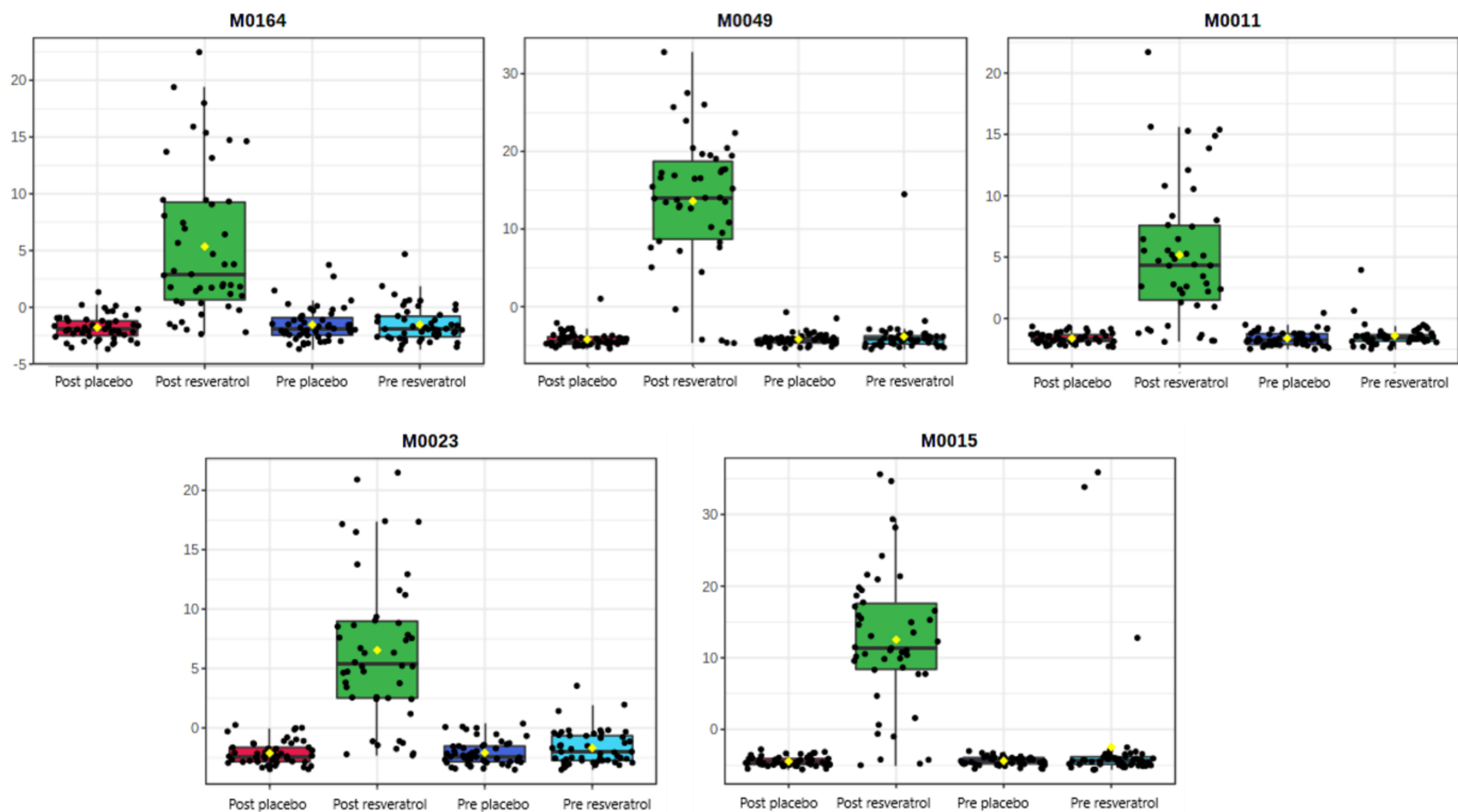

Figure 4.12. Intensity plots of significant features from HILIC Negative analysis. As identified by existing databases, detailed within Table 4.58.

4.3.3.2. Stool samples metabolomics analysis

DNA sequencing and rarefaction analysis data denotes that depth of sequencing is sufficient for robust analysis. The analysis determines that 1.0 x 10^4 reads are needed to reach asymptote. A mean of 3.5 x 10^4 read depth was taken forward for these analyses.

Comparing overall diversity between participants in both treatment groups, the alpha diversity illustrates that there was no statistical change to the bacterial community diversity within the gut of both observed ASV (p =.48) and Shannon Diversity (p=>1).

Comparing Beta diversity, mapping of the movement in the Beta diversity between both pre and post intervention was conducted. The PCoAs presented in Figure 4.13 illustrate that there was no statistical difference based on any test variable (study arm p=.71, participant p=.074, individual sample p=1). Whilst the study arm is not attributed to differences in community dissimilarity, there is a high degree of dissimilarity, with the largest difference based on the individual sampled.

Two differentially abundant taxa were observed following intervention with Placebo, these were Ruminococcaceae and ACK-M1 (Figure 4.14), with a reduction in Actinobacteria (ACK-M1) and an increase in Clostridia (Ruminococcaceae) observed following intervention with placebo. No differentially abundant taxa was observed following intervention with resveratrol.

One differentially abundant taxa was observed between treatment groups following intervention, with more Bacteroidia (Barnesiellaceae) observed following resveratrol supplementation when compared with placebo (Figure 4.15).

One differentially abundant taxa was observed between treatment groups prior to receiving any supplementation, with a greater abundance of Acidimicrobiia (C111) observed prior to resveratrol supplementation compared with the pre placebo group (Figure 4.16).

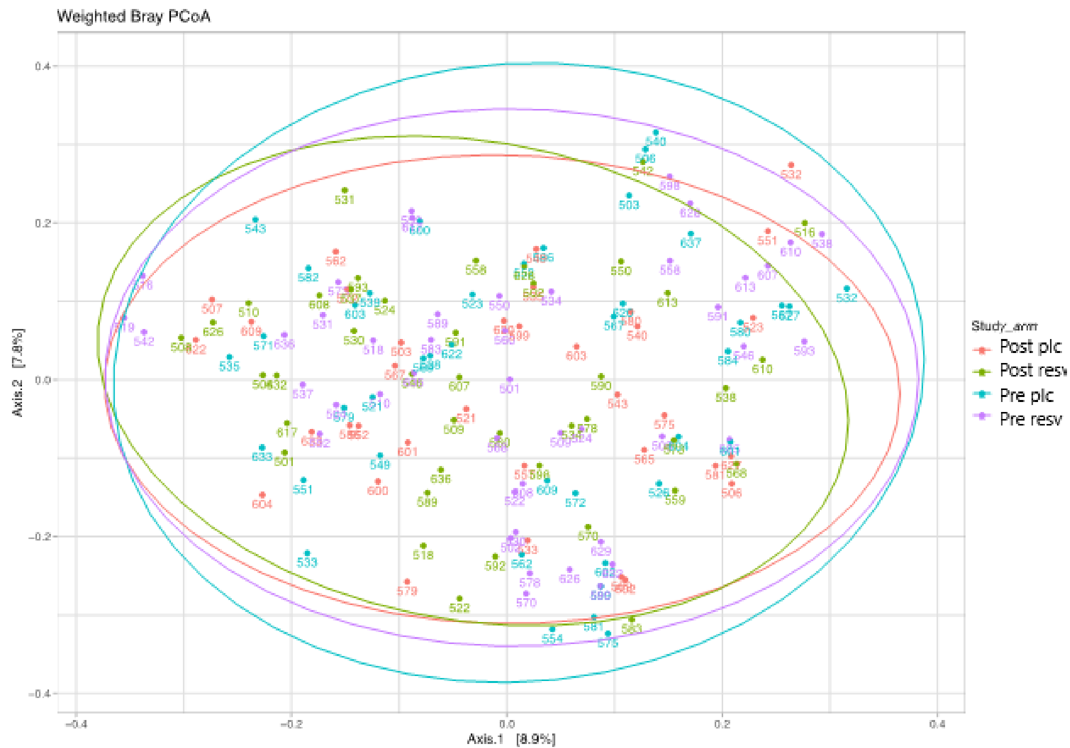

Figure 4.13. Principle coordinates analysis ordination of Bray-Curtis dissimilarity. Between pre and post intervention samples of subjects receiving placebo and resveratrol supplementation. Each data point represents an individual sample.

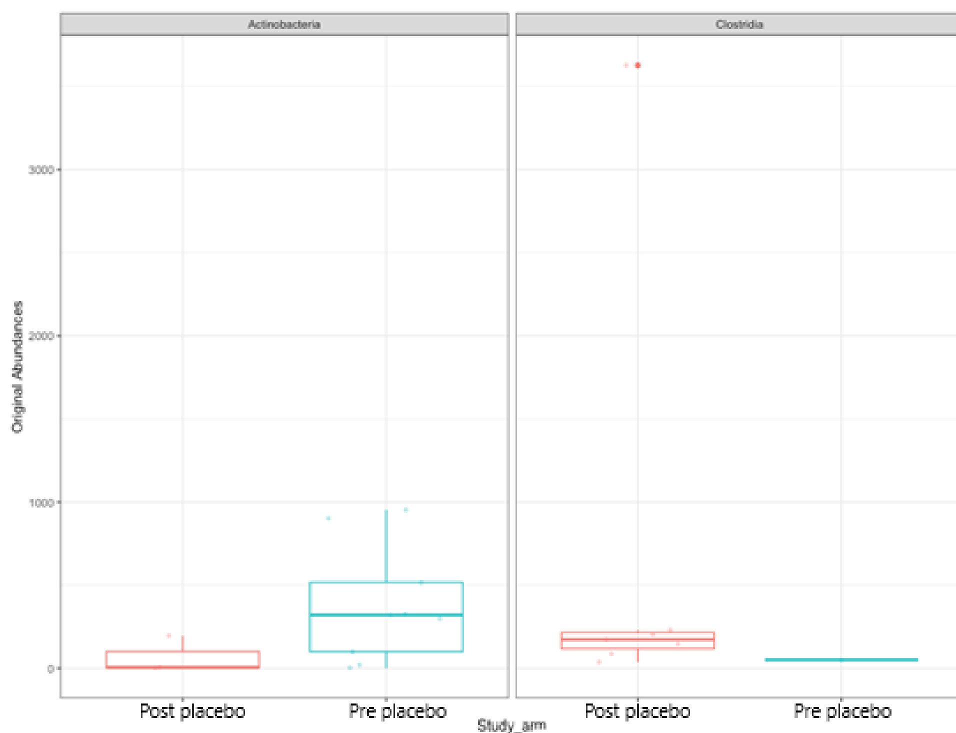

Figure 4.14. Placebo intervention effects on specific bacterial strains. A reduction in Actinobacteria (ACK-M1) and an increase in Clostridia (Ruminococcaceae) observed following intervention with placebo.

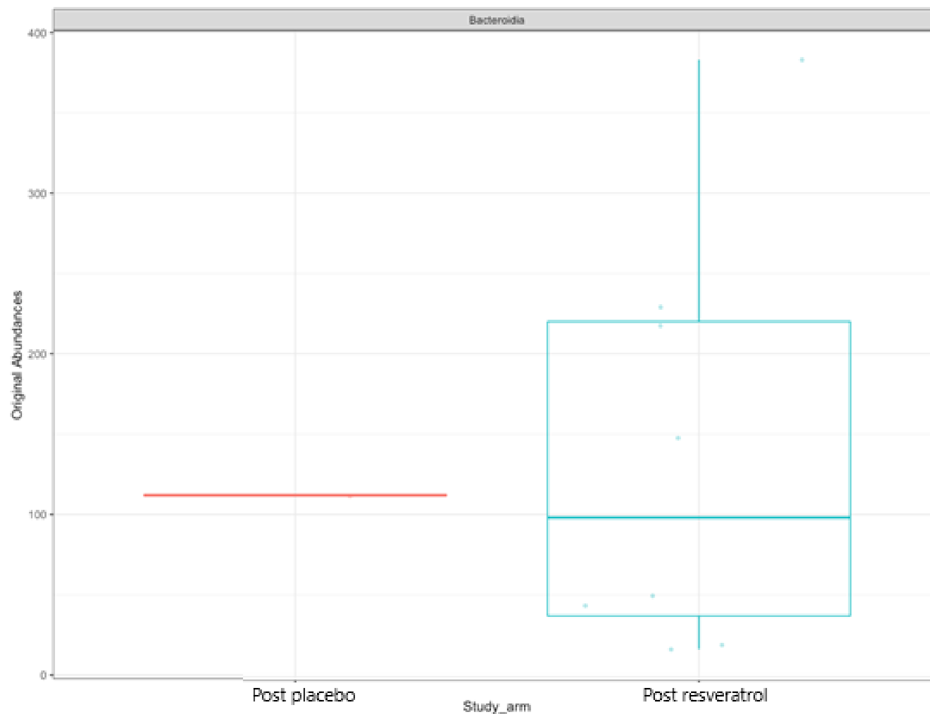

Figure 4.15. Resveratrol intervention effects on specific bacterial strains. An increase in Bacteroidia (Barnesiellaceae) was observed following resveratrol supplementation when compared with placebo.

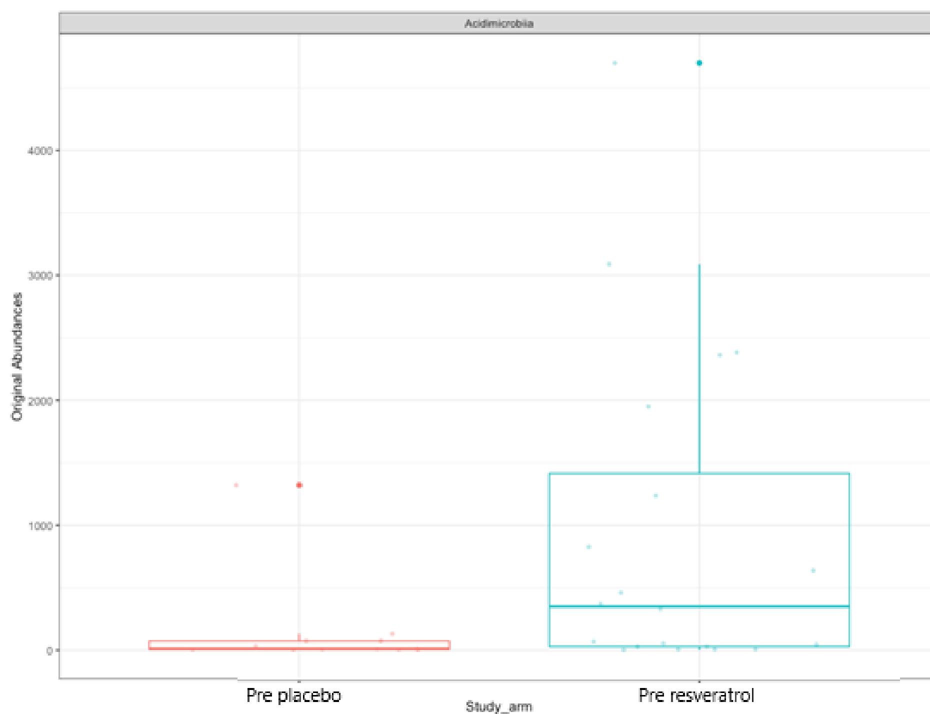

Figure 4.16. Comparison between treatment groups. An increase in Acidimicrobiia (C111) was prior to supplementation in the resveratrol group when compared to the placebo group.

4.3.3.3. Blood biomarker samples analysis

Analysis of blood biomarkers indicated a significant acute effect of treatment on triglycerides concentration on Day 1, [$F\,(1,\,42) = 4.50$, $p = .04$, $d = .68$], where concentrations were lower following resveratrol supplementation (mean 158.75 ng/mL) in comparison to placebo (mean 226.14 ng/mL).

Additionally, a pure chronic effect of treatment was observed on total cholesterol concentrations at the Day 84 PM sample, [F (1, 22) = 7.29, p = .013, d = 1.15], where concentrations were lower following resveratrol supplementation (mean 161.36 ng/mL) in comparison to placebo (mean 199.48 ng/mL).

No effect was observed on any other biomarker and no further effects were observed at any other time point, as presented in Table 4.59 and Table 4.60.

Table 4.59. Blood biomarkers on Day 1 and Day 84. Outcomes for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard deviation (SD) are presented with F and p values of treatment effects from ANCOVA analysis.

		Baseline			Post-dose		Main Effects	
		n	Mean	SD	Mean	SD	F	p
		Day 1 Acute						
Cholesterol	Placebo	20	181.66	51.70	180.95	26.68	.09	.75
	Resveratrol	24	162.41	30.71	166.13	35.00		
CRP	Placebo	20	73.10	42.46	107.31	68.56	.43	.51
	Resveratrol	22	66.33	25.99	80.43	36.57		
FRAP	Placebo	19	62.01	9.97	61.52	9.57	1.61	.21
	Resveratrol	20	63.52	12.47	65.82	7.69		
Glucose	Placebo	20	95.82	33.87	120.60	37.62	2.05	.16
	Resveratrol	22	95.31	31.43	106.59	29.38		
HDL	Placebo	22	20.55	28.96	26.61	18.39	.00	.97
	Resveratrol	22	15.05	14.74	27.29	28.23		
IL-6	Placebo	21	.20	1.04	.55	1.70	.64	.42
	Resveratrol	24	.38	.14	.44	1.88		
LDL	Placebo	20	156.21	60.22	136.03	47.75	1.08	.30
	Resveratrol	23	153.94	74.91	161.69	68.10		
Resveratrol-3-O-D-glucoside	Placebo	24	136.83	66.98	148.99	103.79	.00	.98
	Resveratrol	26	155.68	34.33	178.91	89.33		
Resveratrol	Placebo	25	246.71	186.90	257.08	201.83	1.36	.24
	Resveratrol	27	215.10	166.94	242.73	184.21		
Resveratrol-3-0-sulfate	Placebo	26	39.86	37.98	47.87	50.12	1.56	.21
	Resveratrol	27	38.55	41.87	64.55	71.23		
Resveratrol-4-O-D-glucoronide	Placebo	22	81.67	72.28	96.31	85.82	.06	.79
	Resveratrol	26	86.55	75.68	104.09	107.86		
Triglycerides	**Placebo**	**20**	**190.54**	**97.29**	**226.14**	**101.59**	**4.50**	**.04***
	Resveratrol	**22**	**153.30**	**99.49**	**158.75**	**52.49**		
		Day 84 Acute						
Cholesterol	Placebo	12	176.88	42.64	187.37	57.24	.42	.52
	Resveratrol	14	177.29	41.01	170.77	31.45		
CRP	Placebo	12	61.32	22.34	103.34	69.47	.44	.51
	Resveratrol	14	63.25	27.97	77.86	38.23		
FRAP	Placebo	11	68.40	15.48	68.51	13.38	.70	.41
	Resveratrol	9	69.43	20.19	60.28	5.62		

Glucose	Placebo	12	81.17	32.44	96.85	36.97	1.39	.24
	Resveratrol	14	77.79	35.74	108.79	12.65		
HDL	Placebo	10	16.99	13.32	33.39	30.81	.67	.42
	Resveratrol	14	17.53	13.63	25.17	12.01		
IL-6	Placebo	13	.20	.20	.52	1.90	1.35	.25
	Resveratrol	15	.08	.41	.04	.14		
LDL	Placebo	12	143.64	38.80	132.09	37.05	2.01	.17
	Resveratrol	13	161.63	58.93	175.94	98.17		
Resveratrol-3-O-D-glucoside	Placebo	13	162.30	58.95	168.98	74.65	.71	.40
	Resveratrol	17	165.32	62.55	151.93	64.33		
Resveratrol	Placebo	14	210.12	164.95	209.88	116.76	.00	.92
	Resveratrol	17	176.77	87.14	186.86	107.31		
Resveratrol-3-0-sulfate	Placebo	14	34.11	34.23	55.83	57.75	.68	.41
	Resveratrol	17	31.71	36.19	58.15	103.60		
Resveratrol-4-O-D-glucoronide	Placebo	14	66.53	63.84	63.37	65.44	.19	.66
	Resveratrol	17	65.49	64.46	66.66	78.88		
Triglycerides	Placebo	12	138.65	71.45	237.78	97.09	.62	.43
	Resveratrol	13	144.97	77.86	203.21	80.11		

Table 4.60. Blood biomarkers Pure Chronic analysis. Outcomes for placebo and resveratrol treatment groups. Baseline raw scores and post-dose estimated marginal means and standard error (SE) are presented with F and p values of treatment effects from pure chronic analysis.

			Baseline (V1 AM)			Post dose		Main effects	
			n	Mean	SE	Mean	SE	F	p
Cholesterol	V2 AM	Placebo	12	181.66	7.97	197.63	13.64	.94	.34
		Resveratrol	13	162.41	5.19	178.66	13.07		
	V2 PM	**Placebo**	**12**	**181.66**	**7.97**	**199.48**	**9.87**	**7.29**	**.01***
		Resveratrol	**13**	**162.41**	**5.19**	**161.36**	**9.46**		
CRP	V2 AM	Placebo	12	73.10	6.55	63.62	5.19	.13	.71
		Resveratrol	13	66.33	4.33	66.28	4.98		
	V2 PM	Placebo	12	73.10	6.55	91.18	8.51	.00	.96
		Resveratrol	13	66.33	4.33	91.78	8.17		
FRAP	V2 AM	Placebo	11	62.01	1.63	68.14	3.74	2.47	.13
		Resveratrol	8	63.52	2.24	58.86	4.41		
	V2 PM	Placebo	11	62.01	1.63	68.43	3.46	2.31	.14
		Resveratrol	8	63.52	2.24	60.13	4.08		
Glucose	V2 AM	Placebo	12	95.82	5.22	98.09	10.64	.24	.62
		Resveratrol	13	95.31	5.23	90.73	10.23		
	V2 PM	Placebo	12	95.82	5.22	96.85	8.04	1.20	.28
		Resveratrol	13	95.31	5.23	109.08	7.73		
HDL	V2 AM	Placebo	10	20.55	4.41	19.77	4.35	.68	.41
		Resveratrol	13	15.05	2.52	24.67	3.79		
	V2 PM	Placebo	10	20.55	4.41	31.08	7.11	.16	.69
		Resveratrol	13	15.05	2.52	27.17	6.19		
IL-6	V2 AM	Placebo	12	.20	.16	.28	.14	.07	.78
		Resveratrol	14	.38	.25	.34	.13		
	V2 PM	Placebo	12	.20	.16	.33	.08	.43	.51
		Resveratrol	14	.38	.25	.25	.07		
LDL	V2 AM	Placebo	12	156.21	9.18	151.44	14.25	.05	.82
		Resveratrol	12	153.94	12.84	156.05	14.25		
	V2 PM	Placebo	12	156.21	9.18	133.14	20.80	2.89	.10
		Resveratrol	12	153.94	12.84	183.26	20.80		
Resveratrol-3-O-D-glucoside	V2 AM	Placebo	12	136.83	9.87	157.97	20.54	.23	.63
		Resveratrol	16	155.68	10.30	171.04	17.79		
	V2 PM	Placebo	12	136.83	9.87	157.40	19.30	.02	.87
		Resveratrol	16	155.68	10.30	153.28	16.71		
Resveratrol	V2 AM	Placebo	13	246.71	27.55	235.99	43.29	.58	.45
		Resveratrol	16	215.10	26.73	191.41	39.01		

	V2 PM	Placebo	13	246.71	27.55	202.72	30.81	.04	.84
		Resveratrol	16	215.10	26.73	194.44	27.77		
Resveratrol-3-0-sulfate	V2 AM	Placebo	13	39.86	5.54	48.28	10.24	.94	.34
		Resveratrol	16	38.55	6.79	34.86	9.22		
	V2 PM	Placebo	13	39.86	5.54	41.21	21.98	.55	.46
		Resveratrol	16	38.55	6.79	63.22	19.81		
Resveratrol-4-O-D-glucoronide	V2 AM	Placebo	11	81.67	10.89	88.73	24.45	.18	.67
		Resveratrol	16	86.55	12.11	75.05	20.26		
	V2 PM	Placebo	11	81.67	10.89	67.68	23.96	.00	.97
		Resveratrol	16	86.55	12.11	68.58	19.85		
Triglycerides	V2 AM	Placebo	12	190.54	15.01	173.95	22.45	.05	.82
		Resveratrol	11	153.30	16.81	166.60	23.45		
	V2 PM	Placebo	12	190.54	15.01	236.70	25.58	.27	.60
		Resveratrol	11	153.30	16.81	217.30	26.72		

4.4. Discussion

The aim of the present study was to further the literature suggesting that resveratrol may have the most potential as a cognitive enhancer when administered to more compromised demographic groups, rather than the healthy, young adults previously targeted. The overweight and obese population utilised in this study were hypothesised to be ‘compromised’ in a multitude of ways, each of which resveratrol had the potential to influence. Firstly, within an overweight-obese population, we would typically anticipate elevated levels of systemic inflammation, triggered partially by high-fat diet induced alterations to the intestinal barrier. Importantly, this is related to microbial dysbiosis which exacerbates the pro-inflammatory response and this is, in turn, linked to disruptions in cognitive performance. Given the potential prebiotic-like abilities of resveratrol, it was hypothesised that chronic (12-week) resveratrol supplementation, may be a potential therapeutic method in this interaction. Specifically, by promoting the growth of anti-inflammatory related gut bacterial strains, resveratrol would be anticipated to reduce inflammation and potentially improve cognitive function via the gut-brain axis.

When considering the effects on cognitive performance, the findings from this study overall indicate no support of resveratrol as a cognitive enhancer within this demographic, with results for all outcomes summarised within Table 4.61. Despite this conclusion, limited significant effects of resveratrol were observed on numeric working memory accuracy (pure chronic effects), aspects of picture recognition accuracy and reaction time (Day 1 and pure chronic effects on day 84); improved delayed word recall performance (Day 1) and the working memory cognitive domain (Day 1). Resveratrol supplementation also increased participant subjective ratings of vigour/activity; as assessed by POMS (pure chronic effect on day 84). However, almost overwhelmingly, analysis indicated a reduction in cognitive performance

following resveratrol, in comparison to placebo, potentially suggesting an unexpected detrimental cognitive effect of resveratrol, within this population. Here, placebo supplementation resulted in quicker performance on numeric working memory (on Day 84); increased total (pure chronic) and correct (Day 1 and pure chronic) serial subtractions of threes, alongside reduced subtraction errors (Day 1 and pure chronic). Similarly, improvements in total (Day 84 and pure chronic) and correct (Day 84 and pure chronic) subtractions of sevens were observed and reduced false alarms during rapid visual information processing (pure chronic). These placebo-induced enhancements on the cognitive demand battery were also observed during NIRS task performance. Here, increased total (Day 84) and correct (Day 84) SS3s, and improved RVIP accuracy (Day 1 and Day 84) and reduced false alarms (Day 1 and Day 84) were observed. In addition, quicker performance (pure chronic) and trends towards reduced errors (Day 1) were observed for Peg and Ball and a trend towards improved performance on choice reaction time (pure chronic effect) was evinced. Likewise, trends towards improved accuracy (Day 1 and Day 84) and quicker performance (Day 1 and pure chronic) on name-to-face recall was reported and improved accuracy (Day 1, Day 84 and pure chronic effects) for word recognition, alongside increased correct (Day 84) and reduced errors (pure chronic) on immediate word recall was seen. Participants also reported findings the tasks less difficult following placebo supplementation (Day 84 and pure chronic). However, no significant effects of treatment were observed during the completion of corsi blocks task, subjective ratings of mental fatigue and during the NIRS post-dose assessment of serial seven subtractions. In addition, no significant effects were observed for Overall Speed and Accuracy of Attention cognitive domains.

These placebo favouring enhancements may be most apparent when considering the analysis of cognitive domains data as, the benefit of consolidating all appropriate task outcomes like this, is in cutting through some of the noise which results when interpreting single task sub-measure outcomes like above. Here, participants performed quicker for the speed of attention (trend Day 84 and pure chronic) and speed of memory (trends Day 84) cognitive domains and had improved performance on episodic memory (Day 1 and Day 84) and overall accuracy (Day 1 and Day 84).

Table 4.61. Summary of study findings. Summarising all significant and trending towards significant findings for all outcome measures from the study. Split by results in favour of resveratrol and placebo treatment groups. ↑ = increased score. ↓ = reduced score. * = significant (p<.005). t = trend towards significant

Outcome measure	500 mg Resveratrol	Placebo
	COMPASS Assessments	
Numeric Working Memory	↑ Overall Accuracy Day 84 A1 (Pure Chronic)* ↑ 'Yes' Accuracy Day 84 A1 (Pure Chronic)* ↑ 'No' Accuracy Day 84 A1 (Pure Chronic)*	↓ Overall RT Day 84 (Acute) A3* & A2^t ↓ Correct RT Day 84 (Acute A3* ↓ 'Yes' RT Day 84 A3*
Choice Reaction Time	/	↑ Accuracy Day 84 A2 (Pure Chronic)^t
Serial 3 subtractions	/	↑ Total Subs - Day 84 A3 Rep 2*, A2 Rep 3^t, A1 Rep 3^t (Pure Chronic) ↑ Correct Subs - Day 84 A3 Rep 2*, A1 Rep 3*, A2 Rep 3*, A3 Rep 3* (Pure chronic) ↑ Correct Subs – Day 1 A2 Rep 3* (Acute) ↓ Errors – Day 84 A1 Rep 1* (Pure chronic) ↓ Errors – Day 1 A2 Rep 3* (Acute)
Serial 7 subtractions	/	↑ Total subs – Day 84 A2 Rep 2*, A1 Rep 3*, A3 Rep 3* (Pure Chronic) ↑ Total subs – Day 84 A2 Rep 2*, A3 Rep 2* (Acute) ↑ Correct subs – Day 84 A2 Rep 2*, A2 Rep 3*, A3 Rep 3* (Pure Chronic) ↑ Correct subs – Day 84 A2 Rep 2*, A3 Rep 2* (Acute)
Rapid Visual Information Processing	/	↓ False Alarms – Day 84 A3 Rep 3*, A1 Rep 3^t (Pure chronic)
Peg and Ball	/	↓ Thinking RT – Day 84 A1^t, A2^t (Pure chronic) ↓ Errors – Day 1 A2^t (Acute)
Name to face Recall	/	↑ Overall accuracy – Day 1 A3* & A2^t (Acute) ↑ Correct Forename % - Day 1 A3^t (Acute) ↑ Correct Surname % - Day 1 A3*, Day 84 A2* (Acute) ↓ Forename Correct RT – Day 84 A1^t (Pure Chronic) ↓ Surname Correct RT – Day 84 A1^t (Pure Chronic) ↓ Overall Correct RT – Day 1 A3* (Acute)
Picture Recognition	↑ Correct 'Yes' % – Day 84 A3* (Pure Chronic) ↑ Correct 'Yes' % Day 84 A3* (Acute)	↑ Overall Accuracy – Day 1 A3^t (Acute) ↑ Correct 'No' % - Day 84 A3* (Acute)

	↓ ‘Yes’ RT – Day 84 A1* (Pure Chronic) ↓’Yes’ RT – Day 1 A3* (Acute)	
Word Recognition	/	↑ Overall % - Day 84 A2* (Pure Chronic) ↑ Correct ‘No’ % - Day 84 A2* (Pure Chronic) ↑ Overall % - Day 84 A2*, Day 1 A2^{t} (Acute) ↑ Correct ‘No’ % - Day 1 A3*, Day 84 A2* (Acute)
Immediate Word Recall	/	↑ Correct – Day 84 A2^{t} (Acute) ↓ Errors – Day 84 A1* (Pure Chronic)
Delayed Word Recall	↑ Correct – Day 1 A3* (Acute)	/
Task difficulty VAS	/	↓ Task difficulty – Day 84 A2 R2*, A3 R2^{t}, A1 R3^{t} (Pure Chronic) ↓ Task difficulty – Day 84 A2 R1^{t} (Acute)
Mental fatigue VAS	/	↓ Mental fatigue – Day 84 A2 R1* (Pure chronic) ↓ Mental fatigue – Day 84 A2 R1^{t} (Acute)
	COMPASS Cognitive Domains	
Speed of Attention	/	↓ Day 84 A2^{t} (Pure chronic) ↓ Day 84 A2^{t} (Acute)
Working Memory	↑ Day 84 A1* (Pure chronic)	/
Speed of Memory	/	↓ Day 84 A2^{t} & A3^{t} (Acute)
Episodic Memory	/	↑ Day 1 A2* (Acute) ↑ Day 84 A2* (Acute)
Overall Accuracy	/	↑ Day 1 A2* (Acute) ↑ Day 84 A2* & A3* (Acute)
	NIRS COMPASS Task Performance	

Serial 3 subtractions	/	↑ Total Day 84 Rep 2* and 3* ↑ Correct Day 84 Rep 2* and 3*
Rapid Visual Information Processing	/	↑ Accuracy Day 1 Reps 1*, 2*, 3* ↑ Accuracy Day 84 Reps 2* & 3* ↓ False Alarms Day 1 Rep 1* & 3* ↓ False Alarms Day 84 Rep 2* & 3*
	Mood	
Profile of Mood States	↑ Vigour-Activity – Day 84 PM[t] (Pure chronic)	/
	Physiological Measures	
Oxygen saturation	↓Interaction effect Tmt*Day (Day 84)*	
Total Haemoglobin	↑ Main effect* ↑ Day 84 Planned comparisons*	/
Oxygenated Haemoglobin	↑ Main effect* ↑ Day 84 Planned comparisons*	/
Deoxygenated Haemoglobin	↑ Main effect*	/
Blood pressure and heart rate	/	↓ Diastolic BP – Day 1 A3* (Acute) ↓ Heart rate – Day 1 A3* (Acute)
	Biological Samples	
Urinary metabolites	↑ Sulfonic acid ↑ Dihydroxy-oxo-sulfanylium ↑ Dihydroresveratrol 4'-sulfate ↑ Trans-Resveratrol 3,4'-disulfate ↑ Oxidanesulfonic acid	/

Stool metabolomics	↑ Bacteroidia (Barnesiellaceae)	↓ Actinobacteria (ACK-M1) ↑ Clostridia (Ruminococcaceae)
Cholesterol	↓ Day 84 PM* (Pure chronic)	/
Triglycerides	↓ Day 1* (Acute)	/

The cognitive findings of this study are directly contradictory to what was anticipated to be observed within this population. Where previous work has indicated that resveratrol supplementation did not enhance cognitive performance in young, healthy adults (Wightman et al., 2019), this conclusion was made in the absence of significant results in favour of either treatment group. And, as previously suggested, the lack of effects within younger demographics has consistently been explained by participants being 'too healthy' and at the peak of their cognitive performance; resulting in them not benefiting from resveratrol supplementation. Moreover, many of these previous studies employed acute methodological designs, where chronic supplementation may be required to exert beneficial effects (Wightman et al., 2014). Additionally, many employed short cognitive paradigms, which were hypothesised to be insufficiently challenging to observe the subtle behavioural effects of resveratrol, particularly within a young, healthy population. Whereas, more prolonged, cognitively demanding paradigms are hypothesized to increase mental fatigue and potentially mitigate the performance ceiling effects observed in this population.

Certainly, when considering work in older, more compromised populations, chronic resveratrol supplementation appears to have a more beneficial effect. For example, 90-day supplementation of 1000 mg resveratrol improved psychomotor speed on the Trail making test in older, overweight adults (Anton et al., 2018). Likewise, 26-week co-supplementation of resveratrol and quercetin improved word recall task performance in older adults (Witte et al., 2014). Within post-menopausal women, 14-week resveratrol supplementation significantly improved overall cognitive performance and the verbal memory cognitive domain (Evans et al., 2017). Similar improvements are seen in cognitive flexibility and processing speed cognitive domains observed within post-menopausal women who received 12-month resveratrol supplementation (Zaw et al., 2020a, 2020b). Despite appearing more promising than work in younger demographics, it is crucial to acknowledge that many of these studies assessed very limited areas of cognitive performance, employing paradigms often incorporating few cognitive tests. Moreover, where resveratrol-induced cognitive enhancements are observed, these are repeatedly limited to just one outcome of a task. More consistent, widespread findings across multiple tasks or domains would provide a more convincing, positive effect of resveratrol.

Based on these potential methodological shortcomings, the present study aimed to employ a design which might increase the likelihood of observing subtle resveratrol-induced cognitive improvements, if they were there to observe. Specifically, this involved utilising a demographic sample who were likely compromised in terms of cognitive abilities; due to an expected exacerbated inflammatory status induced by high-fat dietary intake. Additionally, as a chronic

intervention period had been suggested to be most effective (specifically over 10 weeks supplementation (Asgary et al., 2019)), this study employed a 12-week intervention period. Further, a cognitively demanding paradigm was employed (~60 minutes in length, including 3 consecutive repetitions of the Cognitive Demand Battery), which participants completed three times at each of their testing visits. As such, it was theorised that the subtle behavioural effects of resveratrol would be able to be detected in an already compromised participant sample, then placed under high cognitive demand. However, not only did the current study fail to observe positive effects of resveratrol on cognition here, the current findings appear to imply a potentially detrimental effect of resveratrol supplementation on cognitive function. It is difficult to understand why this might have been the case within this study and, indeed, no explanation could really account for why negative effects were observed in contrast to the null findings that have previously been observed in healthy populations.

It is tempting here to suggest that this completely unanticipated effect of resveratrol on cognition was due to some catastrophic effect of the study investigational product but this argument is not supported by other study outcomes which did demonstrate the typical pattern of effects for resveratrol; namely cerebral blood flow. Here, the current study supports previous findings that resveratrol consistently modulates cerebral blood flow within the frontal cortex. Specifically, here resveratrol-induced increases in total haemoglobin concentration, oxygenated and deoxygenated haemoglobin were observed following 12-week supplementation. Contrary to previous work however, post-hoc analysis indicated that these treatment mediated modulations were only observed on Day 84, where past work has shown acute modulation just 45 minutes following resveratrol intake (Kennedy et al., 2010; Wightman, Haskell-Ramsay, Reay, et al., 2015).

Methodological variations here may explain the lack of detection of any acute enhancements of CBF. For example, post-dose CBF assessments took place at ~115 and ~155 minutes post dose, longer than the traditional 45-minute absorption period noted above, to allow for a full cognitive assessment to take place at 45 minutes post dose. Despite this, the observed chronic modulation of CBF is mostly consistent with previous work; which consistently observed increases in total haemoglobin concentration during task performance (Kennedy et al., 2010). Given that total haemoglobin concentration is calculated by summing concentrations of oxygenated and deoxygenated haemoglobin, it is apparent that increases in ThC would be observed during neural demand. However, variations in oxygenated and deoxygenated Hb concentrations have been observed, where increased deoxygenated haemoglobin was observed following acute supplementation of resveratrol (Kennedy et al., 2010) and increases in oxygenated haemoglobin were observed following co-supplementation

of resveratrol and piperine (Wightman et al., 2014) and 4-week supplementation of resveratrol (Wightman, Haskell-Ramsay, Reay, et al., 2015).

With a typical neural response to demand, anticipated changes consistent with neurovascular coupling mechanism would be increases in oxygenated haemoglobin with concurrent reductions in deoxygenated haemoglobin (Denfield et al., 2016). This response is indicative of the need for more oxygenated haemoglobin during neural demand. Conversely, increases in deoxygenated haemoglobin, has previously been suggested to be suggestive of enhanced oxygen extraction and utilisation during task performance (Kennedy et al., 2010). The findings of the current study, which indicate concurrent increases in oxygenated, deoxygenated, and total haemoglobin, are potentially more indicative of a resveratrol mediated increase in cerebral blood volume, rather than a neurovascular coupling mechanism. However, given the discrepancies in findings it is difficult to pinpoint exactly what these CBF modulations might imply, and certainly warrants further investigation, perhaps utilising supplementary neuroimaging techniques.

Moreover, an important methodological discrepancy in the area is differences in NIRS equipment employed. Where most of the previous work employed continuous wave-NIRS that can only measure relative changes in cerebral activation (Kennedy et al., 2010; Wightman, Haskell-Ramsay, Reay, et al., 2015; Wightman et al., 2014); this study utilised frequency domain (FD) NIRS. This FD NIRS allows for the measurement of quantifiable, absolute amounts of haemoglobin, which allows for assessment of changes throughout the supplementation period. Of the previous work, only Eschle (2017) employed the FD NIRS, during observation of acute effects of resveratrol supplementation in older adults, where they did not observe any effects on NIRS parameters. As such, the differences in methodology may explain the variations in haemodynamic response noted to date. Even so, the current study supports the conclusion previously established that resveratrol has the ability to modulate CBF, irrespective of enhancing cognitive performance; further consolidating the notion that any potential cognitive-enhancing effects of resveratrol likely occur via mechanisms other than CBF.

Prior to discussing the findings from the biological samples analyses, a potentially important consideration is that analyses of all samples within this chapter were significantly delayed due to the Covid-19 pandemic, with some samples stored for about 3 years prior to analysis. Whilst to the best of our current knowledge and technical advice has not indicated this; prolonged storage at -20°C (urine) and -80°C (plasma, serum and stool) may have impacted sample stability and therefore, findings from this analysis.

Whilst the microbiota literature to date appears characterised by vast changes in bacterial species abundance and shifts in microbial composition, this is certainly not the case for the findings of this trial. However, this is not necessarily a detrimental finding. Indeed, whilst analysis indicated that treatment intervention did not result in any significant difference in alpha diversity of the microbiota, this indicates that it did not impact upon the overall diversity of the gut. As increased microbial diversity, alongside bacterial richness, is considered a key marker of gut health (Cotillard et al., 2013b; Rinninella, Raoul, et al., 2019); we certainly wouldn't expect to, or indeed want to, see a difference in alpha diversity. This is particularly the case following a relatively small dietary intervention, over a quite short time period, within a healthy population (irrespective of overweight status). Here a shift in alpha diversity would be indicative of an antimicrobial effect, and a marker of intestinal disequilibrium. When considering beta-diversity, no effect of treatment was observed here either. However, a great change was observed in individual variations. These said inter-person variation also likely explains the observed slight taxa changes, where the distribution skews the findings to appear more meaningful than they are. Indeed, to actually quantify if these findings are evident of an actual increase, or just relative, further analysis, specifically a quantitative PCR, would be required. However, with such limited shifts observed it is unlikely that additional analysis would have yielded more fruitful findings. Nevertheless, supplementary work should consider additional analyses to better understand the role of dietary intervention on microbial composition.

When considering the treatment related taxa changes, resveratrol supplementation appeared to result in increased abundance of Bacteroidia (Barnesiellaceae); a bacterial strain belonging to the Bacteroidetes phylum (García-López et al., 2019). To the best of our knowledge, despite considerable interest in the Bacteroidetes phylum, this bacterial strain has not previously been associated with red wine, grape or resveratrol supplementation. However, increased abundance of *Barnesiellaceae* has previously been observed following 4-week supplementation with California strawberry powder in healthy adults (n = 15) (Ezzat-Zadeh et al., 2021). These authors also observed significant increases in various bacterial strains, including *Christensenellaceae,* that they report are associated with lean body weight and improved host health. Of interest for the current study, it has previously been suggested that abundance of Bacteroidia negatively correlated with serum LDL cholesterol and triglycerides (Parkar & Blum, 2022); where this study indicated lower serum triglycerides (Day 1) and total cholesterol (pure chronic effect). Taken together these findings tentatively suggest the potential of health benefits to the host, however supplementary work is required to understand this further.

As this microbial analysis appears to show a large distribution between individuals, it is difficult to establish if said minor bacterial changes are actual effects or are due to distribution within the sample utilised, or potential outliers within the sample. Moreover, it is well known that microbial variations between individuals are primarily impacted by age, BMI and lifestyle factors including exercise frequency and diet (Rinninella, Raoul, et al., 2019). Potential methods to reduce baseline differences within the sample include conducting research in groups of similar people, such as from the same small area. For example, recent research indicates that spouses have more similar bacterial taxa and microbial composition in common than siblings (Dill-McFarland et al., 2019). A secondary method, is to employ a 'wash in' diet to participants prior to intervention, as employed by Ezzat-Zadeh et al. (2021). Here participants consumed a 2-week run in period consuming a low fibre, low polyphenol 'beige diet', which they then continued throughout the 4-week intervention period. Whilst this is a useful method in reducing noise from baseline variations in human intervention trials, it can be argued that this artificial environment would lead to entirely different microbial response than in day-to-day life presented with these compounds in a typical diet with other macro- and micro-nutrients.

Another interesting point here is that, despite the recent gut-microbial dominance of the nutritional research literature area, there is still a relatively limited amount of published work in the area. This is especially the case when considering the lack of human intervention trials; in spite of increasing interest and importance being placed on this area of research. Of the trials published, very few have concurrently measured changes in microbial composition whilst also assessing cognitive function. Further, of the trials published, as previously mentioned, the majority are characterised by vast changes in taxa abundance, which could potentially indicate publication bias in the area; where studies observing null microbial findings are not being distributed.

Urinary metabolomics were employed here to enhance mechanistic evidence of health-promoting effects of resveratrol; a technique that has recently been employed in nutritional intervention trials (Beckmann et al., 2016; Navarro et al., 2019; Wang, 2021). Here, findings from the current study indicated some modest effects of resveratrol supplementation on the urinary metabolome. Here, when compared to placebo, the variation was described as 8% and 16%, for positive and negative ionisation modes, respectively. These variations are thought to be typical of human metabolome data which can be impacted by host demographics (BMI and age) and diet (Slupsky et al., 2007); as well as individual variations in resveratrol metabolism. Considering the spread of participant demographics employed within this study,

high interindividual difference likely explains the limited variance between treatment intervention groups. Nevertheless, the current study was able to identify discriminatory features that increased in intensity following resveratrol supplementation, relative to placebo. Specifically, resveratrol metabolites Dihydroresveratrol 4'-sulfate and Trans-Resveratrol 3,4'-disulfate, were observed alongside sulfonic acid derivatives. However, to understand the impact that these mass spectral features had, supplementary analysis would be required, which is beyond the scope of this investigation. Future work should aim to conduct tandem mass spectrometry (MS/MS) to confirm the identity of the mass spectral features.

Interestingly, when considering the blood biomarker analyses, there were no significant treatment related differences on any resveratrol biomarkers analysed. There are several explanations for the lack of findings here. Firstly, a potential methodological issue where, due to technical advice, only serum was obtained during this study. This is contradictory to the previous trial, in which we collected both serum and plasma, as much of the literature supports plasma as is more appropriate for detecting resveratrol metabolites. Additionally, no limitations were placed on participants diet during the supplementation period, which may explain the limited difference in resveratrol metabolites observed between the treatment groups. However, the surprising lack of resveratrol markers here could also speak to the unanticipated cognitive outcomes discussed above. An absence of resveratrol in the circulatory system would also preclude any anticipated effects elsewhere. As such, it may be the case that resveratrol wasn't directly responsible for the seemingly negative effects of this intervention on cognition and that this was, instead, the result of some other unknown biomarker. This undetermined marker could be an indirect product of resveratrol metabolism, or something completely unrelated, but it, nevertheless, represents a really interesting potential explanation for those cognitive outcomes. The detection of it in future is problematic though as analytical techniques typically require fore-knowledge of the marker in order to be able to measure it.

When considering supplementary biomarkers, as with findings within Chapter 2, no significant effects were observed on systemic inflammation markers (CRP and IL6). Nor on FRAP, glucose, HDL or LDL cholesterol. However, significantly lower concentrations of triglycerides were observed following resveratrol supplementation on Day 1; and a pure chronic effect of reduced total cholesterol concentrations was observed on Day 84 following resveratrol supplementation. This reduction in triglycerides is consistent with findings within Chapter 2 and also a large number of previous work (Andrade et al., 2014; Cho et al., 2012; Most et al., 2016; Qureshi et al., 2012; Simental-Mendía & Guerrero-Romero, 2019; Timmers et al., 2011; Tomé-Carneiro et al., 2012). Additionally, this study indicated a reduction in total cholesterol, that was not observed in Chapter 2, however coincides with previous findings (Bhatt et al.,

2012; Simental-Mendía & Guerrero-Romero, 2019). This suggests a potentially small improvement to host health following resveratrol supplementation, however in the absence of clear enhancements of biomarkers, it is difficult to extrapolate these findings further.

The null findings observed on inflammatory markers within this study can potentially be explained by the participant demographic utilised or, more accurately, the assumptions which were held about them. In this study, although the sample demographic of overweight and obese participants was deliberately targeted, this was with the assumption that these participants would be 'compromised' in terms of inflammatory status, gut microbial composition and cognitive performance (when compared with previous work in typically healthy, normal-weight participants). A limitation of this assumption is that participants eligibility was based solely on their BMI classification. As previously discussed, there are inherent issues with BMI; namely that the measurement cannot differentiate between body fat mass and lean body mass, leading to incorrect classification of individuals (Flegal et al., 2009). Further, participants who consumed more than five portions of fruit and vegetables per day were excluded. However, this was based solely on participant self-report and future work should incorporate additional dietary information (such as food frequency questionnaires) to better understand participants diet and baseline health status. In addition, in future work to determine that sample populations are 'compromised' it would be useful to employ eligibility criteria based on more concrete measures of metabolic health. This could include the use of techniques such as DXA scanning, which have the ability to determine fat mass; or categorising participants based on baseline inflammatory status would be more effective in establishing compromised individuals who might benefit more from phenolic intervention.

Additionally, due to difficulty accessing veins within this overweight and obese cohort, a reduced number of blood samples were obtained, resulting in a reduced sample size, which may account for the null findings within the biomarker analysis. Moreover, given the likely, large interindividual variation in metabolism of resveratrol, it is apparent that a larger sample size may be necessary to understand the impact that resveratrol supplementation can have on various biological outcomes. Specifically, for many years researchers have been informally discussing the potential for variability in polyphenol absorption and distribution to divide cohorts into 'responders' and 'non-responders'; i.e. those for whom effects of resveratrol could be anticipated and those where this simply couldn't be the case as resveratrol simply isn't processed by the gut bacteria and the ensuing metabolites circulated into the blood stream. Specifically, research has indicated that specific bacterial strains have the ability to metabolise resveratrol (*Slackia equolifaciens, Adlercreutzia equolifaciens* and *Eggerthellla lenta)* (Bode et al., 2013; Jung et al., 2009). Therefore, it is probable that differences in abundances of

these bacterial strains could result in differential metabolism of resveratrol; consequently impacting upon various host-health outcomes. Supplementary trials aiming to identify if 'responders' and 'non responders' exist within resveratrol work, would certainly prove an interesting avenue of work.

Regardless, there are a number of strengths to this trial. Firstly, it's novelty to the research area, where to date there is very limited work investigating the effects of phenolic intake on microbial composition in human intervention trials. Considerably less have also considered how this might impact on host health (including inflammation, cholesterol and blood pressure), alongside cognitive performance. The current study also built upon methodological limitations of previous resveratrol work; including investigating a more compromised participant demographic, over a prolonged intervention period, and whilst measuring cognitive performance during sustained cognitively demanding conditions. Additionally, measurements of cerebral blood flow, urinary metabolome, inflammatory biomarkers and gut microbial composition aimed to enhance understanding of mechanistic effects of resveratrol on host health. Despite limited findings here, this study adds support for additional investigation of interactions between inflammation, gut microbiota and cognitive performance; specifically, how these interrelations can be modulated by resveratrol or phenolic intervention. In addition, these findings taken together with the current body of literature, provide further support for resveratrols inability to enhance cognition in healthy adults. At this point, it seems that the literature has exhausted all potential methodologies that could be employed to observe this effect, and therefore it is highly likely that an absence of positive effects are accurate. As such, future investigations may produce more tangible interrelated effects when considering other polyphenol interventions, or indeed when considering diet as a whole.

CHAPTER 5
GENERAL DISCUSSION

5.1 Summary of objectives

The aims of this thesis were to investigate the effects of resveratrol supplementation on cognitive function, cerebral blood flow, inflammation and gastrointestinal microbiota in healthy-weight, overweight and obese healthy adults. This programme of work was conducted in response to previous resveratrol work which, despite significant modulation of cerebral blood flow, consistently observed null effects on cognitive performance, in young, healthy adults. Specifically, it was proposed that the young, healthy demographic employed in these studies may not be prime candidates for resveratrol-induced cognitive enhancements, for several reasons. Firstly, this population are likely at the peak of their cognitive performance and therefore the probable modest cognitive enhancing effects, may be undetectable. Additionally, methodological limitations including sample size and supplementation duration, might have contributed to these null findings.

It has been suggested that resveratrol supplementation may be more beneficial in more compromised demographic groups, such as older adults or overweight-obese individuals. Pertinent to this thesis, obese individuals are characterised by a multitude of health concerns, including sustained inflammation, elevated cholesterol levels and excessive fat accumulation (Esser et al., 2014; Malik et al., 2013); each of which likely contributes to the pathogenesis of metabolic disease (Abete et al., 2011). Given the widespread biological properties of resveratrol, supplementation within this population likely results in beneficial health effects via anti-inflammatory, antioxidant, and cardiovascular protective mechanisms. Moreover, interaction with these pathways, may induce positive effects on brain function, which is often disrupted in obese individuals, partially due to neuroinflammation (Miller & Spencer, 2014).

Indeed, when considering more recent evidence, findings appear more positive when considering the cognitive enhancing effects of resveratrol supplementation in compromised individuals (Anton et al., 2018; Evans et al., 2017; Scholey et al., 2014; Witte et al., 2014; Zaw et al., 2020a, 2020b). However, this area of work is in its infancy and plagued with methodological discrepancies; notably, supplementation length, demographic group, dosage and cognitive tasks or paradigms employed. Additionally, often any benefits to cognitive performance are observed on singular tasks or task outcome measure, where more convincing beneficial effects would be evidenced by consistent improvements over multiple tasks or cognitive domains. This emphasises the need for supplementary work in the area to

clarify to what extent resveratrol may be able to exert cognitive enhancing effects within this compromised cohort.

Moreover, a recent shift in literature focus has emphasised the importance that gut microbial composition has on host health, including cognitive performance. Dysbiosis of the gut microbiota is associated with disruption to the intestinal barrier, pro-inflammatory response and the pathogenesis of metabolic diseases, including obesity. Diet, including polyphenolic intervention has been demonstrated to provide beneficial modulatory effects, with corresponding improvements to metabolic health outcomes also observed. However, this work, particularly in humans is very limited. Furthermore, given the ability of the microbiota to interact with cognition and mood, via the gut-brain-axis, the health promoting effects of polyphenolic-modulation of the microbiota, likely also extend to cognitive-enhancing effects. Given the limited human trials investigating the effect of resveratrol on these factors, particularly concurrently, further study was warranted to address the methodological limitations and expand the field of research.

Consequently, to address this, Chapter 2 aimed to investigate the effects of chronic (500 mg/ day/ 4 weeks) resveratrol supplementation in a wider demographic than previously studied. Specifically, it was theorised that the study of a broader age and weight range would encompass participants of varying inflammatory statuses, where resveratrol supplementation may be more beneficial than previous young, healthy participants employed. Chapter 4 then comprised the first RCT to simultaneously measure the effect of resveratrol supplementation (500 mg/ day/ 12 weeks) on cognitive performance, cerebral blood flow, systemic inflammation and gastrointestinal microbiota and was also novel in terms of the overweight-obese middle-aged adult population utilised.

5.2 Summary of empirical findings

5.2.1 Cognitive function

The intervention studies detailed within this thesis were conducted to assess if resveratrol supplementation would provide more fruitful cognitive benefits in compromised (overweight and obese) individuals, in contrast to previous null findings in healthy, young cohorts. However, no clear effects of acute (40 minutes) or chronic (4 week) resveratrol supplementation was observed in Chapter 2. In Chapter 4, resveratrol supplementation did not enhance cognitive performance in overweight and obese adults, with participants in the

placebo condition performing more accurately and quickly across much of the cognitive paradigm.

These findings are in support of previous findings in young, healthy individuals (as reviewed in Wightman et al. (2019)), who reported no cognitive benefits of acute (between 45 – 90 minutes) resveratrol supplementation. Similar null effects have also been observed in older (aged 40-80 years) type-2 diabetics, where acute administration of resveratrol (75, 150 and 300 mg dosage) did not result in any significant changes in cognitive task performance. Likewise, 6-week supplementation of 75 mg resveratrol did not have an effect on cognitive performance in obese adults (Wong et al., 2013). Although the seemingly negative results of resveratrol on cognition in chapter 4 were surprising, there is previous limited evidence to suggest a potential detrimental effect of resveratrol supplementation. However, this seems to be limited to just 1 trial, within older (aged 50-69 years) adults, where acute supplementation of 500 mg resveratrol resulted in less accurate and slower performance on just 1 task; the rapid visual information processing task (Eschle, 2017).

In contrast, improvements in select aspects of cognitive performance has previously been observed. For example, psychomotor processing speed was improved on a Trail Making Task, following 90-day supplementation with 1000 mg resveratrol in older (aged 65-93 years), overweight adults (Anton et al., 2018). Similarly, 26-week co-supplementation of resveratrol and quercetin improved delayed retention of words in overweight, older adults (aged 50-80 years) (Witte et al., 2014). Post-menopausal women have received increasing interest as a model of compromise within the literature. Here, improved performance has been observed on all four tasks within a neuropsychological test battery following 14-week supplementation with 150 mg resveratrol (Evans et al., 2017). Moreover, limited cognitive improvements have been observed following 12-month supplementation with 150 mg resveratrol; notably an improvement on pattern comparison speed test and improvements in processing speed and cognitive flexibility (Zaw et al., 2020a, 2020b).

It could be argued, however, that the above studies citing cognitive benefits from resveratrol often appear to over-interpret their findings. Specifically, cognitive enhancements are often reported where positive findings have only been observed on one task or outcome measure. For example, within Zaw et al. (2020a), analysis indicated that whilst overall cognitive performance was significantly improved following resveratrol supplementation, analysis of the twelve individual cognitive tasks indicates only significant effects were observed on two of these (dimensional change card sort test and forward spatial span test). No enhancements were observed on any of the other cognitive domains. To illustrate a more robust cognitive

enhancing effect, we would surely anticipate clearer positive findings across multiple individual tasks, or cognitive domains. Moreover, several of the above studies employed short cognitive paradigms, comprising of few individual cognitive tasks; meaning limited cognitive domains were assessed and making it difficult to extrapolate cognitive benefits beyond said specific task.

The current programme of work aimed to observe clearer cognitive findings by including a wide range of individual cognitive tasks, which loaded onto various cognitive domains. Moreover, within Chapter 4 in particular, the paradigm was specifically designed to be cognitively demanding with assessments lasting ~60 minutes each and repeated three times over the course of the day. Here it was hypothesised that beneficial cognitive effects would be more clear during cognitive demand.

Overall, the findings observed throughout this thesis indicate that, as in healthy, young adults, resveratrol supplementation does not induce cognitive benefit in overweight-obese individuals. Moreover, we could tentatively suggest that it may result in cognitive impairment, when compared with placebo intervention. Therefore, based now on consistent null or very small beneficial findings in the literature, it is difficult to conclude that resveratrol has a potential to improve cognitive function within numerous demographics. The exception here being that work in post-menopausal women appears the most promising. Therefore, within this demographic, employing a study design similar to that within this thesis might be most promising; particularly if various additional measures such as gastrointestinal composition and a more comprehensive range of blood biomarkers are also measured simultaneously.

5.2.2. Cerebral blood flow

The study presented within Chapter 4 partially aimed to investigate if resveratrol supplementation would modulate cerebral blood flow; as is consistently observed in the healthy, young adults within previous research. This is of particular interest here as it has been indicated that overweight status negatively impacts cerebral blood flow in a similar way to ageing (Knight et al., 2021). This suggests, based on past consistent modulation of CBF, that resveratrol supplementation within the population employed in this study, may be able to mitigate CBF deficits induced by overweight status.

The findings of the current study observed clear modulation of cerebral blood parameters. Here, planned comparisons indicated that Total haemoglobin and Oxygenated haemoglobin was increased during all epochs on Day 84. Likewise, relative to placebo, resveratrol

supplementation resulted in increased deoxygenated haemoglobin on Day 84 during serial three subtractions and RVIP, with trends towards increased concentration during serial subtractions of sevens. These findings clearly illustrate the ability of 12-week resveratrol supplementation to modulate CBF and are in partial agreement with previous work. Here, much of the previous work measured acute changes in blood flow (Eschle, 2017; Kennedy et al., 2010; Wightman et al., 2014), whereas this study did not observe any significant modulation following just one dose of resveratrol.

Methodological discrepancies potentially explain these differences in acute effects, notably the device employed. Where much of the previous work has utilised continuous wave NIRS, which measures relative changes in CBF over a continuous period; the present study used frequency domain NIRS, which measures absolute quantities of haemoglobin. One of the key advantages of frequency domain NIRS is that it is better suited to chronic paradigms, where gross changes in haemoglobin can be measured throughout the supplementation period. In contrast, continuous wave NIRS is only fully suited to acute paradigms, as the data collected is baseline-adjusted to the concentration reading of the first data point in the recording session. However, one key difference in this methodological approach is that, due to the measuring differences, continuous wave NIRS requires the headband to be fitted for the duration of the testing session; whereas frequency domain NIRS data is collected throughout the necessary post-dose assessment.

This factor, in part, dictated the testing time-frame of this previous research. The comfort of participants limited the maximum time of NIRS recording to approximately 2 to 2.5 hours and so the absorption period was restricted to approximately 45 minutes in length. However, the paradigm restrictions of chapter 4 here, meant that post-dose effects of resveratrol weren't assessed until ~115 or ~155 minutes post dose. As such, the discrepancy in timeframes may account for the lack of acute findings observed within Day 1, where CBF modulation may be more apparent earlier. An alternative methodology here would be for participants to be fitted with the NIRS headband throughout the first full post dose assessment. This would both provide CBF data during a more demanding, varied cognitive paradigm, but also alleviate said discrepancies. However, this methodological change would drastically reduce testing feasibilities, resulting in just one participant being able to be tested each day, due to the availability of equipment. As small sample sizes are a likely methodological flaw in previous research that concurrently measured cognitive performance and cerebral blood flow, adapting the methodology to the detriment of sample sizes feels counterintuitive in this situation.

Despite the null findings on Day 1, the chronic modulation of CBF observed on Day 84 is mostly consistent with previous findings. Here, increases in concentrations of total haemoglobin and oxygenated haemoglobin were observed throughout all timepoints on Day 84; consistent with previous work (Kennedy et al., 2010; Wightman, Haskell-Ramsay, Reay, et al., 2015; Wightman et al., 2014). Given that total haemoglobin is calculated as a sum of oxygenated and deoxygenated haemoglobin concentrations, it seems clear that a total haemoglobin increase would be observed when oxygenated haemoglobin was increased in response to neural demand. However, in a typical neurovascular coupling response we would anticipate increased concentrations of oxygenated haemoglobin to be paired with decreased deoxygenated haemoglobin. However, this study also observed increased deoxygenated haemoglobin during certain aspects of task performance. Increased deoxygenated haemoglobin has been observed previously following acute supplementation with 500 mg resveratrol (Kennedy et al., 2010); here the authors suggest this might be indicative of enhanced utilisation and oxygen extraction during task performance. However, contrary to present findings, in this work, increases in deoxygenated haemoglobin were observed with no significant effect on oxygenated haemoglobin. It is difficult to infer why concurrent increases in oxygenated and deoxygenated haemoglobin would be observed; however, it is tentatively suggested that this may be indicative of resveratrols ability to increase cerebral blood volume. In support of this, similar results have been observed in healthy young adults, where acute exercise (cycling) resulted in increased oxygenated and deoxygenated haemoglobin in the prefrontal cortex (Giles et al., 2014); which the authors suggest indicates increased total blood flow. These discrepancies in findings, however, certainly warrant further investigation; here additional neuroimaging techniques such as multi-channel NIRS or fMRI may provide useful insight into cerebral haemodynamic response within additional brain regions beyond the prefrontal cortex.

5.2.3. Inflammation

Resveratrol has continually been illustrated to exert anti-inflammatory effects, specifically via interaction and inhibition of pro-inflammatory signalling pathways including the NF-kB and JAK/STAT pathways (Renaud & Martinoli, 2014). Results include the reduction of pro-inflammatory cytokines such as IL-6, IL-12 and TNF-α (Meng et al., 2021). Given the detrimental impact of sustained inflammation on various aspects of host health, including cognitive function via neuroinflammation, the pathogenesis of metabolic diseases including obesity and, also, the close link with microbial dysbiosis, it was hypothesised that resveratrols ability to interact with inflammatory pathways would likely improve host health via a multitude of mechanisms.

The findings of the experimental chapters however did not support a beneficial effect of resveratrol on inflammatory biomarkers. Within Chapter 2, no significant effects were observed on either CRP or IL-6. However, we suggested this was likely due to employing too healthy a population; with change in these markers often only manifesting in disease states or more extreme models of damage. Despite this, within a more compromised demographic as in Chapter 4, still no significant effects were observed on either inflammatory marker measured.

However, similar null effects have been observed previously and help to shed light on these null findings. Specifically, in post-menopausal women, a 12-week supplementation of resveratrol (75 mg) resulted in no change to inflammatory biomarkers (Yoshino et al., 2012). And further, in patients with metabolic syndrome, 16-week supplementation of high (1000 mg) and low (150 mg) doses of resveratrol had no effect on CRP levels or inflammatory gene expression (Kjær et al., 2017). In fact, in healthy participants, administration of a single 5 g dose of resveratrol resulted in an increase of TNF-α and NF-kB activation (Gualdoni et al., 2014); although this pro-inflammatory response is likely explained by the high dose administered, particularly when comparing that with previous work in healthy adults that employed much lower doses.

Whereas beneficial anti-inflammatory effects have previously been observed, these are predominantly in models of anti-inflammatory disease, where resveratrol may be able to exert a more beneficial response due to higher baseline inflammatory levels. Specifically, 60-day co-supplementation of resveratrol (20 mg) and calcium fructoborate (112 mg), resulted in reductions in high sensitivity C-reactive protein in patients with angina pectoris (Militaru et al., 2013). Moreover, in patients receiving oral implantology, 4-week resveratrol supplementation (2 mg/kg) reduced serum levels of IL-1β, IL-17A and TNF-α (BaGen et al., 2018). Similar beneficial effects have also been observed in vascular inflammatory disease and ulcerative colitis (Samsami-Kor et al., 2015; Shi et al., 2017). It makes sense to give the final word here to a recent meta-analysis, comprising 35 RCTs, who concluded that resveratrol supplementation was capable of reducing levels of CRP and hs-CRP following ≥10 week supplementation of ≥500 mg/day. However, when considering the demographics of individual studies included, each were some form of compromised population, where exacerbated inflammatory response may be more likely (7 diabetes, 5 overweight, 5 metabolic syndrome, 4 angina, 3 coronary artery disease, 2 cardiovascular disease and the remaining included NAFLD, ulcerative colitis, hypercholesterolemia, arthritis, PCOS and post-menopausal women). Given the exacerbated chronic inflammation typically observed within these disease

models, comparison with our relatively healthy sample is difficult. However, this potentially accounts for the lack of findings in our cohorts; where elevated inflammation due to overweight status is unlikely to be as excessive as within these disease models. Again, this suggests that resveratrol supplementation may be most beneficial in highly compromised models.

Nevertheless, as previously mentioned, it is crucial to recognise the methodological limitations within these experimental chapters. Firstly, due to difficulty accessing veins in overweight-obese participants, the inability to collect intravenous blood samples resulted in a reduced sample size for serum and plasma biomarker analysis. Here, said reduced sample size likely underpins the null findings observed. To address this limitation, in the future, venous assessment should be included as part of the screening process and participants should be excluded where the phlebotomist is unable to obtain a baseline sample. A secondary consideration is to assess a wider inflammatory profile. It was suggested that the decision to measure IL-6 might have been an inadequate marker within a healthy population, where inflammation might not be sufficiently compromised to exert an IL-6 response. This was particularly observed in Chapter 2, where analysis was unable to detect the low IL-6 concentrations within the sample. Further work should employ supplementary biomarker analysis such as TNF-α, high-sensitive CRP or a wider range of interleukins, where a more pronounced anti-inflammatory effect might be observed. Moreover, as discussed in more detail within Section 5.3., the sample population utilised within this work may not have been as compromised as they had been assumed to be and so this factor should be confirmed prior to randomisation in future.

5.2.4. Gut microbiota

With the advent of more accessible microbial assessment techniques, researchers now have the capability to determine whether nutritional intervention is regulated by the gut microbiome; with the assumption for a long time, that it has the ability to create so-called ‘responders’ and ‘non-responders’. This is true also of the effect of polyphenols; with many surmising that the naturally low bioavailability of polyphenols, like resveratrol, is due to an inhospitable host environment. Here, it was hypothesised that, particularly within the overweight-obese population employed in Chapter 4, resveratrol supplementation would have the capability to modulate microbial composition. Here, obesity is associated with disruptions to the gut barrier and dysbiosis of the microbiota composition; resulting in exacerbation of the pro-inflammatory response typically observed in this population. Here, the potential prebiotic-like effects of resveratrol are considered to benefit host health, by also modulating inflammatory response, positively benefitting host health via the aforementioned mechanisms. It was also of interest

to investigate whether the very act of consuming resveratrol over a significant period of time, 12-weeks, could alter the host microbial environment; thus creating a microbial profile which was better able to transform resveratrol for absorption rather than excretion.

With such limited previous work investigating resveratrol supplementation on microbial composition, it was difficult to hypothesise what microbial shifts we might observe. When considering dietary intervention work, the literature typically reports vast changes in specific bacterial strains and shifts in microbial composition. However, this was not observed in our findings within Chapter 4. Whilst at first glance it might be tempting to interpret this as a negative finding, actually observing a great change in alpha diversity would be indicative of an antimicrobial effect of the intervention. Which, within a healthy cohort, as employed within this study, would likely result in detriments to health. As such, the observed no change in alpha diversity should be considered favourable, particularly when considering the design of the study (a relatively small dietary intervention of just one phenolic compound, over a short time frame) and the demographic of healthy individuals free of chronic disease (regardless of weight status).

Additionally, no change in beta diversity was observed following treatment intervention. However, analysis indicated a great spread in individual microbial compositions and these said individual variations likely explain the small shifts in bacterial taxa that were observed following treatment administration. Notably, resveratrol supplementation appeared to result in increased abundance of Bacteroidia (Barnesiellaceae); an effect which has previously been observed following 4-week supplementation with California strawberry powder (Ezzat-Zadeh et al., 2021). Pertinent to the sample utilised, this bacterial strain has previously been associated with improvements in host health, including lean body weight and lower serum cholesterol and triglycerides (Parkar & Blum, 2022). However, additional analysis beyond the scope of this investigation would be required to quantify said shifts in bacterial species and provide greater insight into the effect resveratrol supplementation has on individual microbial profiles.

Given that the microbial analysis indicates a large distribution between individuals in this chapter, it is difficult to ascertain if the small bacterial shifts are actual effects of treatment or, more likely, are due to the distribution of composition within the sample employed. Recent evidence indicates that an individual's baseline gut composition likely has an impact on their individual response to dietary intervention. As such, it is crucial that future work considers factors that likely impact the degree of response. Specifically, the degree of microbial plasticity is considered a key determining factor in the level of response and this is impacted by baseline

microbial populations (Zmora et al., 2018). Here, rodent research indicates that baseline compositions with reduced microbial diversity may be associated with impaired responsiveness to dietary interventions (Griffin et al., 2017). Additionally, animal work indicates that ancestral dietary history is likely to both effect microbial composition and the responsiveness to intervention. It has been illustrated, in mice, that following several generations consuming a diet low in microbiota-accessible carbohydrates (MAC) (which is typically observed in Western diets and sourced from dietary fibre) results in reduced diversity. Whilst the impact is largely reversible within a single generation, over several generations, a low-MAC diet results in progressive loss of diversity which is not recoverable by reintroduction of dietary MACs (Sonnenburg et al., 2016). Although this work illustrates a role of dietary history and baseline composition on microbial response to dietary intervention, to date, the contribution of these factors is unclear; with ongoing work aiming to investigate this further and underpin the complex relationship of these factors (Yang, Ye, Yan, He, & Xing, 2019).

It has further been suggested that certain baseline gut microbiota profiles may express an inherent resistance to change and increase their resilience toward dietary modification (Healey et al., 2017). As previously mentioned, it has been suggested that baseline microbial diversity and gene richness are both associated with individualised gut microbial response (Salonen et al., 2014; Tap et al., 2015). Salonen et al. (2014) conducted a study in overweight men (n=14) and identified participants as responders and non-responders based on microbial community stability during 3 dietary interventions (resistant starch, non-starch polysaccharide and weight loss interventions). They demonstrated that responders have gut microbiota communities that were unstable and non-responders as having gut microbiota communities that were more stable in response to the dietary interventions. They further showed that responders had significantly lower baseline alpha diversity scores than non-responders. Similarly, it has been shown that individuals with higher alpha diversity at baseline were more resilient to change when given a high dietary fibre intervention (Tap et al., 2015). Microbial gene richness has also been shown to influence gut microbiota responsiveness, with one study in overweight and obese individuals illustrating that individuals with high bacterial gene richness were less likely to experience a change in gene richness, but individuals with low bacterial richness has a significant increase in gene richness in response to the dietary intervention (Cotillard et al., 2013a). This highlights that greater microbial diversity and gene richness may lead to a gut microbiota profile that is more resilient to dietary change.

Despite this, there is little conclusion in determining what constitutes a resilient gut microbiota profile and it is further suggested that gut microbial resilience likely will differ depending on numerous factors; including host characteristics such as age, sex and habitual dietary intakes

of the study cohort, alongside the dietary intervention being studied (Healey et al., 2017). With this area of work in its infancy it is difficult to predict microbial response to dietary intervention and how that may impact host health. Therefore, to develop understanding further it is imperative that supplementary work is conducted assessing the microbial response to various dietary interventions in differing demographic groups. This is particularly the case in those where we would anticipate low microbial diversity, such as obese individuals, to further understand what a resilient microbiota may consist of and investigate the potential of microbial ‘responders’ to resveratrol intervention.

5.2.5. Physiological metabolic assessments and biomarkers

Research is pretty clear on the ability of resveratrol to exert cardiovascular protective effects; including reductions in cholesterol levels and improving glucose sensitivity (Lagouge et al., 2006; Xie et al., 2014). As such, the experimental chapters analysed blood biomarkers related to cardiovascular health and more widespread health markers to assess the effects of resveratrol supplementation. However, with the exception of cholesterol biomarkers, no significant effects were observed at any time point during either trial. As above, the lack of findings within these biomarkers are likely explained due to difficulty obtaining blood samples, resulting in reduced sample sizes. Whilst it is difficult to ascertain if any significant effects would have been observed if blood samples were obtained from all participants, the current findings do not suggest any effect of resveratrol supplementation on ferric reducing antioxidant power (FRAP) or glucose.

However, when considering biomarkers of cholesterol, several significant effects were observed across the trials. For example, within Chapter 2, a trend towards a significant reduction of triglycerides was observed on Day 1 and the same effect became significant on Day 28. Additionally, a trend towards increased total cholesterol concentration was observed on Day 1, and a trend towards a reduction in HDL cholesterol, following resveratrol supplementation, was reported on Day 1 also. Within Chapter 4, a significant reduction in triglycerides concentration was observed on Day 1, following resveratrol supplementation, and a significant pure chronic effect was also observed. Here, total cholesterol concentrations were lower following resveratrol on Day 84, at the afternoon sample time-point.

These mixed findings are consistent with the literature to date that has also observed varied effects of resveratrol supplementation on cholesterol biomarkers. Both of the current studies observed reductions in triglycerides which is consistent with previous work in animals (Andrade et al., 2014; Cho et al., 2012) and also in humans, including following 30-day

supplementation with 150 mg resveratrol in healthy, obese men (Timmers et al., 2011). The same triglyceride lowering effect has been observed in healthy adults with a diagnosis of dyslipidaemia, following 2-month supplementation with 100 mg resveratrol (Simental-Mendía & Guerrero-Romero, 2019) and within several other human trials that presented resveratrol as a nutraceutical formula (alongside grape extracts, quercetin and epigallocatechin-3-gallate (Most et al., 2016; Qureshi et al., 2012; Tomé-Carneiro et al., 2012). Here, a typical beneficial cholesterol lowering response would be reductions in triglycerides, LDL and total cholesterol, alongside an increase in HDL concentrations (Arsenault et al., 2009). When considering both current trials, we observe polarised findings on total cholesterol; with a trend towards increased concentrations observed in Chapter 2 and a significant reduction in Chapter 4. Previous work has observed reductions in total cholesterol for example, following 3-month resveratrol supplementation (250 mg) in patients with type 2 diabetes (Bhatt et al., 2012). This was observed also within the above trial in patients with dyslipidaemia (Simental-Mendía & Guerrero-Romero, 2019). Despite beneficial findings observed in these trials, supplementary work has not observed any cholesterol lowering effects (Dash et al., 2013; Haghighatdoost & Hariri, 2018; Sahebkar, 2013; van der Made et al., 2015; Javid et al., 2017). Given the varied findings within the literature and the current studies in this thesis, it is difficult to understand the effect of resveratrol supplementation on lipid profiles in healthy populations. The need for additional work to further clarify this can be the only response here.

As additional assessments of metabolic health, both experimental studies measured blood pressure following each cognitive assessment and also change in weight/BMI throughout the course of the supplementation period. No significant effects on weight or BMI were observed in either study. Further, whilst Chapter 2 noted no effect on blood pressure at any timepoint, Chapter 4 indicated that, on Day 1, diastolic blood pressure and heart rate were significantly lower following placebo, when compared to resveratrol supplementation. This limited, negative effect of resveratrol supplementation on blood pressure is contradictory to previous work which has observed blood pressure lowering effects following resveratrol supplementation in animal models (Cheng et al., 2014; Gordish & Beierwaltes, 2016; Mozafari et al., 2015). However, in human trials, results are less convincing. Here, several studies, mostly in participants with metabolic disease, have observed reductions in systolic and diastolic blood pressure (Bhatt et al., 2012; Heebøll et al., 2016; Imamura et al., 2017; Timmers et al., 2011). With a recent meta-analysis comprising 17 studies, observing favourable, but non-significant blood pressure lowering effects of resveratrol on systolic blood pressure, but no effect on diastolic blood pressure (Fogacci et al., 2019). However, additional studies have not observed any beneficial effects of resveratrol supplementation on blood pressure (Faghihzadeh et al., 2015; Kjær et al., 2017; Poulsen et al., 2013; Wong et al., 2013; Zamora-Ros et al., 2012; Zaw et al., 2020b).

Several other meta-analyses have also indicated no beneficial effects of resveratrol supplementation on blood pressure (Liu et al., 2015; Sahebkar et al., 2015) but, some individual trials have noted increases.

For example, in line with the findings in Chapter 4, an observation trial in Iranian adults reported that the highest quartile of stilbene intake 0.054 mg/day) was positively associated with high blood pressure (Sohrab et al., 2013). Similarly, diastolic blood pressure was observed to be increased following acute supplementation of 250 mg resveratrol and 20 mg piperine in healthy, young adults (Wightman et al., 2014). The same increase in diastolic blood pressure was observed following 28-day supplementation with 500 mg resveratrol (Wightman, Haskell-Ramsay, Reay, et al., 2015). Given the mixed findings in the literature this far it is difficult to determine the effects of resveratrol supplementation on blood pressure. However, results from the current work indicate a potential blood pressure increasing effect and it is tempting to link this to the co-occurring negative effects on cognition also observed in chapter 4; although the mechanism for this is not apparent. Additional work measuring blood pressure more regularly throughout the supplementation period and testing visits, particularly via the use of 24-hour blood pressure monitors, would likely provide more informing data on this effect.

5.3. Limitations

The experimental studies within this thesis have addressed multiple limitations associated with previous work investigating the effects of resveratrol supplementation on cognitive performance. Notably, they employed considerably larger sample sizes than much of the previous work and employed chronic paradigms assessing prolonged (4-week and 12-week) supplementation on cognitive performance. In addition, building upon recent work indicating that resveratrol supplementation is likely most beneficial in compromised demographic groups; this program of work targeted participants who are likely compromised due to overweight status. This provided the additional benefit of researching a group which is hugely underrepresented in the field of nutrition research more generally; those outside of the 'healthy' BMI window. Moreover, the consideration of the impact that resveratrol can have on microbial composition and resulting health outcomes, is a relatively novel addition to the research area. The employment of novel methodologies to the research area, such as concurrent measurement of cognitive performance, cerebral blood flow, gastrointestinal microbiota and of various biomarkers, also allowed development of understanding of resveratrol supplementation in healthy adults of varying weight ranges. However, as

discussed within previous chapters, it is crucial to recognise the methodological limitations that might have impacted the findings within this thesis.

Firstly, we must consider the populations utilised within this body of work. Although it has been stated many times, it's worth reiterating that the literature to date has indicated that resveratrol supplementation appears largely ineffective in a young, healthy population. More promising findings have recently been observed in populations considered 'compromised', be that via ageing, disease or overweight status. Therefore, the current body of work aimed to investigate these effects specifically in participants compromised by overweight status; where said individuals likely have exacerbated systemic inflammation, disruptions to intestinal barrier and dysbiosis of the microbial composition – each of which is related to metabolic dysfunction and disease. Within Chapter 2, a wide range of participants were employed, both in terms of age and weight status and the hypothesis was that this would result in a wider range of inflammatory statuses, irrespective of weight status. It was further hypothesised that an individual with a more compromised inflammatory status would benefit more from resveratrol supplementation.

There were several flaws within this assumption. Firstly, due to the low number of blood samples obtained, issues with IL-6 analysis sensitivity and a small inflammatory profile measured; we cannot determine if a range of inflammatory statuses was indeed captured within the sample population. Here, a greater understanding of baseline inflammatory status is necessary, which would be particularly useful in stratifying recruitment into the study, allowing a guaranteed distribution. Not only would this be useful in understanding the effects of resveratrol in varying inflammatory states, but if detailed dietary information was collected in conjunction, an interesting avenue of research would be to investigate in more detail potential 'protective' elements of diet. Here recent work has considered the inflammatory potential of individual diets, where detailed dietary intake information collected via food frequency questionnaires or 24-hour diet diaries can be measured using the dietary inflammatory index (Shivappa, Steck, Hurley, Hussey, & Hébert, 2014). Moreover, pertinent to this area of work, collection of said dietary intervention lends itself to calculation of daily phenolic intake via the Phenol-Explorer database (Neveu et al., 2010). Whilst using FFQs and dietary diaries comes with its own limitations, particularly reporting bias, recent developments in web-based and smartphone applications FFQs, appear promising in improving portion size estimation and increasing usability (Ambrosini, Hurworth, Giglia, Trapp, & Strauss, 2018; Jobarteh et al., 2020). Nevertheless, both areas of work would provide greater understanding of the health status of individual participants and moreover, offer better knowledge into the profile of an individual most likely to benefit from resveratrol supplementation.

The same limitation is observed within Chapter 4, where although this aimed to target more 'compromised' individuals by only recruiting overweight and obese participants, there is a number of constraints with the methodology used to do this. One such constraint is that participant eligibility was assessed using just BMI calculations. Given the shortfalls of BMI classification, namely the inability to discriminate between lean muscle and fat mass, there is the potential that participants included within the trial were classified incorrectly and were 'too-healthy' for the demographic targeted (Flegal et al., 2009). The inclusion of waist-to-hip ratio measurement here does provide a measurement of fat accumulation (for example, the average of 0.89 would indicate that participants typically had high abdominal fat accumulation) and so could provide an easy assessment alongside BMI calculation. Nevertheless, a better option to assess eligibility into studies targeting overweight-obese compromised individuals would be do employ methodology such as DXA scanning; which has the capability of assessing fat mass.

Moreover, it was potentially too blunt of an assumption that overweight and obese participants would be 'compromised' in terms of inflammatory status, gut microbial composition and cognitive performance, when compared to young, healthy individuals that previous work has targeted. And again, without a more comprehensive baseline inflammatory profile it is difficult to ascertain this. Furthermore, given that it has previously been suggested that trial volunteers are typically health conscious, with potentially higher nutritional intake than the general population (Morris & Tangney, 2011; Young et al., 2020), a further constraint is that trials of this nature may be unlikely to be able to recruit participants 'compromised' enough to benefit from resveratrol. Furthermore, with a strict exclusion criteria employed within these current trials, it seems probable that any true 'compromised' individuals would be excluded due to concomitant diagnosed medical conditions. Indeed, where previous findings have appeared more promising, these have been observed in compromised demographics primarily with metabolic diseases such as diabetes and metabolic syndrome.

Lastly, there are evident limitations in the microbiota and metabolomic analyses, which unfortunately due to time and financial constraints of this thesis meant follow-up analysis could not be completed. Here, whilst both analyses indicated small treatment related changes in composition, ideally subsequent analysis would have been conducted to quantify said changes. This would provide greater understanding as to how resveratrol supplementation impacted microbial and urinary metabolomic composition within a healthy, overweight cohort. However, despite this being out of the realms of this line of work, based on the limited shifts observed, it is likely that supplementary analysis would not have yielded more fruitful findings.

As such, a more interesting line of work here, would be to consider individual variation in response to resveratrol. Specifically, supplementary research should consider if resveratrol 'responders' can be identified by observing changes in microbial composition. This would be particularly relevant given that the only two previous studies investigating resveratrol supplementation on microbial composition in humans has observed some interesting findings. Notably, 12-week co-supplementation of resveratrol with epigallocatechin-3-gallate resulted in reduction in *Bacteroidetes* and also in *Faecalibacterium prausnitzii* and an increase in fat oxidation, however these beneficial findings were only observed in male participants (Most et al., 2016). Whilst it is not clear why a sex difference would we observed, a potential issue within Chapter 4, is the unbalanced sex split in participants, in favour of females. Here, a recruitment stratification by gender, might allow more insightful changes to microbiota composition. Similar demographic differences were observed following 35-day supplementation with 2 g resveratrol in male participants, where increases in *Akkermansia* abundance was observed alongside insulin sensitivity and glucose homeostasis, only in Caucasian participants (Walker et al., 2019). Additionally, given the known impact of baseline microbial composition on dietary response, it is crucial that further research is conducted to ascertain if a particular baseline microbial profile is associated with response, or conversely resilience to resveratrol supplementation. This interesting area of work will enable more targeted intervention in the future, to specific individuals likely to benefit most from resveratrol supplementation.

5.4. Future research

5.4.1. Improving the bioavailability of resveratrol supplements

One of the potential explanations underpinning the lack of beneficial findings within this thesis and area of literature as a whole, is the poor bioavailability of resveratrol. This is particularly apparent within Chapter 4, where no significant differences were observed on resveratrol metabolites between treatment groups. As such, most recent work in the area has considered methodologies to improve absorption and oral bioavailability. One such example includes using LipiSperse®, which is a new delivery system that is specifically designed to increase the dispersion of lipophilic ingredients, like resveratrol, in aqueous environments (Briskey, Sax, Mallard, & Rao, 2019). A recent trial aimed to compare the oral bioavailability of Veri-te resveratrol (used within this thesis) and resveratrol- LipiSperse® complex (Veri-Sperse®) (Briskey & Rao, 2020). Here healthy adults received either 150 mg Veri-te, 75- or 150- mg Veri-Sperse®, with venous blood samples collected at baseline and then 30 minutes, 1, 2, 3, 4, 5, 6, 8 and 24 hours post dose. Their findings indicated that, when compared with 150 mg

Veri-te, 150 mg Veri-Sperse® resulted in significant improvements in oral absorption; as evidenced by increases in plasma concentrations of resveratrol conjugates.

Additional novel delivery systems include development of solid lipid nanoparticles and nanostructured lipid carriers, which have been observed to slow the rapid metabolism of resveratrol and increase its physical stability, resulting in a controlled release after uptake (Neves, Lúcio, Martins, Lima, & Reis, 2013). Further, self-nanoemulsifying drug delivery systems (SNEDDS) have been observed to be a good methodology for delivering lipophilic compounds; where SNEDDS formulation of resveratrol enhanced oral bioavailability in rats (Yen, Chang, Hsu, & Wu, 2017). The above novel delivery systems, alongside additional examples, are detailed further in Chimento et al. (2019) and provide promise in this research area. However, whist results utilizing these novel delivery systems might inform on past null effects, and give industry better targeted resveratrol in the future, this really doesn't help with bioavailability of naturally consumed resveratrol, and indeed other polyphenols.

Another consideration in modifying resveratrol absorption might be to consider the dietary conditions surrounding the supplementation time; a field which has some promising findings utilizing other nutritional interventions. For example, increased intestinal absorption of supplements including Vitamin D3 and omega 3, has been observed when a high fat meal was consumed immediately following supplement administration (Dawson-Hughes, Harris, Palermo, Ceglia, & Rasmussen, 2013; Maurya & Aggarwal, 2017; Shimada et al., 2017). However, the effects that this has on resveratrol absorption is less clear. Here, consumption of 2000 mg resveratrol, alongside a with a high-fat breakfast (~750 kcal, 45 g fat, 60 g carbohydrates and 30 g protein), resulted in lower area under the plasma concentration-time curve, when compared with a standard breakfast (~500 kcal, 18 g fat, 70 g carbohydrates and 12 g protein) (la Porte et al., 2010). The authors here, suggest that in order to maximise resveratrol exposure, supplementation should take place with a standard breakfast, rather than a high-fat breakfast. Moreover, when compared with a fasting condition, consumption of 400 mg resveratrol alongside a high-fat meal (~850-900 kcal, 53 g fat, 57 g carbohydrates and 40 g protein) delayed the rate of absorption but not the extent of absorption in healthy adults (Vaz-da-Silva et al., 2008).

The continued development of new methodologies to improve bioavailability of resveratrol, provide promising future avenues for resveratrol research. Specifically, where incorporation of new nutraceutical formulations of resveratrol into complex paradigms similar to those within this thesis, may result in more fruitful health-promoting effects.

5.4.2. Moving past resveratrol

It is the spirit of the researcher to focus on avenues which suggest promise; building a rationale on the promising findings of previous research and developing sensible paradigms to extend on these reports. However, researchers have to be equally accepting when this is not the case; and a research avenue has come to an end. Whilst still not a large area of research, the consistent null effects observed within the resveratrol literature, despite various methodological approaches, likely confirms the lack of beneficial effects of resveratrol supplementation here. Where previous work has suggested this in young, healthy adults, despite addressing previous methodological limitations, the present findings appear to confirm that this is also the case in older individuals who are potentially more compromised.

This finality with isolated resveratrol supplementation in such groups does, positively, provide direction to other avenues of research though. For example, given that we do not consume polyphenols in isolation, but instead within diet alongside additional macro- and micro-nutrients, it may be more logical to investigate the effects of whole dietary styles on host health. Indeed, the field of polyphenolic intervention on health also appears to be moving towards this conclusion; with much recent work focussing on the role of the MedDiet, specifically on microbial composition. Most recently, as part of the DIRECT-PLUS study, 294 participants with abdominal obesity, were assigned to one of three dietary intervention styles for 18 months (Meir et al., 2021). Said dietary interventions were healthy dietary guidelines, MedDiet or ‘Green-Med’ where participants in the latter group consumed a diet richer in plants and polyphenols, with restricted consumption of processed and red meat. These participants were provided with 3-4 cups/day of green tea and consumed a ‘green shake’ comprised of *Wolffia globosa* for dinner. Whilst both traditional MedDiet and Green-Med resulted in significant changes to microbial composition, the authors report that Green-Med changes were more prominent (Rinott et al., 2022). Specifically, they observed increases in *Prevotella* and reductions in *Bifidobacterium,* and the diet was also associated with beneficial changes in body weight and cardiometabolic biomarkers (including blood pressure and cholesterol) which correlated with increased plant intake. The findings from this trial certainly suggest potential beneficial effects of intervention with a dietary style rich in polyphenols on microbial composition and related metabolic health outcomes. An interesting area of future research would be to investigate if these beneficial effects extend to cognitive function, as was hypothesised of resveratrol within this thesis.

5.5 General conclusions

The aim of this thesis was to investigate the effects of resveratrol supplementation on cognitive function, cerebral blood flow, systemic inflammation and gastrointestinal microbiota in healthy adults of varying weight ranges. The study presented within Chapter 2 provided support for previous work which indicated no clear effects of resveratrol supplementation on cognitive performance in healthy adults (Wightman et al., 2019). Here, despite including a more diverse participant demographic than previous work, and addressing some previous methodological limitations including sample size and supplementation duration, no clear cognitive-enhancing effects of resveratrol supplementation were observed following acute or chronic (4-week) intervention. Given increasing focus on more 'compromised' demographic groups, where resveratrol supplementation appeared to show more cognitive promise in those compromised via age or disease, it was hypothesised that the lack of findings here were due to participants being 'too healthy'.

As such, the Chapter 4 trial was the first to study, concurrently, the effect of resveratrol supplementation on cognitive function, cerebral blood flow, systemic inflammation and gastrointestinal microbiota. Moreover, the employment of a 'compromised' demographic of overweight-obese individuals provides a novel approach to the research area, where typically young healthy adults have previously been utilised. Consistently, with previous work in the area, this study observed clear modulation in cerebral blood flow during task performance. However, despite this, no improvement was observed on cognitive performance within this cohort, with participants in the placebo condition regularly performing better than those in the resveratrol condition. Moreover, limited treatment-related changes in microbial and metabolomic composition were observed, however these were characterised by high individual differences. Without supplementary analysis, which was beyond the scope of this thesis, it is difficult to interpret whether these effects are due to the distribution in individual compositions or an actual treatment effect. Interestingly, no effect on inflammation was observed within either trial and limited, inconsistent findings were observed on cholesterol biomarkers. However, said limited biomarker findings are likely explained by methodological limitations within these trials. Chief amongst these is that the participant demographic employed was assumed to be compromised due to overweight status, resulting in exacerbated inflammatory response and likely dysbiosis of the gut microbiota. It may be that this assumption was too blunt and here participants were still 'too healthy' to benefit from resveratrol supplementation, which may prove most efficient in models of disease.

Taken together, this thesis demonstrates that whilst resveratrol supplementation clearly modulates CBF in healthy, overweight-obese adults. It also confirms that within a healthy population, resveratrol is unable to exert cognitive enhancing effects. However, it is difficult to draw conclusions on the impact on microbial composition and, therefore, this presents the most promising avenue for future investigations, of either resveratrol, supplementary polyphenols or dietary styles.

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Appendices

Appendix I: Chapter 2 Treatment Guess Questionnaire

Study Code: 52P7 Date: ____/____/____
Subject ID:
Randomisation No.:
Visit:

Which treatment do you think you were administered? (please circle)

1. Placebo (dummy pill)
2. Active (Veri-te^{TM} Resveratrol)

What is your reason(s) for thinking this?

____

____

____

Appendix II: Chapter 2 Treatment Diary

SUBJECT DIARY

The acute and chronic effects of resveratrol supplementation on inflammation and cognitive performance in healthy adults.

Subject ID: |__ |__ |__ |

Random Number: | __ |__ |__ |

Dear Participant,

For the success of the study it is very important that you follow the instructions from the study team very carefully. Therefore please find a short summary of the most important points.

Diet

During the course of the whole study you should not change your dietary habits.

Medication / Therapy or Health Problems or Symptoms

If you take any new medication or therapy or if you have any health problems or unusual symptoms you should document in as much detail as possible in this diary on pages 10 & 11 and talk about it to the researcher during the next visit. You do not need to write down routine medications which have already been discussed at the screening visit.

In general you should avoid, if possible, the use of non-prescription drugs during the study. However, if you intend to use non-prescription drugs within the week preceding the next visit, please contact the research team. There is a chance that the visit might be postponed. Prior to each study day you must:

- Fast for 12 hours prior to attending and consume nothing but water during the morning of your testing visit.
- Avoid caffeine containing foods or beverages for 18 hours and avoid alcohol for 24hrs
- Remember to complete your diary every day and bring it with your last visit
- Avoid over the counter medications for 24hrs and oral antihistamines for 48hrs

You should reschedule the appointment if you are feeling unwell or require medication. If unsure, please contact the researcher

- Please bring details of any changes to, or new, medications or supplements that you may have taken between appointments so we can document details about them (name, dosage etc.)

Taking the Capsules

1 capsule should be taken **30 minutes after your breakfast** and 1 capsule **30 minutes after your evening meal** each day except on testing visits. They should be taken with a standard glass of water. **Do not take your capsule on the day of your next visit as both capsules will be taken in the lab.** If you forget to take your breakfast dose then you should take it with your evening meal and record this time on your diary. If you forget to take your evening capsule then please just note this as a missed dose on your diary- you do not need to make this up by taking a third capsule the following day. Please note that the capsules must not be chewed.

Please bring this diary and all unused capsules and empty containers to the next visit.

If you have any questions or any problems occur please contact:
Ellen Smith (Lead Researcher) E-mail: ellen.smith@northumbria.ac.uk or any member of the research team on 0191 2437252 (office hours).

Please remember to take your 1 capsule 30 minutes after breakfast and 1 capsule 30 minutes after your evening meal each day (except for on study visit days). In the table below, please record the date and times, each day, that you take the capsules. Potential 'surplus' days have been inserted in case of unforeseen circumstances; e.g. ill health prevents you from attending your day 29 lab visit. In this case you must contact the researcher right away to reschedule and confirm that you have sufficient treatment to self- supplement for further days. Any ill health or intake of non-routine medications/therapy should be recorded on pages 10 and 11.

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
1	Acute lab visit (x2 capsules consumed in the lab)					
2	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
3	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
4	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
5	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
6	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
7	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
8	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
9	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
10	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
11	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
12	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
13	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
14	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
15	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
16	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
17	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
18	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
19	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
20	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
21	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
22	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
23	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
24	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
25	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
26	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
27	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
28	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
29	Chronic lab visit (x2 capsules consumed in the lab)					
Surplus day	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken

Surplus day	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
Surplus day	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
Surplus day	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken
Surplus day	(____ / ____ / ____)	x1 capsule 30 minutes after breakfast	Time taken	+	x1 capsule 30 minutes after evening meal	Time taken

Since the last visit, have you taken any medication or dietary supplements?

☐ No
☐ Yes: *please give details in the table below. Please include the product/drug/therapy name in full, the amount (e.g. 1x200mg tablet), the date you took it and what you took it for.*

Product/drug/therapy name	Quantity	Dose	Date	What you took it for?
Example: paracetamol	*2 / day*	*1000mg*	*10/11/2016*	*headache*

Have you experienced any new health problems or unusual symptoms since the last visit?

☐ No
☐ Yes*, please give details in the table below. Please include your symptom(s), the date(s) it/they started and stopped if applicable, its/their severity and any action you took to relieve it/them.*

Symptom	Date symptom started	Please rate the severity of the symptom in terms of how it affects your daily functioning 1: Mild 2: Moderate 3: Severe	If resolved please give the date it stopped	Did you do anything to relieve your health problems/symptoms (like drug, therapy)?
Example: headache	*10/11/2016*	*mild*	*10/11/2016*	*paracetamol*

Appendix III: Chapter 4 Treatment Guess Questionnaire

Study Code: 52P6 Date: ____/____/____
Subject ID:
Randomisation No.:
Visit:

Which treatment do you think you were administered? (please circle)

1. Placebo (dummy pill)
2. Active (Veri-te™ Resveratrol)

What is your reason(s) for thinking this?
____
____
____
____

SUBJECT DIARY
Weeks 1 - 6

The acute and chronic effects of Resveratrol supplementation on cognitive function, gastrointestinal microbiota and cerebral blood flow: a double-blind, placebo-controlled parallel-groups study in healthy, overweight humans

Subject ID: |__ |__ |__ |

Random Number: | __ |__ |__ |

Date Subject Diary dispensed: |__ |__ |- |__ |__ |- |__ |__ |

Treatment Exchange Visit - Week beginning: |__ |__ |- |__ |__ |- |__ |__ |

Dear Participant,

For the success of the study it is very important that you follow the instructions from the study team very carefully. Therefore please find a short summary of the most important points.

Diet
During the course of the whole study you should not change your dietary habits.

Medication / Therapy or Health Problems or Symptoms
If you start a new medication you must contact the researcher, as it may exclude you from the study, any medication taken (including over the counter medication) must be documented in the diary on page 12. If you have any health problems or unusual symptoms you should document then in this diary in as much detail as possible on page 13 and talk about it to the researcher during the next visit. You do not need to write down routine medications which have already been discussed at the screening visit. In general you should avoid, if possible, the use of non-prescription drugs during the study.

Taking the Capsules
1 capsule should be taken with breakfast and 1 with your evening meal each day except on testing visits. They should be taken with a standard glass of water. **Do not take your capsule with breakfast on the day of your final visit as both capsules will be taken in the lab.** If you forget to take your breakfast dose then you should take it with your evening meal and record this time on your diary. If you forget to take your evening capsule then please just note this as a missed dose on your diary- you do not need to make this up by taking a third capsule the following day. Please note that the capsules must not be chewed.

Treatment Exchange Visit
You are required to attend the lab, to collect your next bottle of capsules, during Week 6 of the study. The date of this week is shown on the front of this diary and the researcher will be in contact closer to the time to arrange for you to come in. The appointment just lasts 5 minutes and is flexible based on when you are able to attend during that week.
Please bring this diary and all unused capsules and empty containers to the next visit.

If you have any questions or any problems occur please contact:
Ellen Smith (Lead Researcher) E-mail: ellen.smith@northumbria.ac.uk or any member of the research team on 0191 2437252 (office hours).

Please remember to take your 1 capsule with breakfast and 1 capsule with your evening meal each day (except for on study visit days). In the table below, please record the date and times, each day, that you take the capsules. Potential 'surplus' days have been inserted in case you are unable to attend your treatment exchange visit exactly 42 days.

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
1	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
2	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
3	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
4	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
5	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken

6	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
7	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
8	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
9	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
10	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
11	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
12	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
13	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
14	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
15	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
16	(____/ ____/ ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
17	(____/ ____/ ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
18	(____/ ____/ ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
19	(____/ ____/ ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
20	(____/ ____/ ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
21	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
22	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
23	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
24	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
25	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
26	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
27	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
28	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
29	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
30	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken

31	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
32	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
33	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
34	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
35	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
36	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken

37	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken
38	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken
39	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken
40	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken
41	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken
42	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken

Surplus day	(____/ ____/ ____)	x1 capsule 'with breakfast'	Time taken	**+**	x1 capsule 'with evening meal'	Time taken
Surplus day	(____/ ____/ ____)	x1 capsule 'with breakfast'	Time taken	**+**	x1 capsule 'with evening meal'	Time taken
Surplus day	(____/ ____/ ____)	x1 capsule 'with breakfast'	Time taken	**+**	x1 capsule 'with evening meal'	Time taken
Surplus day	(____/ ____/ ____)	x1 capsule 'with breakfast'	Time taken	**+**	x1 capsule 'with evening meal'	Time taken
Treatment Exchange Visit ~ Day 42						

Since the last visit, have you taken any medication or dietary supplements?

☐ No

☐ Yes: *please give details in the table below. Please include the product/drug/therapy name in full, the amount (e.g. 1x200mg tablet), the date you took it and what you took it for.*

Product/drug/therapy name	**Quantity**	**Dose**	**Date**	**What you took it for?**
Example: paracetamol	*2 / day*	*1000mg*	*10/11/2016*	*headache*

Have you experienced any new health problems or unusual symptoms since the last visit?

☐ No
☐ Yes, *please give details in the table below. Please include your symptom(s), the date(s) it/they started and stopped if applicable, its/their severity and any action you took to relieve it/them.*

Symptom	**Date symptom started**	**Please rate the severity of the symptom in terms of how it affects your daily functioning** **1: Mild** **2: Moderate** **3: Severe**	**If resolved please give the date it stopped**	**Did you do anything to relieve your health problems/symptoms (like drug, therapy)?**
Example: headache	*10/11/2016*	*mild*	*10/11/2016*	*paracetamol*

SUBJECT DIARY
Weeks 6 - 12

The acute and chronic effects of Resveratrol supplementation on cognitive function, gastrointestinal microbiota and cerebral blood flow: a double-blind, placebo-controlled parallel-groups study in healthy, overweight humans

Subject ID: |__ |__ |__ |

Random Number: | __ |__ |__ |

Date Subject Diary dispensed: |__ |__ |- |__ |__ |- |__ |__ | *(dd-mm-yy)*

Final testing visit: |__ |__ |- |__ |__ |- |__ |__ | *(dd-mm-yy)*

Dear Participant,

For the success of the study it is very important that you follow the instructions from the study team very carefully. Therefore please find a short summary of the most important points.

Diet
During the course of the whole study you should not change your dietary habits.

Medication / Therapy or Health Problems or Symptoms
If you start a new medication you must contact the researcher, as it may exclude you from the study, any medication taken (including over the counter medication) must be documented in the diary on page 12. If you have any health problems or unusual symptoms you should document then in this diary in as much detail as possible on page 13 and talk about it to the researcher during the next visit. You do not need to write down routine medications which have already been discussed at the screening visit.

In general you should avoid, if possible, the use of non-prescription drugs during the study. However, if you intend to use non-prescription drugs within the week preceding the next visit, please contact the research team. There is a chance that the visit might be postponed. Prior to each study day you must:

- Fast from 8pm the previous evening – consuming nothing but water.
- Avoid caffeine containing foods and beverages for 18 hours
- Avoid alcohol for 24hrs
- Remember to complete your treatment diary every day and bring it with you at your treatment exchange and final study day visit
- Avoid over the counter medications for 24hrs and oral antihistamines for 48hrs
- Bring with you your completed 4 day diet diary and stool sample

You should reschedule the appointment if you are feeling unwell or require medication. If unsure, please contact the researcher.

Taking the Capsules
1 capsule should be taken with breakfast and 1 with your evening meal each day except on testing visits. They should be taken with a standard glass of water. **Do not take your capsule with breakfast on the day of your final visit as both capsules will be taken in the lab.** If you forget to take your breakfast dose then you should take it with your evening meal and record this time on your diary. If you forget to take your evening capsule then please just note this as a missed dose on your diary- you do not need to make this up by taking a third capsule the following day. Please note that the capsules must not be chewed.

Please bring this diary and all unused capsules and empty containers to the next visit.

If you have any questions or any problems occur please contact:
Ellen Smith (Lead Researcher) E-mail: ellen.smith@northumbria.ac.uk or any member of the research team on 0191 2437252 (office hours).

Please remember to take your 1 capsule with breakfast and 1 capsule with your evening meal each day (except for on study visit days). In the table below, please record the date and times, each day, that you take the capsules. Potential 'surplus' days have been inserted in case you are unable to attend your treatment exchange visit exactly 42 days after starting and in case you are unable to attend your second study day exactly 84 days after your first study day visit.

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
Treatment Exchange ~ day 42					x1 capsule 'with evening meal'	Time taken
Surplus day	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
Surplus day	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
Surplus day	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
Surplus day	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken

43	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken
44	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken
45	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken
46	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken
47	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
48	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
49	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
50	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
51	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
52	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
53	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
54	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
55	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
56	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
57	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
58	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken
59	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken
60	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken
61	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken
62	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	+	x1 capsule ‘with evening meal’	Time taken

Day	Date	Dose 1 instruction	Time taken		Dose 2 Instruction	Time taken
63	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	**+**	x1 capsule 'with evening meal'	Time taken
64	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	**+**	x1 capsule 'with evening meal'	Time taken
65	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	**+**	x1 capsule 'with evening meal'	Time taken
66	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	**+**	x1 capsule 'with evening meal'	Time taken
67	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	**+**	x1 capsule 'with evening meal'	Time taken
68	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	**+**	x1 capsule 'with evening meal'	Time taken

69	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
70	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
71	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
72	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
73	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
74	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
75	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken

76	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
77	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
78	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
79	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
79	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
80	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken
81	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken

82	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
83	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
84	Chronic Visit – **DO NOT** consume treatment on this morning as you will take both tablets during the testing visit					
Surplus day	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
Surplus day	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
Surplus day	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken
Surplus day	(____ / ____ / ____)	x1 capsule 'with breakfast'	Time taken	+	x1 capsule 'with evening meal'	Time taken

urplus day	(____ / ____ / ____)	x1 capsule ‘with breakfast’	Time taken	**+**	x1 capsule ‘with evening meal’	Time taken

Since the last visit, have you taken any medication or dietary supplements?

☐ No
☐ Yes: *please give details in the table below. Please include the product/drug/therapy name in full, the amount (e.g. 1x200mg tablet), the date you took it and what you took it for.*

Product/drug/therapy name	Quantity	Dose	Date	What you took it for?
Example: paracetamol	*2 / day*	*1000mg*	*10/11/2016*	*headache*

Have you experienced any new health problems or unusual symptoms since the last visit?

☐ No
☐ Yes*, please give details in the table below. Please include your symptom(s), the date(s) it/they started and stopped if applicable, its/their severity and any action you took to relieve it/them.*

Symptom	**Date symptom started**	**Please rate the severity of the symptom in terms of how it affects your daily functioning** **1: Mild** **2: Moderate** **3: Severe**	**If resolved please give the date it stopped**	**Did you do anything to relieve your health problems/symptoms (like drug, therapy)?**
Example: headache	*10/11/2016*	*mild*	*10/11/2016*	*paracetamol*