The metabolic, cerebral haemodynamic, and cognitive effects of *trans*-resveratrol in healthy, young and older humans.

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Abstract

The stilbene polyphenol *trans*-resveratrol has shown to interact with several mechanisms that may serve to preserve or even enhance cognitive function, either directly or indirectly. A small, but growing body of research has found resveratrol is capable of nitric oxide (NO)-mediated increases to cerebral blood flow (CBF), which, in turn, serve to increase access to the neural metabolic substrates (oxygen & glucose). To date, research has found increases in CBF and oxygen extraction resulting from oral resveratrol administration, yet these have not been found in conjunction with improved cognitive performance in young, healthy samples; questioning the CBF mechanism of resveratrol as an appropriate means to enhance cognitive performance.

Additionally, it was hypothesized that the CBF effects of resveratrol may provide increased utility in naturally ageing populations, as such cohorts are noted to experience reductions to CBF and neural oxygen metabolism; which, in turn, is an acknowledged contributor of age-related cognition. To link the reduced neural oxygen availability suffered during ageing and subsequent poorer cognitive performance, the current thesis also aimed to test the use of hypoxia as a representative, experimental model for the cognitive ageing process. The purpose of this model was to provide clear and direct evidence that the CBF effects of resveratrol can function to attenuate reductions to cognition imposed via a compromised neural fuel supply. Therefore, the four acute, placebo-controlled, double-blind, crossover investigations of this thesis aimed to assess the efficacy of the resveratrol-mediated CBF effects to engender cognitive enhancement; in both young and older adults.

The key findings from this thesis show that there is merit to a hypoxia model of cognitive ageing, evidenced by clear cognitive deficits that are also commonly observed with ageing cohorts. Furthermore, a single dose of resveratrol showed to increase fuel oxidation during cognitive performance when measured via indirect calorimetry. However, resveratrol was unable to provide increases to CBF (when measured by Near-Infrared Spectroscopy) or cognitive performance in hypoxia or in ageing populations. In fact, resveratrol was found to impair cognitive performance in the latter. The results of this thesis therefore do not support the argument that resveratrol can provide CBF-mediated cognitive enhancement in healthy, young or naturally ageing samples.

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Declaration

I declare that the work contained in this thesis is all my own work and has not been submitted for any other award. I also confirm that this work fully acknowledges the opinions, ideas and contributions of the work of others. All investigations in this thesis have been approved by the Northumbria University Department of Psychology (within the Faculty of Health and Life Sciences) Ethics Committee and were conducted according to the Declaration of Helsinki (1964).

I declare that the word count of this thesis is 76,017 words

Name: Timothy Michael Eschle

Signature:

Date:

List of symbols and abbreviations

ATP	Adenosine tri-phosphate
BBB	Blood brain barrier
Ca^{2+}	Calcium
CBF	Cerebral blood flow
CHO	Carbohydrate
$CMRO_2$	Cerebral metabolic rate of oxygen
CNS	Central nervous system
CO_2	Carbon dioxide
CW-NIRS	Continuous wave NIRS
Deoxy-Hb	Deoxygenated haemoglobin
EE	Energy expenditure
eNOS	Endothelium nitric oxide synthase
FD-NIRS	Frequency domain NIRS
F_iO_2	Fraction of inspired oxygen
Hb	Haemoglobin
ICa	Indirect Calorimetry
NIRS	Near-Infrared Spectroscopy
NO	Nitric oxide
NOS	Nitric oxide synthase
P_aO_2	Partial pressure of oxygen in arterial blood
P_aCO_2	Partial pressure of carbon dioxide in arterial blood
PO_2	Partial pressure of oxygen
O_2	Oxygen
Oxy-Hb	Oxygenated haemoglobin
RER	Respiratory exchange ratio
rCBF	Regional cerebral blood flow
Total-Hb	Total haemoglobin

Chapter 1

General Introduction

1.1 Definition, classification and synthesis of polyphenols

1.1.1 Occurrence and synthesis of polyphenols

Plants are immobile and must be self-nourishing, or autotrophic, to survive. Due to their stationary nature, plants face several challenges including the risk of nutrient deprivation, exposure to ultraviolet radiation and numerous environmental stressors (namely pathogens, competing plants and herbivores) (Bennett & Wallsgrove, 1994; Kennedy & Wightman, 2011). Consequently, plants synthesize a wide range of phytochemicals that perform key roles in enhancing survival and providing protection against environmental stressors. It is worth noting that these metabolites do not contribute to the plant's primary physiological or metabolic functioning (such as growth or photosynthesis), but rather enhance the survival of the plant, and aid in its ability to overcome challenges in its immediate environment (Kennedy & Wightman, 2011). Thus, these phytochemicals have been termed 'secondary metabolites' (Crozier et al., 2006). The role of these compounds within plants has been found to include attraction of pollinators and seed-dispersing animals, protection against microbial infection and herbivore attack, and also as signalling molecules for interactions between other plants and herbivores (Pichersky & Gang, 2000; Kennedy, 2014). There are three main categories of secondary metabolites: terpenes, alkaloids and phenolics. The latter of these, will be the sole focus of this thesis.

Phenolics are initially synthesized from shikimic acid via the shikimate pathway, although the acetate malonate pathways can provide components of more complex phenolic structures (Kennedy, 2014; pp 143). Some simple phenolic acids can be produced directly from shikimic acid but, in most cases, this process then proceeds with the essential amino acid L-phenylalanine. This is subjected to hyrdroxylation and methylation to form cinnamic acid, which forms the basis of all polyphenols. The synthesis of stilbene polyphenols occurs via the phenylpropanoid / malonate pathway. Initially phenylalanine, the enzyme phenylalanine ammonia lyase, transforms phenylalanine into cinnamic acid. Further action from 4-hydroxylase and 4-coumarate CoA ligase convert cinnamic acid to produce P-Coumartl-CoA (Lijavetzky et al., 2008).

Stilbene synthase then converts this into the building block for all stilbene polyphenols, including resveratrol.

1.1.2 Classification, definition and sources of polyphenols

Possessing a molecular weight of ≥30,000 Da (Bravo, 1998), polyphenols are an extensive family of phytochemicals that encompass a diverse range of further sub-categories. By strict definition, a polyphenol is a compound that structurally contains two or more phenolic rings. As phenolic acids possess only one phenol ring, and therefore not a polyphenol, the description of these compounds will be omitted from this section. Polyphenols are amongst the largest group of secondary metabolites (Castellano et al., 2012); with more than 10,000 structures now identified. Polyphenols can be divided into structural categories based on the number of phenol rings that they contain and the basis of structural elements that bind these rings (Li et al., 2014). The first categorisation of polyphenols is the partition of flavonoids and non-flavonoids – see Figure 1.0. The separation of flavonoids is indexed by their shared structure (C_6–C_3–C_6), comprising two 6-carbon molecule rings with a further 3-carbon molecule bridge (Kennedy & Wightman, 2011). As Figure 1 demonstrates, this classification can be further sub-grouped into: flavonols (partitioned further into: flavan-3-ols and proanthocyanidins), flavones, flavanols (or catechins), flavanones, isoflavones and anthocyanins.

Arguably the most common of these sub-groups is the flavanols (Crozier et al., 2006), found abundantly in fruit and vegetables (Aherne, & O'Brien, 2002), with notable examples including quercetin and myricetin. Flavones are found less commonly than flavonols in fruit and vegetables (Manach et al., 2004), despite their similar structure. Flavones such as apigenin exist in their highest quantities within fresh celery (75 mg/100 g) and a handful of herbs, such as parsley (1484 mg/100 g fresh matter) (Hanske et al., 2009). Flavanol catechins like epigallocatechin-3-gallate (EGCG) exist in their most prevalent quantities in sources such as green tea, with a standard (250 ml) brewed beverage containing around 50–100 mg catechins (Khan & Mukhtar, 2007; Rains et al., 2011). Other noteworthy flavanols such as (-)-epicatechin, are found in large amounts (158.30 mg/100 g) within cocoa (Rothwell et al., 2013).

Flavanones exist within in a few aromatic plants (e.g. mint), but only citrus fruits appear to contain quantities which could be regarded as high (El Gharras, 2009). Examples such as hesperidin are found in average quantities of 25.85 mg/100 ml in orange juice (Rothwell et al., 2013) with lemons and other citrus fruits providing somewhat smaller

quantities. Usually the fruit itself contains up to 5 times more than its juice, which is mainly due to the albedo (the white spongy film between the skin and the flesh) and the membranes separating the segments holding a high proportion of flavanones (El Gharras, 2009). Isoflavones generally originate from (although not limited to) soy sources in relatively high concentrations (Setchell, 2000). Most notable examples include daidzein and glycitein, with almost all isoflavones found exclusively in legume plants (El Gharras, 2009). Finally, anthocyanins are perhaps one of the most widespread of the flavonoids, which provide the blue, purple and red colour of many fruits, vegetables, and flowers (He & Giusti, 2010). In terms of human consumption, one of the most commonly consumed anthocyanins is cyanidin; with most anthocyanin food sources consisting of fruits (Horbowicz et al., 2008), particularly berries (up to 611 mg/100 g) (Koponen et al., 2007).

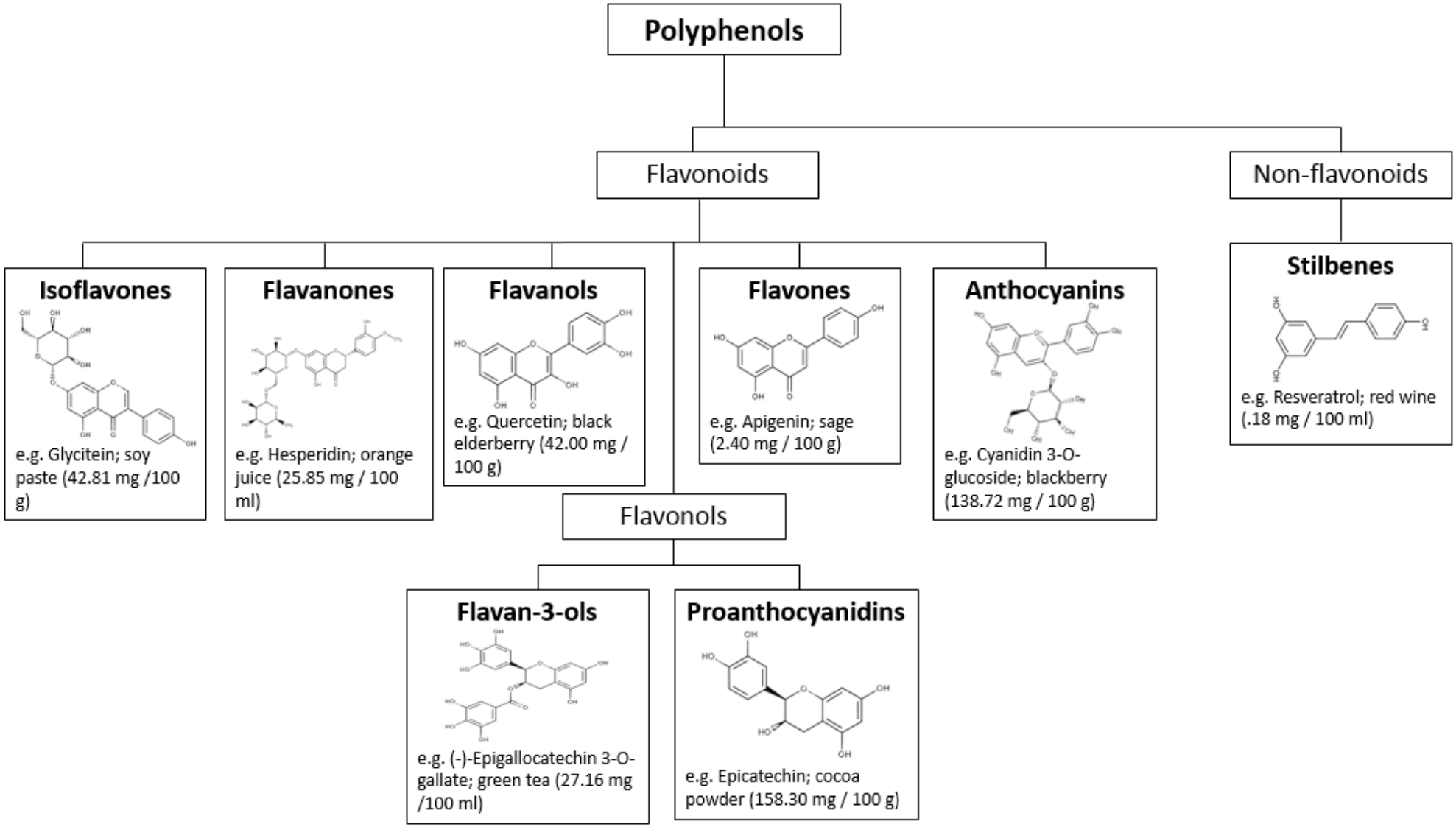

Figure 1.0 The main sub-groups of polyphenols with dietary examples and the average amount of content (in mg/ g /ml). Examples, dietary information and chemical structures in this diagram were adapted from phenol-explorer version 3.6 (Rothwell et al., 2013).

1.1.3 Classification and sources of resveratrol

Figure 1 also details one non-flavonoid group, the stilbene polyphenols. These are particularly notable due to their similar structure to flavonoids (C_6–C_2–C_6). The most commonly referenced stilbene polyphenol is the phytoalexin resveratrol (3, 4', 5 trihydroxystilbene), and is the polyphenol under investigation in this current PhD thesis. Resveratrol was first identified in the 1940's from the roots of white hellebore (*Veratrum grandiflorum*) (Takaoka, 1940) and, later, in Japanese Knotweed (*Fallopia japonica*) (Nonomura et al., 1963). Since then, resveratrol has been found to exist in a number of dietary sources including nuts, berries, soy and Itadori tea; yet is most notably associated with its presence in red grapes and in turn, red wine (Burns et al., 2002). Resveratrol typically exists in two stereoiso-forms, *cis* or *trans*; with the latter being the focus of most research to date (Giovinazzo et al., 2012). This is potentially due to the *trans*-isoform being more biologically active than *cis*- (Mukherjee et al., 2010). Consequently, the supplementation of the *trans*-resveratrol isoform will be the focus of this PhD thesis (and will be referred to hereafter simply as 'resveratrol'). Resveratrol has been reported to be found in average quantities of .27 mg/ 100 ml in alcoholic red grape wines (Rothwell et al., 2013) although these quantities can vary largely according to the colour, type and maturity of the grape.

1.2 Absorption and bioavailability of polyphenols

Much has been learned about the possible mechanisms of action of polyphenols and potential health benefits once consumed. Yet, how polyphenols and their dietary glycoside forms reach their multiple intended sites of action in humans (if at all) is complex and multifactorial (Walle, 2004). Therefore, before outlining evidence of the bioactivity of polyphenols through epidemiological studies and controlled trials, it is first important to outline their absorption, metabolism and distribution. Assessing these factors is instrumental in understanding the bioavailability of polyphenols and how this impacts their biological activity *in vivo*.

1.2.1 Absorption, metabolism and bioavailability of polyphenols

It is first important to consider that even the most common polyphenols in the human diet are not necessarily active, with the majority being unable to be absorbed by the small intestine effectively. This is largely due to many dietary polyphenols being regarded as xenobiotic (foreign) to the human body and thus, the rapid excretion of these compounds is a protective mechanism aimed to prevent potential damage (Li et al., 2014). Polyphenols are primarily present as either esters, polymers or glycosides (D'Archivio et al., 2007), yet only aglycones (with the exception of a few intact glycosides) are believed to be absorbable by the body (Williamson & Manach, 2005). The bioavailability of individual polyphenols differs even though their metabolism occurs, in general, by a common pathway. Polyphenols must be released from their native matrix to pass through the intestinal barrier (Valdés et al., 2015). Thus, once orally ingested, polyphenols are first hydrolysed by intestinal enzymes to allow for absorption by the small intestine where they are subject to absorption via passive diffusion. Here, phenolics undergo extensive phase I metabolism (oxidation, reduction, & hydrolysis) in the jejunum and ileum of the small intestine. Polyphenols which are not absorbed by the small intestine (for example, anthocyanins), reach the large intestine where they are metabolised by the microbiota into a wide array of low molecular weight phenolic acids (Scalbert et al., 2002). Alternatively, microbial glucuronidases and sulphatases deconjugate the remaining polyphenols to allow for the reuptake of aglycones (Lampe, 2009; Valdés et al., 2015).

Once the final aglycone (or its derivative) has been absorbed, it then undergoes phase II metabolism at an enterocyte level (Marín et al., 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 their elimination through bile and urine; although enterohepatic reuptake increases the presence of polyphenols within the body (Manach et al., 2004). From here, polyphenol metabolites are then available to be transported into bile, urine or tissues.

The bioavailability varies greatly between individual dietary polyphenol classes, potentially due to their glycosylation structure and the degree of polymerization (Manach et al., 2005; Pandareesh et al., 2015); impacting the rate and extent of absorption in the small intestine and colon (Cartea et al., 2010). Manach et al. (2005) reported that plasma concentrations of total metabolites ranged from 0 to 4 mol/L after an intake of 50 mg aglycone equivalents. Moreover, it was found that the urinary excretion values ranged from 0.3% to 43% of the intake, which exhibits the variability in the bioavailability of individual polyphenol classes. Isoflavones were found to be the best absorbed flavonoids with maximal plasma concentration (C_{max}) values of approximately 2 mol/L. In contrast, proanthocyanidins, (galloylated) tea catechins and anthocyanins, were found to have the poorest rate of absorption of the flavonoids examined.

1.2.2 Absorption, metabolism and bioavailability of resveratrol

The stilbene resveratrol is well tolerated in both animals and humans, even in quantities far beyond those usually consumed in the diet (Crowell et al., 2004; Boocock et al., 2007; Brown et al., 2009). Due to its chemical structure, resveratrol has a low water solubility (<0.05 mg/ mL) and this is believed to be a key factor inhibiting its absorption *in vivo* (Gambini et al., 2015). Despite this, the oral absorption of resveratrol has been estimated to be relatively high in humans (approximately 75%) (Walle, 2011; Sergide et al., 2016).

Resveratrol, in its free (aglycone) state, is taken up by the enterocytes and is conjugated with glucuronic acid and sulfates (Brown et al., 2009) thus decreasing circulating levels of free *trans*-resveratrol. Resveratrol undergoes rapid and extensive phase I (oxidation, reduction, & hydrolysis) and phase II-pass (glucuronic acid, sulfate, & methylconjugations) metabolism (Neves et al., 2012), which results in glucuronide resveratrol being the predominant form in plasma following oral dosing; equating to approximately 95–99% of total plasma resveratrol (Brown et al., 2009). However, it should be noted that metabolism of resveratrol still allows for small quantities of free *trans*-resveratrol in the plasma to be delivered to tissues and this has been found to be

increased with higher doses of resveratrol (Boocock et al., 2007). Despite this proportion being also present in the excretion of the metabolites, glucuronic and sulfate conjugates of resveratrol in human urine and faeces following oral consumption equates to approximately 71–98% (Walle et al., 2004); demonstrating that the circulating form of resveratrol is principally the modified metabolite (not the original aglycone) (Neves et al., 2012).

This rapid biotransformation means that resveratrol concentrations have been initially identified after around 30 minutes in humans, with plasma levels peaking after one hour (Walle et al., 2004). On review of the literature, peak plasma concentrations have been shown to occur between 30-90 minutes post oral administration in humans (Walle et al., 2004; Vaz-da-Silva et al., 2008; Almeida et al., 2009; Kennedy et al., 2010; Sergides, et al., 2016), from a range of dosages (25 mg – 500 mg) with a half-life of between 1-3 hours from a single dose (Almeida et al., 2009). In addition, due to hepatic and enteric recirculation, conjugated metabolites of resveratrol are reabsorbed following intestinal hydrolysis; resulting in a final plasma peak at approximately 6 hours post-consumption (Walle et al., 2004; Amri et al., 2012). Repeated resveratrol consumption has also been found to increase levels of circulating metabolites in comparison to single doses. For example, Wightman et al. (2015) found that a 28-day supplementation of 500 mg resveratrol resulted in a significant increase in total metabolites levels in comparison to day one baseline levels, before and after the final treatment administration. This may suggest that resveratrol concentrations can increase from regular exposure; yet, whether this can then result in an increase in biological activity of resveratrol is still a matter of debate.

The current thesis takes note of the above pharmacokinetics properties and proposes that a 500 mg dose of resveratrol seems to evince a promising profile. Sergides, et al. (2016) assessed the absorption and bioavailability of a single, oral dose of 500 mg of resveratrol in 15 fasted participants. Peak plasma concentrations were achieved at around 1.3hrs post administration and, in line with other investigations within the literature, the predominant conjugates identified were glucuronated (62.53%) and sulphated resveratrol (22.73%), with small amounts of free resveratrol (0.28% of the total resveratrol) detected. The authors concluded the plasma concentrations of resveratrol seen here were above the proposed range to promote the pharmacological and biological activities of resveratrol *in vitro*. The following sections will demonstrate that 500 mg resveratrol is capable of eliciting significant psychophysiological effects, supported by controlled human trials.

1.2.3 Polyphenols, brain localisation and the blood brain barrier (BBB)

With much evidence now demonstrating that polyphenols and their metabolites can enter systemic circulation, investigations have also directed interest at the ability of polyphenols to reach the central nervous system (CNS). Indeed, a number of epidemiological investigations have found polyphenol rich diets can have a beneficial impact on human cognition and brain function (Letenneur et al., 2007; Kesse-Guyot et al., 2011; Devore et al., 2012). Although, this may be the result of both direct and indirect interactions with the CNS, the capacity by which some polyphenols can exert such benefits on brain tissue appears dependent upon their individual ability to traverse the brain blood barrier (BBB). Evidence of polyphenols penetrating the BBB in animal models is well documented (El Mohsen et al., 2002; Andres-Lacueva et al., 2005; Ferruzzi et al., 2009; Janle et al., 2010; Janle et al., 2014). It is notable however, that the quantities of polyphenols and their metabolites found within brain tissue is significantly lower than the quantities found in other tissues. As an example, supplementation of the flavanol quercetin showed its metabolites to be at their lowest concentrations within the brain (~0.12 nmol/g tissue), in comparison to other tissues such as the kidney (11.6 nmol/g tissue) and the lung (15.3 nmol/g tissue) within rodents (de Boer, 2005).

This appears to be a common finding as, on review of the literature, polyphenols have been found to localise within brain tissue at levels below 1 nmol/g (Schaffer & Halliwell, 2012). The degree to which polyphenols can cross the BBB appears dependent upon the lipophilicity of individual polyphenols and their capacity to interact with efflux transporters (Youdim et al., 2004; Spencer, 2010; Vauzour, 2012). With regards to the former, despite glucuronides being found to traverse the BBB (Aasmundstad et al., 1995), the Youdim et al. (2003) *in vitro* model demonstrated that less polar O-methylated metabolites have a higher permeability through the BBB, in comparison to their more polar glucuronidated conjugates. Interestingly, despite evidence suggesting prolonged polyphenol administration may lead to an increase in circulating levels of metabolites (Wightman et al., 2015), a handful of chronic studies have suggested that polyphenol metabolites may not necessarily accumulate in higher concentrations within the brain from regular exposure. For example, chronic supplementation of quercetin failed to accumulate in higher concentrations within the brain when compared with a single dose in pigs (Bieger et al., 2008) whilst, despite readily crossing the BBB, the activity of resveratrol within the brain has been found to last up to only 4 hours in gerbils (Wang et al., 2002). This may indicate that concentrations of polyphenols and their metabolites

reflect the last meal and may be unable to accumulate in the brain over time (Rendeiro et al., 2015).

Despite some authors finding accumulation in the brain in a non-specific regional manner (Janle et al., 2010), polyphenols and their metabolites have been found to accumulate at different concentrations in different brain regions (Janle et al., 2014). This includes several key areas of the brain such as the hippocampus (Tsai et al., 2011) and cerebral cortex (Peng et al., 1998). Indeed, anthocyanins have been found to localise in various brain regions important for learning and memory, which may allow them to exert their benefits centrally (Andres-Lacueva et al., 2005). Moreover, levels of flavonoids and their metabolites have been reported to be sufficient to exert pharmacologic effects, even when present at low concentrations (10–300 nM) (Spencer, 2010). Collectively, sufficient evidence exists regarding the ability of polyphenols to transverse the BBB and localise within brain tissue in quantities sufficient to exert neurological actions.

1.3 Polyphenols as cell signalling molecules: the potential for improved health and brain functioning.

The consumption of polyphenols is almost unavoidable given their abundant nature in plant tissue (Kennedy, 2014). Although not considered 'essential', polyphenols make up an inevitable part of the human diet and can be considered phytonutrients. Habitual polyphenol consumption has attracted great interest, namely due to increasing evidence from epidemiological correlations suggesting that polyphenol rich foods can exert beneficial effects on human health (D'Archivio et al., 2010) and aspects of cognitive functioning (Nurk et al., 2009; Lamport et al., 2014).

1.3.1 Polyphenol consumption and health: epidemiological correlations

Recent meta-analyses have revealed total flavonoid intake can significantly reduce all-cause mortality (Ivey et al., 2015; Liu et al., 2017). Moreover, as cardiovascular disease (CVD) and hypertension are the largest causes of mortality in the UK (Bhatnagar et al., 2015), it is noteworthy that recent epidemiological investigations have also revealed an inverse relationship between total polyphenol consumption and the incidence of CVD (Wang et al., 2014; Tresserra-Rimba et al., 2014; Medina-Remón et al., 2015). Conversely, individuals who consume low quantities of flavonoids have been found to display a higher number of non-fatal cardiovascular events in comparison to those who consume high amounts of flavonoids (Ponzo et al., 2015). This may imply that the regular consumption of polyphenol-rich foods and beverages can lead to cardiovascular benefits. As an example, The Mediterranean diet is one that is characterised by copious consumption of plant foods (fruit, vegetables, beans, nuts & seeds), whilst consuming moderate amounts of dairy products, fish and poultry and low quantities of red meat (Willett et al., 1995); this diet therefore, is naturally high in polyphenols. Consequently, a series of systematic reviews and meta-analyses have revealed that adherence to the Mediterranean diet may reduce mortality and protect against vascular disease (Sofi et al., 2008; Sofi et al., 2010; Grosso et al., 2015).

Similarly, habitual consumption of individual flavonoid rich foods such as chocolate (Buitrago-Lopez et al., 2011), tea (Arab et al., 2013), fruits and vegetables (Wang, et al., 2014; Aune et al., 2017) have all been linked with reductions in CVD and its associated biomarkers. Red wine has also been credited with cardiovascular benefits and is associated with the 'French paradox'. This is the observation that certain populations

(such as the eponymous French) have a low incidence of coronary heart disease (CHD) despite a diet rich in saturated fat (Kopp, 1998; de Leiris & Boucher, 2008); this is assumed to be due to protective dietary factors, such as moderate consumption of red wine. Indeed, consumption of moderate quantities of red wine has been found to lead to improvements to blood pressure, endothelial functioning and platelet aggregation (Saleem & Basha, 2010). Equally, habitual consumption of grape juice and grape derived foods have also shown to significantly reduce the risk of CVD (Dohadwala & Vita, 2009). This demonstrates that the cardiovascular benefits of red wine are not the sole result of its alcohol content and is likely to be the result of overall grape phenolic content and/or the efficacy of individual polyphenols such as resveratrol (Catalgol et al., 2012).

Alongside the increased risk of CVD, ageing also brings with it an increased risk of neurodegenerative diseases and cognitive impairment (Fratiglioni & Qiu, 2009; Bishop et al., 2010). Consequently, interest is growing in therapeutic nutritional interventions which may offer protection and/or a reduction in risk factors and biomarkers of these neurological disorders. Indeed, evidence has also suggested that flavonoid intake may contribute to a significant reduction in the risk of dementia (Commenges et al., 2000), Parkinson's (Checkoway et al., 2002) and Alzheimer's disease (AD) (Dai et al., 2006). Moreover, older adults who regularly consume moderate quantities of polyphenol-rich red wine have shown a reduced incidence of developing AD, vascular dementia and ischemic stroke (Orgogozo et al., 1997; Djoussé et al., 2002).

Epidemiological correlations have also revealed that dietary polyphenol intake may delay the effects of cognitive ageing and maintain brain function. In a longitudinal investigation, Letenneur et al. (2007) reported that elderly populations (aged 65≥) in the upper quartile for flavonoid consumption scored significantly better on cognitive tasks in comparison to those in the lower quartile. On a 10 year follow up, the same authors found that those in the lower quartile showed a greater reduction in performance on the tasks in comparison to those in the upper quartile. Similarly, a further cross-sectional study of participants aged between 70-74 years, revealed that those who habitually consumed a combination of chocolate, tea and wine demonstrated significantly superior mean cognitive test scores (Nurk et al., 2009). Interestingly, these effects were found to be dose dependent for both chocolate (~10 g/day) and wine (75-100 ml/day); with this effect being more pronounced for the latter. Habitual tea consumption alone has also been significantly associated with a lower prevalence of cognitive impairment (Ng et al., 2008) and associated with lower risk of neurocognitive disorders in a dose and duration dependent manner in elderly

women (Feng et al., 2016). Additionally, in a recent longitudinal prospective investigation, regular consumption of chocolate was shown to reduce global cognitive decline in a cohort of 531 participants aged ≥65 on a 2 year follow up (Moreira et al., 2016). This effect was only observed in individuals who consumed <75 mg of caffeine daily. Crichton et al. (2016) also found that more frequent chocolate consumption in older adults was significantly associated with better performance on a wide range of cognitive domains and assessments. Here, participants who reported to consume chocolate more than once a week were found to score significantly better on the Global composite score, visual-spatial memory and scanning and tracking. Interestingly, the increases observed in cognitive performance in this study were still present even when independent of cardiovascular, lifestyle and dietary factors. However, despite white, milk and dark chocolate all containing varying amounts of polyphenols (Marsh et al., 2017), the authors did not measure the type of chocolate consumed by the participant; making it is difficult to identify the exact relationship of polyphenols consumed and the cognitive benefits observed.

The above briefly outlines the potential cardiovascular and cognitive benefits from habitual consumption of flavonoid-rich food. However, such results should be interpreted with caution as epidemiological evidence is naturally limited due to its susceptibility to confounding variables (e.g. social economic status, lifestyle & unknown dietary factors); therefore, one can only infer the relationships between average polyphenol consumption and their contribution towards health claims and reported mortality. Furthermore, as a polyphenol-rich diet also provides high levels of vitamins, minerals and fibre, it becomes difficult to determine the efficacy of phenolics alone as these compounds all contribute to energy metabolism (Huskisson et al., 2007), brain function (Haller, 2005; Kennedy, 2016) and are essential for normal cellular functions (Said, 2011). Nevertheless, the above has prompted an extensive literature of controlled intervention trials which will be outlined further in the subsequent subsections.

1.3.2 Polyphenols as cellular signalling molecules

The initial attention of polyphenols from the scientific community was based on their innate activity as antioxidants based on their ability to scavenge free radical species (e.g. reactive oxygen species (ROS)) or their potential ability to influence intracellular redox status (Rice-Evans et al., 1995; Rice-Evans, 2001). Given that ROS related mechanisms have been indicated as the precursors of several human pathologies (Aruoma, 1998;

Moskovitz et al., 2002), this was initially thought to be the underlying basis of all health claims of polyphenols. However, despite promising *in vitro* support for polyphenols to function as hydrogen donating antioxidants, there has been little compelling evidence to support this *in vivo* (Halliwell, 2013). This is further reinforced by the observation that flavonoid concentrations within plasma and tissues may be too low to effectively reduce ROS in humans (Brunetti et al., 2013). This is particularly evident within the brain, where concentrations of phenolics and their metabolites fail to localise in large quantities (Spencer, 2008; 2010).

More compelling evidence has suggested that polyphenols and their metabolites are more likely to exert their biological activity through their capacity to directly interact with cellular transduction and signalling cascades that mediate cellular responses to stressors (Kennedy, 2014; pp 162). These survival pathways are a vital mechanism for life, particularly for inflammatory, cardiac, and neurological functions (Mansuri et al., 2014). Equally, their dysfunction is associated with the pathogenesis of cerebrovascular and neurodegenerative diseases (Williams & Spencer, 2012; Kennedy, 2014). The most notable of these signalling cascades includes the mitogen-activated protein kinase (MAPK) pathway, phosphatidylinositide 3-kinase (PI3K) Akt/protein kinase B (Akt/PKB) and protein kinase C (PKC). MAPK in particular, plays a key role in transducing various extracellular signals (inclusive of the mitogenic extracellular signal-regulated protein kinase (ERK) pathway and the stress activated c-Jun N-terminal kinase (JNK) and p38 cascades) into intracellular responses to downstream transcription factors, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and cAMP response element-binding protein (CREB).

In the first instance, polyphenols can initiate cellular signalling at the cell membrane. Here, polyphenols can interact with extracellular signalling molecules (namely growth factors and hormones) or bind directly to extracellular receptors imbedded within the cell membrane (e.g. Tyrosine kinase and G protein receptors), causing a change in the conformation of the receptor. This enables the relevant protein receptor to transduce its activation into the cell and consequently trigger a signalling cascade encompassing the numerous protein kinases (Aiyer et al., 2012; Kennedy, 2014); ultimately leading to the activation of downstream transcription factors which drive gene expression (Vauzour et al., 2010). Polyphenols and their metabolites can also be taken up into intracellular compartments including the cytosol and mitochondria, where they can mediate additional biological actions. For example, polyphenols such as EGCG have shown to interact

within the cytosol via increases to other intracellular second messengers such as calcium (Ca^{2+}) or through binding to nuclear receptors (Kim et al., 2014).

In addition, polyphenols and their metabolites can also alter the phosphorylation state of the components of these cellular signalling cascades (Hou & Kumamoto, 2010; Mansuri et al., 2014). This can ultimately result in the removal of proteins attached to transcription factors in the cytosol allowing them to translocate to the nucleus, or alternatively, interactions with transcription factors within the nucleus (Kennedy, 2014). This may suggest that polyphenols can interact selectively within signalling cascades (Spencer, 2008) as polyphenolic compounds have been found to suppress the phosphorylation of various protein kinases with differing affinity and selectivity (Hou et al., 2012). Finally, the nature of nuclear receptor ligand-binding may also facilitate flavonoid mediated transcriptional regulations (Avior et al., 2013). For example, the flavanone naringenin has been found to bind to and activate estrogen receptor α and induce anti-estrogenic effects (Ruh et al., 1995).

1.3.3 Molecular targets of resveratrol

Resveratrol has also been found to interact within cellular transduction and signalling cascades. Evidence shows resveratrol can increase or inhibit phosphorylation of components of signal transduction pathways (Kumar et al., 2016; Jin et al., 2016), or alternatively, mediate effects via modulation of cellular Ca^{2+} homeostasis (Chang et al., 2013). Indeed, resveratrol has been found to interact with a number of sirtuins, kinases, transcription factors, and multiple other protein targets (Kuršvietienė et al., 2016). Most notably, resveratrol has been found to activate sirtuin 1 (SIRT1), a protein that plays a key role in the regulation of mitochondrial respiration, metabolic homeostasis and the aging process (Cantó & Auwerx, 2009; 2012). Interestingly, activation of SIRT1 has also been found to mimic the benefits of calorie restriction (Cantó, 2016); an acknowledged means of preventing chronic disease and ultimately extending the lifespan in animal models (Bordone & Guarente, 2005). Consequently, administration of resveratrol has shown promising results in improving the lifespan and health of metabolically compromised mammals (Bhullar et al., 2015). As an example, chronic resveratrol administration has shown to extend the lives of mice fed a high fat diet (Baur et al., 2006). Such effects may also have ramifications for brain functioning, as supplementation of resveratrol in addition to a calorie restricted diet has shown to significantly improve cognitive performance in primates (Dal-Pan et al., 2011).

SIRT1 is also a precursor for the activation of peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α); a gene which modulates mitochondrial biogenesis. As a result, resveratrol consumption has been suggested to mimic the effects of aerobic exercise (Narkar et al., 2008). In support, resveratrol activates SIRT1, heightening PGC-1α activity and in turn, increases mitochondrial function, improves aerobic capacity and subsequently increases energy expenditure (EE) in rodent models (Lagouge et al., 2006). It is noteworthy that some authors have questioned the capacity of resveratrol to module SIRT1 directly (Baur, 2010). However, it has been proposed that resveratrol can active SIRT1 indirectly by binding to phosphodiesterase and triggering cAMP signalling which consequently stimulates 5' adenosine monophosphate activated protein kinase (AMPK) (Price et al., 2012). AMPK itself is a key regulator of energy sensing procedures (especially under physiological conditions of energy stress, such as exercise and hypoxia), and subsequently whole-body metabolism (Kulkarni & Cantó, 2015). Once activated, AMPK has been shown to regulate mitochondrial biogenesis (Reznick et al., 2007), glycaemic response and energy homeostasis (Hardie et al., 2012). The capacity for resveratrol to interact with these energy signalling cascades and proteins may allow for whole body and perhaps even cerebral metabolic effects. In support, resveratrol (150 mg /day) has been found to improve mitochondrial function in the skeletal muscle and reduce basal EE after 30 days administration in obese, but otherwise healthy men (Timmers et al., 2014). While, Most et al. (2016) found a 3 day supplementation of a combination of both resveratrol (150 mg) and EGCG (300 mg) was capable of increasing both fasting and postprandial EE at rest. As EGCG was unable to increase EE alone, the researchers concluded either resveratrol alone or a synergistic effect had taken place. This highlights that resveratrol has the ability to influence metabolic changes in EE during a fasted state, even after short term supplementation.

1.3.4 Polyphenols and brain function

The capacity of polyphenols to exert their multiplicity of effects on the CNS appears to be dependent upon their interactions with numerous protein and lipid kinase signalling cascades (Williams et al., 2004; Vauzour, 2017). Here, polyphenols can improve brain functioning by the inhibition/attenuation of neuroinflammation, protecting neurons from injury and thereby preserving cognitive functioning. Alternately, polyphenols may promote memory, learning and cognitive functioning through direct neuronal activation, synaptic strengthening and the modulation of cerebral blood flow (CBF) (Spencer, 2010).

1.3.4.1 Polyphenols and neuroinflammation

Inflammation is the principal, localized bodily response to injury or infection. Chronic systemic inflammation, however, involves the overproduction of pro-inflammatory mediators (such as cytokines). The transcription factor NF-kB has been shown to lead to the expression of cytokines and additional inflammatory molecules such as cyclooxegenase-2 (COX-2), tumour necrosis factor α (TNFα) and inducible nitric oxide synthase (iNOS) (Karunaweera et al., 2015; Lawrence, 2009; Bhat et al., 2002), making NF-kB central to both pro and anti-inflammatory responses (Lawrence, 2009; Karunaweera et al., 2015). A growing wealth of literature shows that polyphenols can inhibit this signalling cascade and reduce pro-inflammatory cytokines in animal models (Goya et al., 2016). For example, the flavonoid quercetin has been shown to suppress NF-κB translocation leading to the inhibition of iNOS and COX-2, within RAW 264.7 cells *in vitro* (Endale et al., 2013). Moreover, resveratrol has also been found to block NF-kB activation and subsequent COX-2 expression in mouse skin *in vivo* (Kundu et al., 2006). Despite exploration being somewhat more difficult in humans, a series of reviews have shown that polyphenols can reduce inflammatory biomarkers and anti-inflammatory effects in humans (Joseph et al., 2016; Goya et al., 2016). The efficacy of polyphenols to inhibit inflammatory mediators, may present a cogent therapeutic technique to reduce systemic inflammation and its associated risk factors (Gomez-Pinilla & Nguyen, 2012).

Even ‘normal’ brain aging is associated with elevated levels of neuroinflammation (Simen et al., 2011). However, over expression of these pro-inflammatory mediators leads to an increase in the risk factor in age-related pathologies (Cevenini et al., 2013) including neurodegenerative diseases (Santangelo et al., 2007). Moreover, evidence has also suggested that neuroinflammation can obstruct the physiological processes of memory and learning and, in turn, may play an important role in the pathogenic mechanism underlying many cognitive and behavioural disorders (Scapagnini et al., 2011). Building on promising epidemiological data, and the ability of polyphenols to interact with these inflammatory signalling cascades, it is proposed that polyphenols can attenuate inflammation and thus provide support for protection of brain function. Indeed, blueberry polyphenols have been found to attenuate learning impairments following the insult of neurotoxicity in rodent models via the inhibition of pro-inflammatory mediators in rodent models (Shukitt-Hale et al., 2008). Moreover, resveratrol administration can

prevent elevations of inflammatory cytokines (TNFα & IL1β) leading to a reversal in age-related impaired cognitive functions in male Wistar albino rats (Gocmez et al., 2016).

1.3.4.2 Polyphenols and neuroplasticity

As polyphenols have the capacity to cross the BBB, they also possess the ability to directly modulate brain plasticity via modulation of receptor activity (Spencer, 2010; Rendeiro et al., 2015). Moreover, evidence has also suggested that flavonoids can exert such effects on brain plasticity via modulation of individual cascades such as CREB signalling; a transcription factor that can promote gene expression associated with synaptic plasticity and memory (Spencer, 2012; Spencer et al., 2012). Conversely, disruption to CREB obstructs the formation of memory, particularly long-term memory (Bourtchuladze et al., 1994). Xu et al. (2010) found that 7 weeks administration of procyanidins (50-100 mg/kg body weight) from lotus seedpod resulted in declines in phosphorylation to CREB in the hippocampus of cognitively impaired aged rats. Moreover, Williams et al. (2008) demonstrated that supplementation with a (flavonoid-rich) blueberry diet for 12 weeks, was capable of improving memory performance after only 3 weeks, which remained until the end of the testing period in rats. Interestingly, this increase in cognitive performance was found alongside the activation of CREB. Although no causal relationship can be made, this would certainly imply that there is potential for flavonoids to induce direct modulation of cognitive performance.

The capacity of polyphenols to interact with CREB can also lead to direct protein synthesis. Indeed, consumption of polyphenols has been found to elevate levels of brain derived neurotrophic factor (BDNF) (De Nicoló et al., 2013). BDNF is a protein that is associated with both short term and long-term memory formation (Lu et al., 2014), while also being a critical component in synaptic plasticity and long-term potentiation (Baudry et al., 2015). Consequently, increased circulating levels of BDNF following chronic administration of polyphenols may explain subsequent improvements to memory and global cognitive functioning in healthy individuals (Neshatdoust et al., 2016). Intriguingly, circulating BDNF levels have also been found to increase after one hour following administration of a flavonoid-rich blueberry drink in humans (Dodd, 2012). Bell et al. (2015) note that if BDNF levels can increase in a short period of time following flavonoid administration, it is unlikely that this facilitation would result in direct global cognitive effects after only 1–2 hours. However, the authors do argue that this could result in cognitive improvements at later time points.

1.3.5 Polyphenols and nitric oxide (NO) signalling

An alternative cognitive-enhancing mechanism attributed to polyphenols pertains to their ability to induce endothelial vasodilation. Several flavonoids have been found to interact with the PI3K/ Akt pathway and modulate intracellular Ca^{2+}, leading to an increase in endothelial nitric oxide synthase (eNOS) expression (Vauzour et al., 2010). eNOS is required for the release of nitric oxide (NO); a cell signalling molecule that modulates the vasodilatory response, and therefore regulation of vascular tone and blood flow (Tsai et al., 2007; Xia et al., 2014). Cocoa-flavanols in particular have been found to bolster NO production and endothelial functioning (Fraga et al., 2010). In support, Schroeter et al. (2006) found that oral ingestion of a flavanol-rich cocoa drink (containing 917 mg of flavanols) lead to an increase in the concentration of NO-derived species in the plasma and urine of healthy subjects. The subsequent vasodilatory actions of acute flavanol-rich cocoa are also significantly blunted with the introduction of NG-nitro-L-arginine methyl ester (a NO inhibitor) (Fisher et al., 2003).

Resveratrol has also been credited with the capacity to increase eNOS expression and NO production (Xia et al., 2010). In a similar manner to cocoa-flavanols, the ability of resveratrol to initiate eNOS-dependent vasodilation results from its capacity to cause an initial increase in intracellular Ca^{2+}. This Ca^{2+} then binds to the calmodulin protein-binding site that initiates the NOS enzyme (Figure 1.1). Elíes et al. (2011) demonstrated that resveratrol induced a concentration-dependent increase in cytoplasmic Ca^{2+} levels within human endothelial cells, *in vitro*. Interestingly, the authors found that this effect was diminished with the introduction of Ca^{2+} channel blockers, inhibition of intracellular Ca^{2+} release and inhibition of eNOS. The authors argue that resveratrol does not modify eNOS activity directly, suggesting instead that the observed inducement of NO generation ensues via resveratrol mediated mobilisation of Ca^{2+}.

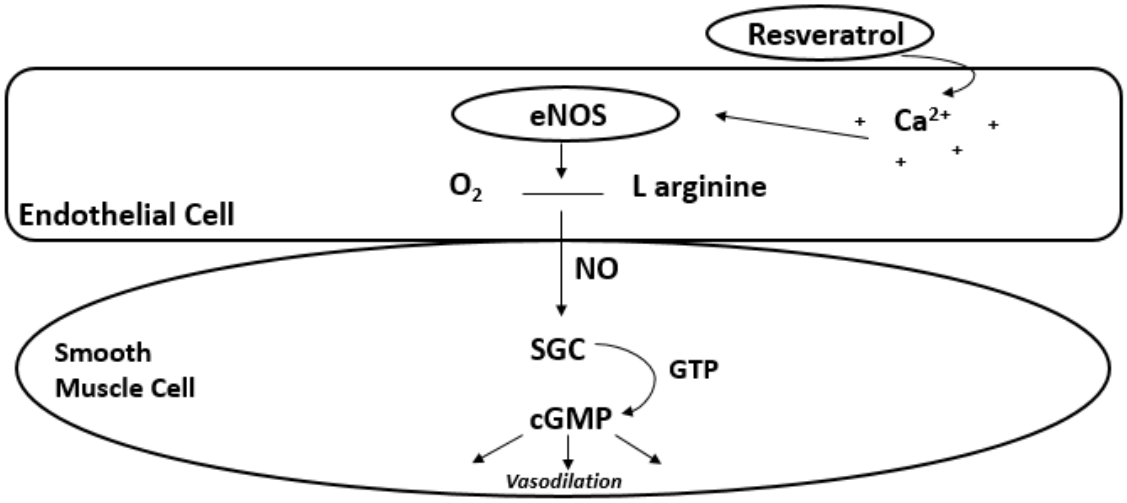

Figure 1.1. The resveratrol interaction with nitric oxide (NO)-dependent endothelium vasodilation. The initiation of eNOS is calcium (Ca^{2+}) dependent. Resveratrol can incite this initial influx of Ca^{2+}, which binds to the calmodulin protein binding site which initiates the NOS enzyme. This then catalyses oxygen and the amino acid L arginine, which instigates NO production. This then diffuses into the smooth muscle cell from the endothelium, activating soluble guanylyl cyclase (SGC) which, in turn, actives cyclic guanosine monophosphate (cGMP). This then stimulates downstream protein synthesis, ultimately resulting in smooth muscle relaxation and vasodilatation. Figure based on Klabunde (2016).

eNOS is also the isoform found in the endothelium of cerebral blood vessels (Peterson et al., 2011) and mediates the movement of blood through the cerebral arteries and veins supplying the brain; playing a key role in maintaining CBF and preventing neuronal injury (Greco, 2017). This suggests that the ability of polyphenols to modulate the expression of eNOS could result in vasodilation within the brain and, consequently, augmented CBF. Considering the above evidence of polyphenol accumulation within specific brain regions, this mechanism may lead to the induction of angiogenesis and new cell growth in keys areas of the brain associated with learning and memory; such as the hippocampus (Spencer, 2010). The capacity of polyphenols to induce modulation of NO-dependent vasodilation has also sparked wide interest as an acute method of cognitive enhancement. In part, this is due to the hypothesized improved delivery and availability of blood borne neural fuels, namely oxygen (O_2) and blood glucose, to the brain, via increases in CBF.

1.4 The use of polyphenols for cerebrovascular and neurocognitive enhancement.

The above has briefly outlined the ability of polyphenols to interact with signalling pathways within the CNS and how this may potentially offer direct improvements to brain functioning via neuronal activation, synaptic strengthening and the inhibition/attenuation of neuroinflammation (Spencer, 2010; Rendeiro et al., 2015). Equally, evidence is growing to suggest that the capacity of polyphenols to interact with eNOS expression which can lead to the modulation of peripheral (in the body) and cerebrovascular blood flow. Effective functioning of the endothelium is critical in mediating vascular tone, regulating blood pressure, general vascular homeostasis and the avoidance of both cardiovascular and cerebrovascular disease (Xia et al., 2014; Bleakley et al., 2015; Chistiakov et al., 2015). Moreover, an uncompromised delivery of blood to the cerebral vasculature is critical for brain health and functioning (Chen et al., 2014). Therefore, given the link between vascular health and cognitive functioning (Dinges, 2006), the ability of polyphenols to promote increased blood flow capacity through the modulation of endothelial functioning may offer an indirect mechanism by which dietary polyphenols may exert their effects on brain function and cognition (Rendeiro et al., 2015).

1.4.1 Polyphenols and cardiovascular health

Building on the above epidemiological evidence, which indicated an inverse association between polyphenols and CVD (see section 1.3.1), a series of controlled intervention studies have demonstrated that administration of individual and a mixture of polyphenols can also improve several aspects of cardiovascular functioning. Meta-analyses and systematic reviews of randomised, controlled, human trials have revealed both acute and chronic consumption of cocoa-flavanols can reduce systolic blood pressure in healthy and hypertensive cohorts (Reid et al., 2017). Similarly, chronic supplementation of resveratrol in doses of >150 mg/day have been reported to induce a reduction in blood pressure in healthy populations with higher doses evincing the greatest consistency across the literature (Liu et al., 2015).

In addition to elevated blood pressure, CVD is also associated with several risk factors including increased lipid levels, impaired glucose metabolism and endothelium dysfunction (Mozaffarian et al., 2016; Ginsberg, 2000; Brown & Hu, 2001). Interestingly, administration of individual polyphenol subclasses including flavanols, flavanones, stilbenes and anthocyanins have resulted in significant improvements to these

cardiovascular risk factors (Cassidy et al., 2016). For example, on review of randomised trials, resveratrol has been found to significantly lower systolic blood pressure, total cholesterol and fasting glucose when consumed regularly at doses of ≥300 mg/day (Huang et al., 2016). A review of 42 acute and sub-chronic (<18 weeks) studies also found that chocolate or cocoa can significantly improve insulin resistance and reduce diastolic blood- and mean arterial pressure (Hooper et al., 2012). However, only marginally significant effects were found for a reduction in low-density lipoprotein (LDL) and increases in high-density lipoprotein (HDL) cholesterol. In a more recent review of the literature, Vlachojannis et al. (2016) reported that cocoa-flavanol consumption can result in moderate improvements to blood glucose and lipid metabolism in healthy individuals, with even stronger associations seen in increased flow-mediated vasodilatation (FMD); a quantitated index of endothelial function.

1.4.2 Vasodilatory and blood flow effects of polyphenols

The above sub-sections demonstrate the ability of polyphenols to interact with eNOS expression and, in turn, to enhance the vasodilatory response and endothelial functioning. Controlled intervention trials with cocoa-flavanols support such mechanisms in humans. For example, Fisher et al. (2003) found that supplementation with 821 mg of flavanol-rich cocoa, across four consecutive days (in daily 230 ml drinks), significantly increased peripheral FMD. Moreover, an acute dose administered on the fifth day had an even more pronounced effect within the young, healthy cohort. Meta-analyses and systematic reviews since have shown that FMD can be improved after both chronic and acute supplementation with cocoa-flavanols, with optimal effects resulting from 500 mg, 2 hours after consumption (Hooper et al., 2012). Additionally, citrus fruit flavanones such as hesperidin have also demonstrated a capacity to bolster NO production and endothelial vasodilatation (Rizza et al., 2011) and has been causally linked to the microvascular endothelial reactivity effects of orange juice (Morand et al., 2011). Likewise, tea polyphenols have shown to increase FMD from acute and chronic dosing in controlled human trials (Grassi et al., 2008; Duffy et al., 2001; Hodgson, et al., 2002).

Endothelial function is a key indicator of cardiovascular health and its dysfunction can lead to an impaired vasodilatory response and a reduction in blood flow capacity. Several lifestyle factors have been shown to compromise the endothelium in the vasculature, including smoking and obesity (Verze et al., 2015; Park & Park, 2015). This is attributed, in part, to both smoking and obesity reducing NO bioavailability and consequently

hindering the vasodilatory responsiveness of the endothelium (Messner & Bernhard, 2014). Interestingly, it has been proposed that the ability of polyphenols to increase blood flow via NO-dependent signalling may provide an increased utility in such cohorts with endothelial dysfunction. In support of this, Heiss et al. (2006) demonstrated that acute administration with a flavanol-rich cocoa beverage (176-185 mg) was capable of improving NO bioavailability and consequently endothelium-dependent dilation in 11 smokers. Further improvements to blood pressure and endothelial functioning have been observed from acute supplementation of cocoa-flavanols in obese cohorts (Shiina et al., 2009) and chronically with other populations with CVD risk factors such as hypertension (Grassi et al., 2005).

As the aging process is usually accompanied by some degree of endothelial dysfunction and arterial stiffness (Lee, 2010; Sun, 2015), it is intuitive that the peripheral vasodilatory response of polyphenols may be able to attenuate such declines in function and, as such, may offer more prominent effects in healthy ageing adults. Indeed, a 6 month supplementation of a flavanone-rich grapefruit juice has been shown to reduce carotid-femoral pulse wave velocity (a measure of arterial stiffness) in healthy, postmenopausal women in comparison to placebo (Habauzit et al., 2015). In addition, Fisher and Hollenberg (2006) found that several measures of endothelial function were more pronounced following cocoa-flavanol administration in healthy older participants (>50 years of age) than their younger (<50 years of age) counterparts. Despite enhancing parameters in both groups, cocoa-flavanol administration significantly increased systolic blood pressure and FMD in the older group of participants only. It is important to interpret such results with caution however, as there was no direct comparison made between the two age groups.

Heiss et al. (2015) administered 450 mg of cocoa-flavanols, or a nutrient-matched, cocoa flavanol-free control, to 22 healthy, young (<35 years) and 20 elderly (50–80 years) participants for 2 weeks. FMD was found to improve in both young and older participants with a similar effect size. However, the authors do note that the systolic blood pressure of the elderly participants was elevated at baseline, and that supplementation with cocoa-flavanols led to a decreased aortic augmentation index and consequently systolic blood pressure. This may suggest different mechanisms between the young and old samples with net effects seen in the former but a relieving of age-related stiffness in the latter. Indeed, in a recent meta-analysis, Reid et al. (2017) reports that baseline blood pressure may play a role in the subsequent effects of cocoa-flavanols. Here subgroup analysis of

trials with pre-hypertensive and hypertensive participants revealed a greater blood pressure-reducing effect of cocoa compared to normotensive participants.

1.4.2.1 Vasodilatory and blood flow effects of resveratrol

Given the mutual capacity of resveratrol to interact with endothelial NO (see section 1.3.4), the above evidence would also suggest that resveratrol may also possess the ability to modulate NO-dependent vasodilation. In a meta-analysis, grape polyphenols have been found to significantly increase FMD within two hours after oral ingestion; with peak FMD occurring ~30 min after consumption (Li et al., 2013). Although this does not identify the vasodilatory effects of resveratrol specifically, the fact resveratrol has been shown to increase NO production in endothelium cells *in vitro* (Elíes et al., 2011), may imply a role in these findings. Indeed, in a controlled trial, daily administration of relatively low doses of resveratrol (10 mg) in patients after a myocardial infarction for 3 months, was found to significantly increase FMD and left ventricular diastolic function in comparison to controls (Magyar et al., 2012). Like the aforementioned cocoa-flavanols, evidence of increased blood flow from the vasodilatory effects of resveratrol and other grape polyphenols are claimed to be more pronounced in cohorts with high cardiovascular risk factors (Li et al., 2013).

For example, in a randomised, double-blind, placebo-controlled, crossover human intervention study, Wong et al. (2011) assessed the acute vasodilatory effects of 3 doses of resveratrol (30, 90 & 270 mg) in 15 obese males and 5 postmenopausal women, with untreated borderline hypertension. Here, participants arrived fasted (for at least 4 hours) to consume the treatment for the day before resting for 45 minutes. Participants then had their endothelial function assessed via FMD and provided blood samples for the assessment of plasma resveratrol concentrations. The researchers found that all 3 doses of resveratrol produced a significant increase in FMD following one hour of administration. Moreover, this effect was also reported to be in a significant dose dependent pattern, which coincided with plasma concentrations in a positive linear relationship.

In a follow up chronic investigation, Wong et al. (2012) explored the capacity of resveratrol to improve FMD in a cohort of older (mean age = 61) obese adults, in a randomised, crossover design. Participants were randomised to consume either 75 mg of resveratrol or a placebo (matched for colour) daily for six weeks. Following this six-week treatment period, participants arrived fully fasted for 18 hours before having their BMI,

blood pressure, arterial compliance and FMD assessed. Participants then crossed over to the alternate supplementation arm for another six weeks; returning in week 12 to complete a second assessment session (identical to the first). The results showed 6 weeks supplementation of 75 mg resveratrol resulted in a 23% increase in FMD response in comparison to placebo. The authors argue that as the chronic assessment was measured at least 18 hours since the last administration of the resveratrol supplement, this demonstrates that the effects of resveratrol are not restricted to acute but also chronic vasodilatory effects. Interestingly, it has since been suggested that such vasodilatory mechanisms of resveratrol are not confined to cardiovascular-protection, but may also offer cerebrovascular enhancement and, consequently, cognitive functioning in both young and older adults (Wong et al., 2013).

1.4.3 Polyphenols and cerebral perfusion

As noted previously, eNOS is the isoform found in the endothelium of cerebral blood vessels and mediates movement of blood through the cerebral arteries and veins. Thus, it is of no surprise that polyphenols can consequently increase CBF via interaction with this mechanistic pathway. In support, Francis et al. (2006) supplemented 16 young, healthy adults with flavanol-rich cocoa (150 mg) for 5 days before assessing their cerebral haemodynamic response (via functional magnetic resonance imaging (fMRI)) during cognitive tasks. Although no behavioural effects were observed here, the researchers found that the 150 mg flavanol drink significantly increased CBF during task performance when compared to a low flavanol control. Similarly, in an acute pilot study within the same publication, a single acute dose (450 mg flavanols) of flavanol-rich cocoa could also significantly increase CBF. It was proposed that this increase was mediated by an effect on the cerebral vasculature, rather than a direct effect on neuronal function.

Effects have also been seen here in older adults. For example, using fMRI, Lamport et al. (2015) found significantly increased region-specific perfusion in the anterior cingulate cortex and regions of the parietal lobe (post 2-hour consumption), following a 494 mg cocoa-flavanol drink in comparison to a nutrient matched control (23 mg cocoa flavanols) in subjects aged 50-65 years. Sorond et al. (2008) found that two weeks of daily administration with 900 mg of cocoa flavanols, significantly increased cerebral blood velocity in the middle cerebral artery in comparison to a low flavanol control, within a cohort of (N=34) 59–83 year old adults. In a follow up investigation, increases in cerebral blood flow velocity resulting from administration of cocoa-flavanols (451 mg) were

highly correlated with changes in CBF (measured by fMRI), in a cohort of older adults (62-80 years) (Sorond et al., 2010).

1.4.4 Polyphenols and cognitive performance

In a recent review, Bell et al. (2015) examined 21 acute controlled studies of flavonoid subclasses and concluded that flavonoids may provide cognitive enhancement to several cognitive domains, most notably: attention, working memory, and psychomotor processing speed. Most of these beneficial cognitive outcomes were observed with an acute time frame of 0–6 hours, in a dose dependent manner. One of the first studies to observe the cognitive enhancing qualities of polyphenols was Scholey et al. (2010), who found significant improvements to accuracy on the Rapid visual information processing (RVIP) task from high doses of cocoa-flavanols (994 mg), and on the Serial 3 subtraction task with a lower dose (520 mg) in 30 young, healthy humans. Moreover, Field et al. (2011) demonstrated significant improvements to spatial memory and aspects of the choice reaction time task following 2hrs post consumption of a commercially available dark chocolate bar (containing 773 mg of cocoa-flavanols) in comparison to a white chocolate control (trace cocoa polyphenols).

In a randomized, placebo-controlled, double-blind investigation, Massee et al. (2015) found that acute administration with 250 mg of cocoa-flavanols significantly improved cognitive performance on the Serial 7 subtraction task in the first repetition of the task battery, in 24 young, healthy adults. Single doses of fruit flavanones have also been found to significantly improve cognitive performance. Lamport et al. (2016) supplemented young, healthy adults with a flavanone-rich orange juice (70.5 mg total flavanones) or a nutrient matched control. Following 2 hours administration, a single significant improvement to cognitive performance was observed on a digit symbol substitution task (processing speed) in comparison to controls. Although no cerebral haemodynamics were measured in the aforementioned studies, the research teams of these investigations postulated that these cognitive improvements could be the result of the polyphenol interventions bolstering NO-dependent cerebral perfusion.

1.4.5 The cerebrovascular and cognitive effects of polyphenols

It is notable that trials administrating acute single doses of polyphenols have revealed mixed results in young, healthy samples. Other trials have observed no cognitive benefits

from acute cocoa-flavanol administration (Pase et al., 2013), even when in the presence of significant increases to CBF (Francis et al., 2006; Decroix et al., 2016). As other vasoactive polyphenols, derived from apples, have also shown no behavioural benefits from acute administration in young, healthy cohorts (Bondonno et al., 2014) this may highlight that vasoactive polyphenols may not possess the efficacy to sufficiently enhance cognitive performance in young, healthy individuals and may potentially question the mechanism of augmented CBF as a method of cognitive enhancement in this age group.

In a recent review of the literature, Socci et al. (2017) reported that despite consistent cerebral and peripheral blood flow effects following cocoa-flavanols, such observations are seldom found in conjunction with cognitive findings. However, it was noted that discrepancies in findings may be a result of high variance between protocols, as acute administration appears to provide enhancement to performance in younger cohorts during high cognitive demanding conditions, such as fatigue and sleep loss. Moreover, the authors noted that the current evidence does points towards regular flavanol intake providing beneficial cerebrovascular and cognitive effects. This was proposed to be particularly evident in individuals at risk of cerebrovascular and metabolic dysfunction, thereby protecting human cognition or counteracting different types of cognitive decline. Consequently, as ageing is associated with endothelial dysfunction and therefore a reduction in blood flow capacity, it appears the vasodilatory effects of polyphenols may provide an increased utility in (otherwise healthy) older adults. This is supported by the observation that both arterial stiffness and elevated blood pressure are associated with cognitive decline (Pase et al., 2012; Pase et al., 2013) and the proposition that interventions that can prevent or reduce said arterial stiffness may benefit cognitive functioning in the elderly (Zeki Al Hazzouri & Yaffe, 2014).

In a controlled, parallel-arm study, Desideri et al. (2012) assessed the efficacy of cocoa-flavanols in a range of doses (993 mg, 520 mg, or 48 mg), in 90 older adults with mild cognitive impairment (MCI), for 8 weeks. Both the high and intermediate flavanols groups showed a significant reduction in the time required to complete the Trail Making Test (A–B), in comparison to those assigned to the low flavanol group. Moreover, the high and intermediate flavanol groups also showed a significantly improved verbal fluency test score relative to the low flavanol group. These cognitive improvements were observed alongside decreased insulin resistance, blood pressure, and lipid peroxidation among subjects in both the high-flavanol and intermediate-flavanol groups. Interestingly,

the researchers reported that insulin resistance explained ~40% of the cognitive improvements observed.

In a follow up investigation by the same research team, Mastroiacovo et al. (2015) found that the 993 mg and 520 mg doses showed significant improvements to overall cognitive performance following 8 weeks consumption of cocoa-flavanols in 90 healthy older adults. On comparison between treatments, acute supplementation of both higher doses was found to lead to significantly better performance on the verbal fluency and Trail Making Test (A–B) relative to the lower dose. In addition, significant reductions in systolic blood pressure were found for the higher doses of flavanols in comparison to the low dose; detailing the cardiovascular mechanisms of the polyphenols. The authors again found ~17% of changes in cognitive performance of the overall composite Z scores could be attributed to changes in insulin resistance. Both studies highlight the vasodilatory mechanism of cognitive enhancement of cocoa-flavanols, as a reduced blood flow capacity, commonly associated with ageing, can lead to a compromised supply of insulin and glucose to the brain and, consequently, a reduction in cognitive performance (Messier, 2005; Anstey et al., 2015).

In a double blind, crossover investigation, Brickman et al. (2014) administered 3 months of flavanols (900 mg cocoa-flavanols with 138 mg (−)-epicatechin) or a low flavanol control (10 mg cocoa-flavanols with 2 mg (−)-epicatechin) with or without aerobic exercise, to a healthy cohort, of 50-69 years old adults. The high-flavanol intervention group was found to have enhanced dentate gyrus activation (a region in the hippocampus that is a mediator age-related cognitive decline), when measured by fMRI, while demonstrating improved cognitive performance. Although not measured simultaneously, the authors report that this improvement in regional activation does correlate with improved performance on a delayed retention task, associated with dentate gyrus functioning.

Interestingly, the cognitive effects of polyphenols may even begin to be evident from middle age. In a double blind, randomised, placebo controlled investigation Alharbi et al. (2015) found that a single dose of flavanone-rich (272 mg) orange juice improved executive function and psychomotor speed alongside increased self-reported levels of alertness, relative to a nutrient matched placebo control in a sample of 24 middle aged men (mean age 51 years). A subsequent chronic investigation saw 8 weeks daily supplementation of a high-flavanone (305 mg) orange juice improve global cognitive

performance in 37 healthy older adults (mean age 67 years). However, these benefits were not found in conjunction with any improvements to cardiovascular parameters (Kean et al., 2015).

It is important to note that the majority of published research identifying cognitive benefits of vasoactive polyphenols have done so without the simultaneous measurement of CBF. Therefore, there is a pressing need for future investigations to monitor cerebral haemodynamics in conjunction with cognitive performance to elucidate the efficacy of this proposed mechanism of cognitive enhancement (Bell et al., 2015). Although, the small number of investigations which have simultaneously measured both cognitive and CBF parameters of polyphenols, have shown no clear cognitive benefits despite consistent CBF effects (Francis et al., 2006; Decroix et al., 2016; Kennedy et al., 2010; Wightman et al., 2014; 2015)

1.4.6 Polyphenols and mood

The above demonstrates how polyphenols can interact directly and indirectly with various mechanisms to improve cognition. Although, their interactions with other CNS mechanisms might also suggest effects on mood. Indeed, acute administration of polyphenol rich beverages, such as concord grape juice (136.6 ml/l anthocyanins), have resulted in increases self-reported ratings of 'calm' (Haskell-Ramsey et al., 2017), while, a wild blueberry drink (253 mg anthocyanins) has been found to significantly increase self-reported levels of 'Positive Affect' on the PANAS-NOW mood scale, in both young adults and children (Khalid et al., 2017). Moreover, Watson et al. (2015) found that administration of a blackcurrant extract (525 ± 5 mg polyphenols per 60 kg of bodyweight) resulted in increased self-reported alertness and reduced fatigue but this was only found to be significant at 140 minutes following treatment. With regards to individual administration of polyphenols, a single dose of 300 mg EGCG has been shown to lead to significant improvements to self-reported calmness and stress (Scholey et al., 2012). Interestingly, this improvement in mood was also found in conjunction with increased activation in the frontal gyrus and medial frontal gyrus. The authors therefore proposed that participants who consumed EGCG may have been in a more relaxed and attentive state in comparison to those within the placebo condition.

Cocoa-flavanols have also been found to induce mood enhancing capabilities. In addition to observed cognitive improvements from the single administration of a 520 mg cocoa flavanol drink, Scholey et al. (2010) also found this dose was capable of significantly

reducing self-reported levels of mental fatigue following each of the 6 repetitions of a 10 minute cognition battery (with the exception of repetition 3) in comparison to a nutrient matched control. Improvements to mood have also been observed from chronic cocoa-flavanol consumption. Pace et al. (2013) found that a 500 mg dose of cocoa polyphenols significantly improved self-rated calmness and contentedness following 30 days of supplementation. The authors postulated that cocoa polyphenols may provide a boon in situations where a calm or content temperament is advantageous, i.e. under conditions of high stress or anxiety.

In a randomized, double-blind, placebo-controlled trial, Cox et al. (2015) supplemented healthy older adults aged 60-85 (N=60) with a solid lipid curcumin (a curcuminoid polyphenol) formulation, containing 80 mg curcumin for 4 weeks. In addition to acute and chronic improvements to cognitive performance, the curcumin treatment was found to significantly reduce physical fatigue and provide a greater resistance to mental fatigue (induced by cognitive performance) following 4 weeks administration. Moreover, curcumin administration was found to significantly improve self-reported calmness and contentedness following high cognitive demand. Interestingly, similar findings have been replicated with the polyphenol curcumin in a cohort with depression. Participants (N=56) diagnosed with major depressive disorder were supplemented with either 500 mg of curcumin or placebo for 8 weeks. The results showed that during the second half of the investigation (between weeks 4 to 8), curcumin was significantly more effective in improving several depression-related symptoms in comparison to placebo (Lopresti et al., 2014).

In sum, the capacity of polyphenols to influence human mood states appears to be centred around anxiolytic, anti-depression and fatigue reducing like qualities. This may tentatively present a future for polyphenols as a therapeutic intervention for situations of high stress and or fatigue. The mechanisms of action in which polyphenols exert these mood effects are still unknown, yet, a number of flavonoids have been identified as inhibitors of monoamine oxidase (MOA)-A and MAO-B in animal (Jäger & Saaby, 2011) and human trials (Watson et al., 2015). As MAO is involved in the oxidation of neurotransmitters, including the regulation of serotonin, inhibiting MAO has been suggested as a viable method to reduce anxiety, depression and fatigue (Nemeroff et al., 2002). Thus, the ability of polyphenols to inhibit MAO offers a potential avenue in which polyphenols may promote positive mood states following both acute and chronic supplementation (Pathak et al., 2013).

1.4.7 The cerebral blood flow, cognitive and mood effects of resveratrol

Given that resveratrol has also been established to stimulate NO production pathways within the endothelial cells *in vitro* (Elíes et al., 2011), this is the likely mechanism underlying peripheral blood flow effects in humans (Wong et al., 2011; Wong et al., 2012) and suggests that resveratrol too could be capable of cerebral perfusion and the ensuing cognitive benefits. However, to date, only a limited number of human intervention studies have investigated the cognitive effects of resveratrol following acute and chronic consumption, with the small quantity of research that does exist providing mixed results regrading improvements in cognitive performance.

Kennedy et al. (2010) investigated the CBF and cognitive effects of 250 mg and 500 mg oral resveratrol consumption, in a placebo controlled, repeated measures, double blind, crossover study. The researchers employed functional near infrared spectroscopy (NIRS) to measure localised cerebral haemodynamic response in the prefrontal cortex, whilst assessing cognition via performance on a range of tasks (previously shown to activate the prefrontal cortex). Following a 45-minute absorption period, resveratrol administration instigated a dose-dependent modulation of CBF during post dose task performance. This was indexed by significantly higher concentrations of both total haemoglobin (total-Hb) and deoxygenated haemoglobin (deoxy-Hb) in comparison to placebo. The authors propose that the increase in deoxy-Hb concentrations observed across the post-dose task period demonstrates an increased O_2 extraction and utilization. However, despite the promising resveratrol-mediated cerebral haemodynamic changes observed these were not complimented with any behavioural improvements. The authors hypothesized that the low bioavailability of resveratrol may be a contributing factor for a lack of cognitive benefits.

In a follow up investigation by the same research team, Wightman et al. (2014) utilised 20 mg piperine as a bioavailability enhancer of resveratrol, in order to establish if increasing the bioavailability could amplify the cognitive benefits of resveratrol. Similar to the methodology of Kennedy et al. CBF was also measured throughout the full testing session via NIRS, whilst the bioavailability of the treatments was evaluated in a separate cohort. The results revealed that administration with 250 mg of resveratrol alone had no significant effects on overall total-Hb during the performance of cognitively demanding tasks. However, the same dose of resveratrol, when supplemented with 20 mg piperine, induced a significant increase in CBF across the post-dose task period in comparison to

placebo. The authors therefore suggested that either piperine is capable of CBF modulation itself or amplifies the capacity of resveratrol to do so. The latter explanation is more likely given that the previous CBF findings with resveratrol (Kennedy et al., 2010) and the absence of such evidence with piperine (Wightman et al., 2014). Despite this, pharmacokinetic analysis showed no significant differences in the plasma concentrations of resveratrol and its metabolites between either of the resveratrol treatments; suggesting that the CBF effects of the combined treatment was not due to an increase in bioavailability. Moreover, neither treatment displayed any significant differences in cognitive performance or in self-reported ratings of mood.

As single acute doses had failed to show any benefits to cognitive performance in young, healthy populations, Wightman et al. (2015) proposed that increasing exposure of resveratrol through frequent dosages could improve bioactivity. Here cognitive performance was measured on day 1 and day 28 of supplementation, whilst assessment of CBF and cerebral blood velocity were measured via NIRS and transcranial Doppler respectively. Sleep quality and mood were also measured via questionnaires at weekly intervals. The acute blood flow findings showed that resveratrol administration (with 10 mg of piperine) significantly increased higher total and oxygenated haemoglobin (oxy-Hb) concentrations, across both the absorption and post dose task performance periods, in contrast to placebo. Whilst plasma levels of resveratrol were increased after 28 days of exposure, CBF, subjective sleep quality and health were not modulated. Additionally, no difference in blood flow velocity was observed acutely or chronically. However, self-reported fatigue was reduced following 500 mg resveratrol consumption after 7, 21 and 28 days, with a trending reduction nearing significance after 14 days.

Interestingly, cognitive effects were noticed as a result of both acute and chronic resveratrol supplementation, demonstrating a significant reduction in overall errors made during Serial subtraction tasks in comparison to placebo. However, supplementation with resveratrol was also found to significantly decrease the number of correct responses in comparison to placebo in the Serial 7 subtraction task, which may imply a trade off in performance rather than any treatment-mediated effects. In addition to the modulation of Serial subtraction performance, the authors also found resveratrol increased the number of correct responses on the 3-Back task compared to placebo after 28-days of supplementation. Yet, in the absence of any significant modulation to CBF, this result is somewhat hard to interpret and was viewed as more likely representing a type I error by the authors.

The above studies suggest that acute administration of resveratrol is capable of CBF enhancement and increased O_2 delivery and extraction (indexed by increases in total-Hb, deoxy-Hb and oxy-Hb respectively). However, none of the above studies were able to demonstrate that this increase could translate into cognitive benefit in humans. It is noteworthy that the aforementioned studies all employed young, healthy samples (average age ~20), who may already be in the peak of their cognitive capacities (Rönnlund et al., 2005). Thus, the scope for resveratrol-mediated cognitive improvements in these populations, may be minimal due to their ceiling effects. This argument is supported by evidence from cocoa-flavanol trials which have also shown greater and more consistent improvements within populations who are not at their cognitive peak (i.e older individuals or those with MCI).

To this author's knowledge, there remains only one study that has examined acute doses of resveratrol in ageing populations. Scholey et al. (2014) demonstrated that acute administration with resveratrol-enhanced wine was capable of improving cognitive performance, in comparison to wine alone, in a cohort of older adults (aged 65–78 years). After completing a cognitively demanding battery, wine enhanced with 200 mg of resveratrol was found to improve performance on the Serial 7 subtraction task across all 6 post dose repetitions (but only significantly at repetition 2 & 4). No significant effects on mood were reported. Such findings should be taken lightly however, as there was no dealcoholized control to compare to, nor was there any monitoring of CBF parameters to elucidate the proposed mechanism of CBF. Nevertheless, CBF effects have been observed in cohorts with compromised blood flow capacity. Wong et al. (2016) examined a range of acute doses of resveratrol (75, 150, and 300 mg) in individuals with type 2 diabetes mellitus (aged 49-78 years). All three doses significantly increased blood flow velocity in the middle cerebral arteries, yet, only the lower 75 mg dose was found to additionally increase blood flow velocity in the posterior cerebral arteries.

As a reduction in estrogen within women has been linked with reduced cerebrovascular sensitivity (Penotti, 1996), further support for the vasodilatory mechanism of resveratrol in ageing populations can also be found from investigations into postmenopausal women. Evans et al. (2017) investigated whether 75 mg of resveratrol (administered twice daily over 14 weeks) could improve cognitive performance and mood by enhancing cerebrovascular responsiveness in postmenopausal women. Subjects abstained from the treatment for 12 hours on the day of testing before undergoing cognitive assessments. Resveratrol was found to modulate cerebrovascular responsiveness (assessed via blood

flow velocity in the middle cerebral arteries using transcranial Doppler), during cognitive performance. Despite a consistent lack of significant improvements on individual tasks, overall cognition was significantly improved in participants taking resveratrol in comparison to the control group. In addition, the authors reported treatment related increases in cerebrovascular responsiveness during the cognitive test battery and these were significantly correlated to the improvements seen in overall cognitive performance.

Summarising remarks

The above literature review details how polyphenols can interact with the human CNS via cellular signalling pathways and, subsequently, may offer a means of cognitive enhancement. The stilbene polyphenol resveratrol has shown to induce NO-dependant vasodilation and consequently the capacity to exert positive increases in CBF. This CBF enhancement is hypothesized to provide an increased access to neural metabolic substrates (namely O_2 & glucose) and, in turn, cognitive benefits. Despite this, administration of acute high doses of resveratrol to young, healthy samples have been found to induce the anticipated cerebrovascular benefits, but these have not been found in conjunction with improved cognitive performance. This has called into question the ability of increased CBF alone to provide a viable mechanism of cognitive enhancement. Nevertheless, it is possible that increased CBF may provide more utility in those suffering mild (natural) cognitive decline or a reduction in blood flow capacity, yet further research is needed to elucidate this claim.

1.5 Cerebral metabolism, cognition and ageing.

Despite its relatively small size, the human brain accounts for approximately 11% of total cardiac output, equating to ~20% of overall body metabolism at a resting state (Raichle, 2010). To satisfy this large metabolic demand, the brain requires an abundant supply of O_2. This large utilisation of O_2 is necessary to support the high rate of adenosine tri-phosphate (ATP) production to, amongst other actions, maintain the continual transmission of neuronal signals (Lutz et al., 2003). However, despite its sizable energy consumption, the brain itself cannot store its own energy source. Consequently, a continuous supply of blood-borne metabolic neural fuel substrates is essential for not only cognition, but maintenance and regulation of physiological function (Parrott et al., 2004).

1.5.1 Cerebral metabolism

The key mechanism whereby humans generate metabolic energy is through the process of oxidative phosphorylation of ATP within the mitochondria (Erecińska & Silver, 1989); making ATP the sole cellular energy currency of the brain. The main metabolic fuel of the brain is blood borne glucose. When fully oxidised in the presence of O_2, this produces an increased yield of energy in the form of ATP in the mitochondria (30–36 ATPs) (Magistretti & Allaman, 2015), whereas anaerobic (without O_2) glycolysis produces only 2 ATP. Therefore, the oxidation of glucose is the dominant form of ATP production in the brain. A large proportion of this ATP budget is utilised to maintain and restore the transmembrane Na^{2+} and K^{+} ion channels, which deteriorate with continuous neuronal firing (Attwell & Laughlin, 2001). A comprehensive overview of the oxidation of glucose can be found in more detail elsewhere (Erecińska & Silver, 1989; McLaughlin et al., 2007).

1.5.1.1 Neurovascular coupling

As neural demand increases, the requirement for metabolic resources subsequently increases, leading to an elevation in CBF to ensure an ample supply of neural fuel substrates, namely O_2 and glucose (Parrott et al., 2004; pp. 204). Thus, CBF is adjusted to accommodate the relative increase in cerebral metabolic rate (Tameem & Krovvidi, 2013); this process is referred to as neurovascular coupling. CBF is tightly coupled to the metabolic requirements for neural fuels, notably the cerebral metabolic rate of O_2 ($CMRO_2$) and glucose utilisation (Hyder et al., 1998; Hoge et al. 1999). However,

contrary to common belief, the increased level of CBF during sustained neuronal activity is greater than the rise in both $CMRO_2$ and ATP consumption (Leithner & Royl, 2014; Raichle 2010; Lin et al., 2010). It is proposed that this large volume of blood is necessary to transport the vast quantity of fuel substrates and so even small relative reductions to CBF can comparatively reduce mental performance (Leithner & Royl, 2014).

1.5.1.2 Neural fuel substrates

Despite the brain possessing other high-energy reservoirs (in the form of the phosphate creatinine system), glucose and O_2 almost exclusively meet most of human neural demand (Owen & Sunram-Lea, 2011). Therefore, variations in the availability of both blood-borne fuel substrates can impact brain metabolism and consequently cognitive function. Indeed, it is well established that administration of glucose can enhance aspects of cognitive performance in healthy humans (Gonder-Frederick et al., 1987; Craft et al., 1994; Sünram-Lea et al., 2011). Conversely, in hypoglycaemic populations, reduced circulating glucose leads to cognitive deterioration (Gonder-Frederick et al., 2009), while a blood glucose level of between 2.6–3.0 mmol/l within healthy cohorts has been claimed to impair cognitive function (Warren & Frier, 2005). Fairclough and Houston (2004) report that blood glucose levels are sensitive to 'time-on-task', demonstrating that blood glucose declines in a dose dependent manner the longer participants spend engaging with cognitive activity. Additionally, glucose administration can significantly improve cognitive performance on tasks with higher cognitive load (Kennedy & Scholey, 2000; Sünram-Lea et al., 2001; Scholey et al., 2001). This is hypothesized to be the result of increased mobilised fuel reserves as part of an inherent response to more physiologically demanding tasks (Kennedy & Scholey, 2000).

Like glucose, availability of O_2 within the cerebral vasculature can impact cognitive functioning. Additional supplementation of O_2 improves memory formation and consolidation (Scholey et al., 1998), attention and vigilance (Moss et al., 1998). Blood O_2 saturation has also been found to positively correlate with cognitive performance (Chung et al., 2006). Most cells cannot exist anaerobically for sustained periods and so reductions in O_2 saturation may be more significant during cognitive performance. Indeed, several investigations have demonstrated exposure to hypoxic conditions can lead to cognitive decline (Virués-Ortega et al., 2004; Petrassi et al., 2012). In sum, the relative increase in either or both of these neural fuel substrates allow for enhanced cognitive performance. Conversely, relative decreases in the availability of either of these fuels leads to a

comparative decrease in cerebral metabolism and, consequently, deteriorated cognitive functioning. It is proposed that any substrate or method that may enhance the energy supply of the brain, is capable of cognitive enhancement (Owen & Sunram-Lea, 2011); this may be particularly evident when individuals are depleted or suffer a reduction in these fuels.

1.5.2 Ageing of the cerebral vasculature

With an increasing ageing population, research into both natural and pathological ageing has become of increasing importance. During the ageing progress, the brain undergoes comprehensive changes in its function and physiology (Lu et al., 2010) including regional atrophy (Scahill et al., 2003; Chen et al., 2011), reduction in size (& weight) (Peters, 2006) and deterioration of the neural myelin sheath (Fields, 2008). Cognitive decline as part of the natural process of ageing has been well documented in scientific literature (Harada et al., 2013). However, the causes and mediating factors of cognitive decline are still to be fully elucidated. Advancements in neuroscience have identified the age-related deterioration in the function of the cerebral vasculature as a significant contributor to cognitive decline. Indeed, the reduced efficacy of cerebrovascular functioning is thought to be a significant contributor to several neurodegenerative diseases (ladecola, 2010; Kalaria, 2009) and the associated decline in cognitive functioning (Gorelick et al., 2011). This is perhaps unsurprising given that the brain's requirement for continuous replenishment of neural fuels is totally dependent upon the circulation of blood. Understanding changes in CBF of healthy, older adults, may provide important insight into why cognitive performance naturally declines with age.

CBF is an important biomarker of brain health and function (Durduran & Yodh, 2014) with declining regional CBF (rCBF) being well acknowledged to positively correlate with age (Zemcov et al., 1984; Melamed et al., 1980; Martin et al., 1991; Kalaria, 2009; De Vis et al., 2015) and indeed, negatively correlate with cognitive function (de Eulate et al., 2017). It is important to note that changes in CBF are not uniform across the brain, with subcortical regions remaining relatively unaffected by reduced blood flow (Chen, et al., 2011). Lu et al. (2010) assessed the age-related differences to brain metabolism and cerebral vasculature in a cohort of healthy subjects ranging from 20-89 years. The researchers reported that decline in CBF was widespread across the brain, but increasing age was found to most notably reduce CBF in the prefrontal cortex, insular cortex, and caudate. This is particularly interesting given that older adults have been found to perform

poorer on measures that require frontal lobe function, in comparison to younger adults (West, 1996, 2000; Kievit et al., 2014), whilst both older and younger adults perform very similarly on tasks of non-frontal function (Ardila & Rosselli, 1989). As a reduction in CBF would indicate a decline in cerebral function, age related decreases will lead to a compromised delivery of neural fuels and, consequently, hindered cerebral metabolism.

Given the importance of blood flow to supply these neural fuels, it would be logical to assume that a reduction in CBF would axiomatically lead to a reduction in $CMRO_2$. De Vis et al. (2015) found that CBF was significantly lower, while O_2 extraction fraction (OEF) was significantly higher, in the frontal, temporal and the deep grey matter of the older subjects in comparison to younger participants. The research team also found significantly lower $CMRO_2$ in these regions of the older subjects. This could demonstrate that the reductions in rCBF experienced with age, can lead to an increase in the extraction of O_2 in an attempt to offset the reduction in supply of O_2. However, Aanerud et al. (2012) reported that values of CBF were found to decline to a greater extent than values of $CMRO_2$ during the ageing process. The researchers postulate that the higher O_2 extraction lowers the average tension of O_2 within the cerebral capillaries, disrupting the partial pressure gradient. In turn, this compromises the delivery of O_2 to brain tissue and, subsequently, energy yield. Although the exact mechanism of disruption is perhaps unclear, consistent evidence points towards ageing inflicting an associated reduction in regional blood supply, causing disruption to the energy homeostasis of the brain and, consequently, a gradual decline in energy metabolism.

1.5.3 Cognitive decline in the ageing brain

As several variables play a role in the progression or delay of the ageing process, the age at which cognitive decline significantly progresses is unclear. Salthouse (2009) postulated that cognitive ageing can even begin as young as 20–30 years of age for specific cognitive domains, yet general decline has been estimated to take hold at approximately middle age (Aartsen, et al., 2002; Rönnlund et al., 2005). This would also coincide with the onset of reductions in rCBF (Lu et al., 2010; Aanerud et al., 2012). Cognitive ageing is thought to accelerate from approximately 70 years old (Filley & Cullum, 1994) and, consequently, from this age onward there is a greater risk of pathological and neurodegenerative decline (Howieson, 2015).

With regards to specific cognitive domain decline, the most consistently reported age-related declines to cognition appear generally in speed of processing, executive functioning and memory. Indeed, many subsequent reductions in cognitive performance are thought to be the result of increased speed of processing (Salthouse, 1996). Attention is highly associated with the speed of information processing as tasks associated with cognitive domain typically require periods of sustained attentiveness and responsiveness. As a result, sustaining attention during vigilance tasks (Filley & Cullum, 1994; Berardi et al., 2001) and the more taxing attentional paradigms, such as divided attention (Zanto & Gazzaley, 2014), show a large variance between age groups. These declines in attention can also have consequences for everyday function; for example, increasing age shows a greater risk of car accidents in older adults (Braver & Trempel, 2004).

Older adults commonly report difficulty in remembering or changes in the quality of their memory function. However, with the exception of reductions in episodic memory (Isingrini & Taconnat, 2008; Lundervold et al., 2014), long term memory appears to remain relatively intact with age as procedural, implicit and semantic memory typically show little to no decline (Balota et al., 2000; Craik, 1994). Moreover, most well learned facts and knowledge seems to be highly resistant to age-related deficits (Craik, 1994). Yet, in contrast, processing and recalling new information appears to be more affected (John & Cole, 1986). Regarding the stages of memory function, both acquisition and retrieval of recently acquired information appears to be impacted with age (Duchek, 1984; Craik & McDowd, 1987; Balota, et al., 2000); evidenced by poorer recall (Howieson, 2015). However, retention of information appears to be surprisingly preserved (Kliegel et al., 2000). Finally, short term memory declines more readily with age; with clear observable reductions in working memory (Li et al., 2001).

Not all cognitive domains deteriorate equally however (Glisky, 2007), with speech and language processing remaining largely intact in older adults and extensive vocabulary has been found to improve with age (Salthouse, 2009). This could be result of the ageing process causing regional specific decline to the brain (Burke & Barnes, 2006), leading such areas to be more at risk to the effects of ageing or even pathological deficits. Additionally, accuracy remains relatively intact with age yet, in the presence of increased reaction times, this would clearly imply an age-related difference in speed-accuracy trade-offs (Staub et al., 2013). (The reader is directed to the following texts for a more extensive overview of natural ageing reductions in cognition: Peters, 2006 Glisky, 2007; Salthouse, 2000, 2010; Harada et al., 2013; Hartshorne & Germine, 2015.)

1.5.4 Multifactorial ageing brain: A need to model cognitive ageing

It is clear that the ageing brain is subject to progressive physiological decline with reductions in rCBF and O_2 metabolism contributing to cognitive domain specific age-related reductions. Regarding the current PhD thesis, resveratrol has been identified to increase cerebral perfusion and, consequently, may operate to attenuate reductions in CBF and offer cognitive enhancement as a result. This is hypothesised to be the result of increased access to neural fuel substrates attenuating age-related reductions in rCBF and, in turn, $CMRO_2$. However, despite age-related cognitive decline being well documented within the literature, individual variability of biological (including health), psychological, environmental, and lifestyle factors contribute to the rate and the extent of cognitive decline (Harada et al., 2013).

Research into this area generally assumes the trajectory of ageing is similar for all persons yet, as ageing is a multifactorial biological process, several confounding factors arise when measuring cognitive functioning in 'normal' or 'healthy' older adults. Indeed, the brain undergoes a series of other physiological changes in conjunction with observed reductions to rCBF. Varying levels of regional atrophy, increased neuroinflammation and glucose tolerance are all shown to contribute to declining cognitive functioning with age (Peters, 2006; Cevenini et al., 2013; Lamport et al., 2009). This highlights that there is not one common cause of cognitive decline but a multiplicity of factors. Lifestyle factors such as midlife hypertension, diabetes, smoking, stress and obesity can all increase the progression of cerebrovascular ageing (Debette et al., 2011) while factors such as aerobic exercise contribute to decelerating the cognitive ageing process (Steffener et al., 2016). In support, cognitively demanding tasks have been reported to be sensitive to aerobic capacity, with higher complex cognitive speed performance linked to higher aerobic fitness (van Boxtel et al., 1997).

Cognitive ability is highly diverse in individuals yet variance is thought to increase further in older adults (Jiang et al., 2017). Research has even shown cognitively intact humans aged 100 years (Perls, 2004) demonstrating the diversity which can take place within 'normal' ageing. Individual differences in cognition can also be dependent upon the capacity of an individual to engage in effortful cognitive endeavours (Cacioppo et al., 1996). Indeed, maintaining cognitive activity; including engaging with intellectual activities (e.g. puzzles), has been found to attenuate progressive cognitive decline (Verghese et al., 2003). Furthermore, cognitive ageing seems also dependent upon

cognitive ability in youth (Karama et al., 2014), with higher levels of intelligence and education linked to better cognitive function in older age (Rönnlund et al., 2005; Marioni et al., 2014).

From the above, it would appear necessary to first model the age-related reduction to neural fuels to assess if the mechanism of augmented CBF can improve cognitive performance through the mitigation of such reductions. This would identify that (1) there is merit to the proposed CBF mechanism of resveratrol and (2) the attenuation of rCBF and $CMRO_2$ reductions observed in ageing populations can result in improved cognitive performance; without the interference of other confounding variables, as briefly outlined above, and individual variability. Given the importance of O_2 in cerebral metabolism and consequently cognitive functioning, manipulating the availability of this neural fuel substrate may provide a means of modelling age-related decline in $CMRO_2$. Indeed, the use of hypoxia has been credited as a representative, experimental model for the ageing process (Cataldi & Di Giulio, 2009). Therefore, as reductions in CBF are tightly coupled with the supply and availability of neural fuel substrates, reducing the O_2 supply in healthy, young individuals may provide a means of testing this hypothesis.

1.6 Hypoxia as a model for cognitive ageing

The previous section proposed that disrupting O_2 delivery via an induced state of hypoxia, may offer a representative, experimental model for the ageing process. This would provide a viable method to assess the efficacy of the CBF mechanism of resveratrol (given that CBF enhancement should be expected to increase the depleted O_2), whilst also providing a model of the ageing brain (where O_2 utilization is less efficient). For clarity, the state of hypoxia in this PhD thesis will be simply defined as a reduction in the fraction of inspired O_2 (F_iO_2).

1.6.1 The atmosphere at sea level and altitude

The atmosphere is mainly made up of nitrogen (~78%) and O_2 (~20.93%), with the rest consisting of small amounts of argon (>1%), carbon dioxide (~0.04%) and other gases / water vapour. At sea level, barometric pressure (the weight / force applied to the atmospheric gasses) is approximately 760 mmHg; this allows for 100% of the available atmospheric O_2 to be extracted by the human alveolus. With increasing altitude, barometric pressure reduces, and consequently the partial pressure (the specific pressure applied by a specific gas) also falls; this results in less available O_2 from the atmosphere for extraction and absorption by the body (West et al., 1983; Beall, 2001). As a consequence of the resulting reduction in available O_2, ascent to altitude can cause a variety of impairments to mood, vision and higher mental faculties (Shukitt & Banderet, 1988; Shukitt-Hale et al., 1998; Hornbein, 2001; Yan, 2014; West, 2016), and in more extreme cases, headache, nausea, loss of consciousness and even death (Netzer et al., 2013; Zafren, 2014; Severinghaus, 2015).

1.6.2 Acute physiological response to hypoxia and altitude

The ability of the brain and tissues to respond to lowering levels of O_2 is an essential requirement for survival, therefore exposure to hypoxia instigates a series of physiological responses to maintain O_2 homeostasis. Non-acclimatised individuals who encounter altitude induced hypoxia experience several immediate and gradual adaptations to pulmonary-vascular and metabolic parameters in response to the insufficient supply of O_2. These adaptations are assumed to be beneficial, aiding in protection against disease, cellular dysfunction, and increased O_2 delivery. Indeed, in sporting and athletic contexts, exposure to prolonged bouts of hypoxia have been employed to improve aerobic

performance (McLean et al., 2014); due to hypoxia provoking an increase in erythropoiesis (Garvican et al., 2012; Garvican-Lewis et al., 2015) and, in turn, improving O_2 supply. However, not all adaptations are advantageous. The large stress imposed upon the pulmonary-vascular system from chronic exposure to hypoxia results in structural alterations, leading to a significantly increased risk of pathological diseases, such as pulmonary hypertension (Penaloza & Arias-Stella, 2007) and polycythemia (excessive red blood cells) (Naeye, 1967). The fundamental integrated reflexes induced by acute exposure to hypoxia include changes to ventilation, carriage of O_2 and cardiac output. Understanding these physiological responses and their interactions with one another is critical for the understanding of global and rCBF regulation in hypoxia (Ainslie & Subudhi, 2014). Naturally, the impact of these physiological responses is dependent upon the rate, duration and intensity of exposure to hypoxia, which will be detailed, where appropriate, in this review.

1.6.2.1 Hypoxic ventilatory response

Individuals living at sea level (normoxia) naturally have a low resting ventilation, but this is rapidly increased upon immediate exposure to hypoxic conditions along with a fall in O_2 saturation in the blood (Beall, 2001); referred to as the hypoxic ventilatory response (HVR). The purpose of a HVR is to allow more O_2 to be taken in and be passed through the lungs, offsetting the hypoxic effect. It should be noted however, that this response is not universal; there is considerable variation in the HVR between individuals (Kronenberg & Drage, 1973) with both age and smoking being identified as factors that increase the ventilatory response to hypoxia further (Nesthus et al., 1997; Richalet & Lhuissier, 2015). HVR is particularly noteworthy, as its role is to increase the partial pressure of arterial oxygen (P_aO_2) and reduce that of CO_2 (P_aCO_2), which consequently affects O_2 carriage and CBF.

1.6.2.2 Oxygen carriage

As the circulatory system is the sole supplier of O_2, adjustments to the affinity for O_2 during bouts of hypoxia may contribute significantly to hypoxic adaptation (Samaja et al., 2003). Once inspired, P_aO_2 is approximately 100 mm/Hg in the human alveolus, while P_aO_2 at the capillary is ~40 mm/Hg. Through the process of diffusion (moving from a high concentration to a low concentration), O_2 transfers across the alveolar membrane to bind to the four heme groups present in the haemoglobin (Hb) of the red blood cell. This

allows the transport of O_2 around the body to muscle and other tissues (McLaughlin et al., 2007).

In healthy humans, at sea level, arterial oxygenation within the haemoglobin will be on average 98-100%. This is a result of the high P_aO_2 ensuring almost complete Hb saturation. This amount of O_2 is sufficient to meet most of the metabolic requirements of the body, however, in the event of reduced blood flow, uptake in O_2 becomes proportionate to the rate of delivery (McLaughlin et al., 2007). The oxyhaemoglobin curve (Figure 1.2) depicts the dissociation of Hb saturation at the normal range. As partial pressure begins to fall with ascent to altitude, O_2 extraction becomes compromised at a progressive rate. During moderate hypoxia, this curve can be seen to shift slightly to the right, indicating O_2 is less bound to the heme groups and, thus, is more available to be diffused into the surrounding tissues. However, at higher altitudes this shifts further to the left, indicating a higher retention of O_2, as the body struggles to maintain the demand for O_2.

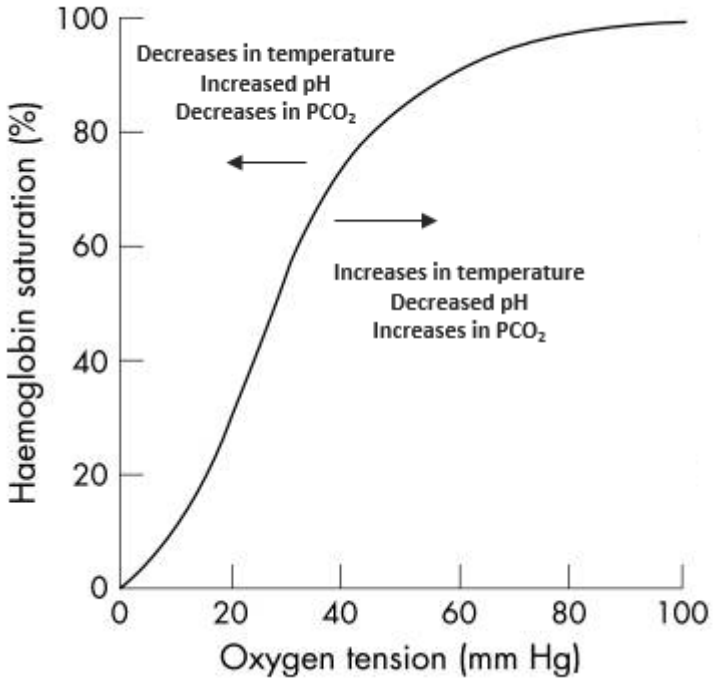

Figure 1.2. The oxyhaemoglobin dissociation curve, with demonstration of the Bohr effect. Adapted from Beasley et al. (2007).

Delivery of O_2 requires a stable microenvironment. Therefore, the binding affinity of O_2 is also dependent upon blood pH and body temperature. The Bohr Effect demonstrates that when the pH of blood is lowered (increased acidity), due to the build-up of excessive carbon dioxide (CO_2) and waste products, dissociation of O_2 occurs at a higher arterial

partial pressure of O_2 (McLaughlin et al., 2007). Core temperature also plays a role in this process, as relative increases in temperature will cause the same effect. This enables a higher dissociation of O_2, leading to increased delivery to the working tissue. In contrast, decreases to core temperature and increases in pH result in the opposite reaction, causing O_2 to be less available to diffuse into the tissues. The Bohr effect emphasises the role which temperature and blood pH can play in the body's reaction to hypoxic conditions. Although the impact of these two variables will be discussed further below, this highlights that the physiological response to hypoxia can be varied and should be considered when adapting the proposed hypoxia model of ageing.

1.6.2.3 Cerebral haemodynamic response

During initial exposure to atmospheric hypoxia, cerebral tissue O_2 saturation decreases which induces an increase in CBF to offset lower cerebral O_2 delivery and subsequently meet O_2 demand (Harris et al., 2013; Brown et al., 1985). This acute compensatory increase in CBF is hypothesized to be a result of NO signalling and NO mediated endothelial vasodilation (Blitzer et al., 1996). In support, evidence from native inhabitants of high altitude where native Tibetan highlanders live at ~4200 m above sea level (85% O_2 saturation) shows significantly (up to 7 times) higher NO transfer from the lung (Hoit et al., 2005). This was reported to give a higher potential for increased vasodilation of the pulmonary blood vessels, compensating for the hypoxic environment. In addition, evidence has suggested that NO inhibitors can blunt CBF and vasodilation in young healthy low landers when exposed to acute hypoxia (Van Mil et al., 2002). Despite an immediate increase in CBF (within the first 12 hours of exposure to high altitude) this ceases after 3–5 days, returning to near sea level values (Ainslie & Subudhi, 2014). This would appear to be due to significant cerebrovascular adaptations taking place, enhancing the carrying capacity of O_2 in the blood after only a few days at altitude (Willie et al., 2014).

As detailed earlier, the physiological responses to acute hypoxia influence one another; hence fluctuations of one consequently dictates the subsequent response. Hypoxia incites stimulation of the peripheral chemoreceptors, leading to hyperventilation, and eventually to hypocapnia and respiratory alkalosis (Petrassi et al., 2012). Both hypocapnia and respiratory alkalosis incite cerebral-vasoconstriction (Raichle & Plum, 1972). Interestingly, extreme hyperventilation has also been found to induce intracellular acidosis, resulting from hypercapnia, which conversely provokes vasodilation (Vavilala

et al., 2002). It is assumed that both hypercapnia and hypocapnia cause fluctuations in P_aCO_2, inducing changes in pH and consequently elicits vasodilation or constriction, respectively (see the above detailed 'Bohr effect'). This emphasises that the response to hypoxia is not straightforward, and individual reactions in hyperventilation may coordinate the CBF response. Although acute exposure to hypoxic conditions increases HVR, this is estimated to reduce within 20 minutes in healthy individuals (Beall, 2001). This may remain elevated for up to 60 minutes in comparison to baseline measures (Huang et al., 1984), but would imply any impact of hypocapnia would subside within a short period of time.

1.6.3 Neurovascular coupling in hypoxia

Davranche et al. (2016) assessed whether specific cognitive processes and cerebral hemodynamics (measured via functional NIRS) correlated during acute and prolonged high-altitude exposure. Eleven healthy males were exposure to 4350 m altitude for 4 days, with measurements for cognitive and CBF taken at initial ascent (3-5 hours exposure) and 2 and 4 days following ascent. After 3-5 hours of exposure, participants displayed a reduction in information processing evidenced by poorer reaction times and an increased number of errors. However, these reductions were attenuated following 2 days exposure suggesting acclimatisation. More importantly however, cerebral haemodynamics were found to correlate with acute cognitive performance on the Simon task[1]. The authors calculated a total oxygenated tissue index, which was found to negatively correlate with speed of reaction time performance, while a significant positive correlation between levels of deoxygenated Hb and reaction time was also found. The results of this study demonstrate that the despite hypoxia leading to decreases in cognitive performance (i.e. slower reaction times), a more effective CBF response can function to attenuate this reduction.

It is noteworthy that the brain is hypothesised to be able to withstand a certain threshold of hypoxia. Mild hypoxic conditions may initiate sufficient compensatory adaptive responses in healthy cohorts which function to attenuate any observable reductions in cognitive performance. Indeed, the stable levels of $CMRO_2$ found consistently during mild / moderate acute hypoxic conditions may suggest that the vasodilatory response is

[1] The Simon task for the present study required participants to respond to either a square or a circle presented either to the left or to the right of a prior presented fixation point. Response pads consisted of left and right response keys corresponding to one of the shapes (e.g. left response for a circle & right for a square) which were counterbalanced across participants, creating congruent and incongruent trials.

sufficient to compensate for the reductions in O_2 (Xu & LaManna, 2006). However, rapid exposure to hypoxia may result in only minimal adaptations to the body, supporting claims that maximal cognitive deficits are more apparent within the early stages of exposure. Therefore, further investigations into mild and moderate altitude are still warranted, if it is employed in short bouts, to enable maximal deficits and minimal adaptation to take place (Petrassi et al., 2012).

1.6.4 Cognitive performance in acute altitude induced hypoxia

As the partial pressure of O_2 (PO_2) decreases with the ascent to altitude, O_2 availability also decreases. This causes an offset in O_2 saturation and exposes vital organs and tissues to hypoxic conditions leading to reduced performance and function. Given that brain function is especially vulnerable to the effects of hypoxia (Larson, et al., 2014); exposure to increasing hypoxic conditions leads to an increasing level of detrimental effects on cognitive performance, yet this is dependent upon the rate and duration of exposure. The impact of altitude-induced hypoxia has been shown to be domain specific, with performance on complex tasks appearing to be more adversely affected at altitude in comparison to more simple ones (Cahoon, 1972; Babbar & Agarwal, 2012). Whilst well learned tasks seem to be more resistant to the effects of hypoxia in comparison to novel tasks, impairments to learning, reaction time, decision-making and (certain aspects of) memory are commonly reported at altitude (Petrassi et al., 2012). Increased error rates are also commonly reported (Wu 1998; Du et al., 1999), despite researchers noting that subjects may slow their responses further as a strategy designed to minimise mistakes (Abraini et al., 1998).

Executive function, memory, and attention have also been found to be impacted by acute exposure to high altitude (Asmaro et al., 2013). Most of these studies suggest that the cognitive impairments are directly related to the severity of hypoxia induced. Nevertheless, there remains a level of ambiguity regarding whether low levels of altitude can impact cognitive domains sufficiently, as most investigations only document consistent domain specific cognitive deficits beginning at around 3000 m above sea level (Bahrke & Shukitt-Hale, 1993). Indeed, it has been proposed that the ‘crucial zone’ for cognitive deficits to take hold is between 4000-5000 m above sea level (equivalent to 12.7-11.2% FiO_2) (Nelson, 1982). However, reductions in mental performance have been reported at levels as low as 1524 m altitude during exercise (equivalent to 84% of the O_2 available at sea level), in which complex reaction time was found to decrease when

exposed to a novel task (Denison et al., 1966). Moreover, learning (Farmer et al., 1992), attention, working memory (Wu et al., 2002) and reaction time (Ando et al., 2010) have all been found to be negatively impacted at low levels of stimulated altitude (2440 m, 2800 m and 2134 m, respectively). Therefore, it does appear that mild or even low levels of hypoxia, can induce behavioural deficits.

1.6.5 Mood in acute hypoxia

It is perhaps unsurprising that hypoxia can also negatively influence mood parameters in addition to cognitive performance. Altitude induced hypoxia has been found to induce a wide range of adverse psychological changes including increased feelings of anxiety, depression, aggression, and fatigue, complemented by reduced feelings of vigour and happiness (Bahrke & Shukitt-Hale, 1993; Shukitt-Hale et al., 1998; Bardwell et al., 2005). Li et al. (2000) explored the effects of acute mild and moderate hypoxia on mood in 18 healthy males, via simulated altitude at 300 m (baseline/ control), 2800 m, 3600 m and 4400 m for 1 hour in a hypobaric chamber. This study demonstrated that acute exposure for as little as 1 hour, to mild hypoxia (2800 m), had a negative impact on mood state in healthy individuals, which seems to further progress with further ascent to altitude. Similarly, Shukitt, and Banderet (1987) found only self-reported 'sleepiness' was found to increase at low levels of hypoxia (1600 m). At 4300 m significant reductions were found to friendliness and clear thinking, whilst significant increases were found to dizziness, sleepiness, and unhappiness in comparison to baseline controls (200 m). Again, the greatest differences in mood were found after only short term (3 hours) exposure to hypoxic conditions.

Legg et al. (2016) investigated complex cognition and mood at 8000 ft (2438 m) and 12,000 ft (3658 m). Despite complex cognition being unaffected by either altitude, self-reported fatigue was increased and vigour was found to be reduced at 12,000 ft. Interestingly, these were found to be restored when subjects were supplemented with 100% O_2. This would support the hypothesis that reduced access to O_2 plays a role in reductions in mood at altitude yet, as few studies have examined the role of mood at hypoxia, it is unclear whether improvements in CBF may also significantly improve mood parameters. However, evidence from studies supplementing additional O_2 at both hypoxia and normoxia would certainly support this proposed mechanism.

1.6.6 General considerations for the use of hypoxia as an experimental model of cognitive ageing.

The rationale behind utilizing the environmental chamber in the current thesis was to remove the extraneous variables that result from the multifactorial nature of cognitive ageing (see section 1.5.4). As the capacity of the aging brain to extract and utilise O_2 and other nutrients declines significantly at the neuron (Campos et al., 2014), this compromises the delivery of O_2 to brain tissue and subsequent energy yield. Similarly, a reduction in F_iO_2 results in a subsequent decrease in maximal uptake and delivery of O_2 to neural tissue allowing for a suitable comparison. Additionally, as section 1.6.4 details, induced hypoxia from 2000-4500 m may reduce cognitive performance on a series of differing cognitive domains including memory, reaction time, and rate of errors. This is of particular interest given that ageing populations tend to display similar reductions to these cognitive domains as detailed previously in this literature review (section 1.5.3). The above would suggest that there is merit in the modelling of cognitive ageing with hypoxia-induced performance deficits, as this not only mirrors the disruption of O_2 that occurs with age, but also the measurable behavioural reductions that might ensue are directly comparable to naturally ageing populations.

It should also be noted that physical activity, ambient temperature, acclimatisation, and individual response variability, all play a role in the relative reduction in behavioural performance within hypoxia. Moderate and extreme reductions / increases in ambient temperature have been reported to negatively affect performance further at hypoxia (Taylor et al., 2016). This would emphasise the need for a controlled environment within hypoxia and utilising temperatures that reflect those at sea level for a fair comparison. Moreover, as physical exercise naturally increases O_2 demand on the body, it is possible that studies which perform behavioural testing alongside, or conjunction with, exercise are biased in demonstrating exaggerated reductions in performance. Indeed, cognitive performance can be impacted at lower altitudes more readily in investigations when combined with strenuous physical exertion (Petrassi et al., 2011). Therefore, it is important to establish cognitive responses at rest and isolate any extraneous or additional demands on O_2 consumption. Additionally, physiological variability from one individual to another can impact adaptation and response to hypoxia. It is of course important to allow such individual differences to represent the natural variance that exist within the population. Nevertheless, variance in responsiveness brought on by lifestyle factors (such as obesity & physical fitness) and pre-acclimatisation must be screened out.

Moreover, it is noteworthy that the individual response of hyperventilation in reaction to immediate exposure to hypoxic conditions can play a large role in the acute cerebral haemodynamic response to hypoxia. HVR may induce cerebral vasodilation as a natural reflex to offset O_2 deficiencies yet, in contrast, rapid hyperventilation may also prompt hypercapnia; stimulating vasoconstriction. However, previous investigations have demonstrated that hypercapnia itself is associated with age related impairment to cognitive performance (Zheng et al., 2008; Mitschelen et al., 2009). Moreover, as resveratrol has been noted to increase CBF via increasing NO bioavailability and NO levels, this will aid in overcoming vasoconstriction or exaggerate a beneficial response of vasodilation respectively, and therefore should present a measurable increase in CBF in either case. In support, evidence from studies administrating nitrate (with evidenced vasodilatory effects (Clements et al., 2014)), have shown beneficial adaptations to physiological function and exercise during moderate / high altitudes via increased modulation of blood flow parameters (Presley et al., 2011; Shannon et al., 2017).

Finally, rapid ascent to altitude results in minimal adaptations to the body, thus, the greatest reductions to cognitive performance arise within the early stages of exposure (Petrassi et al., 2012; Davranche et al., 2016). This would suggest that an effective hypoxic model should only implement short bouts of hypoxia within subjects, to ensure that any measurable effects are not masked by the efficacy of the body's natural adaptations. It is clear from this review that responses to hypoxia are not straightforward, and isolation and control over a number of factors is required to host a reliable hypoxic model of ageing.

1.7 Concluding remarks and the aims of this thesis.

Polyphenols and their metabolites have been found to interact with cellular transduction and signalling cascades, which are proposed to be the underpinning of their numerous health benefits in humans. The non-flavonoid polyphenol resveratrol has shown to modulate NO within endothelial cells, and, in turn, capable of augmenting NO-dependent vasodilation. Interestingly, the capacity of resveratrol to induce vasodilation has not only been found to promote cardiovascular benefits but has also been suggested to offer cerebrovascular enhancement. Indeed, habitual resveratrol consumption has been associated with a reduction the risk of cardiovascular and cerebrovascular disease, while both acute and chronic resveratrol administration have been found to increase peripheral and cerebral blood flow. Intriguingly, the ability of resveratrol to increase CBF has been suggested as an indirect mechanism to provide cognitive enhancement in humans. This is based upon the premise that enhancing the provision of blood-borne metabolic substrates (O_2 & glucose) will subsequently increase the energy yield of the brain and, in turn, cognitive performance in young, healthy humans.

To date, only a limited number of human intervention studies have investigated the CBF and cognitive effects of resveratrol following acute and chronic administration; with the small quantity of research that does exist providing conflicting results. Indeed, despite research demonstrating increases in CBF and O_2 extraction and utilisation (as indexed by increased concentrations of deoxy-Hb), such effects have not been found in conjunction with the hypothesised enhancement to cognitive performance in young, healthy samples. Furthermore, other vaso-active polyphenols such as cocoa-flavanols and fruit flavanones have demonstrated similar inconsistent cognitive benefits in healthy, young cohorts, despite a growing body of literature supporting their CBF effects.

This may suggest the high neurocognitive efficacy of the young, healthy populations employed in previous investigations maybe unable to benefit from the resveratrol-mediated increases in CBF, as such populations are acknowledged to be in the peak of their cognitive abilities and are already possess a sufficient supply of blood to meet demand. It therefore remains to be elucidated whether the vasodilatory effect of resveratrol is an appropriate mechanism to enhance cognitive performance. However, research has demonstrated that resveratrol is capable of enhancing O_2 extraction and utilisation during cognitive performance, while also being found to modulate mitochondrial aerobic respiration and cellular energy production. Examining the

metabolic consequences of resveratrol during high cognitive demand may provide support for the CBF mechanism of cognitive enhancement, as doing so may demonstrate the capacity of resveratrol to modulate the utilisation of the hypothesized heightened supply of neural substrates and / or a subsequent increase in cerebral metabolic output, which comes concomitant with relative increases in CBF.

Given the above, it is also noteworthy that the natural ageing process is associated with reductions to rCBF and subsequent cognitive deficits. It is proposed therefore, that the CBF effects of resveratrol may provide an increased utility in ageing populations, amplifying the delivery of neural fuel substrates and improving cognitive performance. Although, ageing is, of course, multifactorial. The observed reduction in rCBF is only one of many factors that lead to enviable cognitive decline in ageing humans. It is therefore purposed that a model of cognitive ageing will provide a clearer picture of how resveratrol-mediated increases in CBF can improve cognitive performance, before testing these effects directly in a healthy, ageing sample. Given the importance of O_2 to sustain cellular processes, the use of hypoxia has been suggested as a representative, experimental model for the ageing process. This may also be extended to a model of cognitive ageing, due to the relative decreases in delivery and utilisation of cerebral O_2 that occur from reduced rCBF during natural ageing. In further support, these reductions in O_2 have shown to induce deficits to aspects of cognitive performance which resemble those commonly found in naturally ageing cohorts. Thus, utilising a hypoxic model would demonstrate the link between reduced O_2 and poorer cognitive performance, whilst also confirming the CBF effects of resveratrol to attenuate these reductions to cognition directly.

The current aims of this thesis aimed to:

1) Examine the capacity in which resveratrol can impact metabolism and fuel utilisation during high cognitive demand (Chapter 3).
2) Establish the use of mild and moderate hypoxia as a representative model of the cognitive ageing process (Chapter 4 & 5 respectively), to mimic the role of decreased rCBF and subsequent $CMRO_2$ of a naturally ageing sample.
3) Assess the ability of resveratrol to augment CBF within hypoxia, to attenuate consequential reductions in cognitive performance due to a diminished O_2 supply (Chapter 4 & 5).
4) Assess if a single, acute dose of resveratrol can increase CBF and cognitive performance in a healthy, naturally ageing sample (Chapter 6).

Chapter 2: General methods, apparatus and procedures.

The current chapter will explain the general methods and apparatus employed in the following experimental studies. Any subsequent study specific materials or procedures will be expanded upon in the relevant chapter.

2.1 Near-Infrared Spectroscopy (NIRS)

Building on the blood oxygenation level dependent signal, NIRS is a non-invasive brain imaging technique, which exploits the differing diamagnetic and paramagnetic properties of haemoglobin, allowing for the measurement of cerebral hemodynamics within brain tissue. Human tissues are relatively transparent, but natural visible light (~450-700 nm) can only transverse to the depth of around 1 cm. In contrast, infrared light (~700-1000 nm) can travel up to 8 cm (Pellicer & Bravo, 2010), where the light is absorbed by chromphores (pigment compounds), namely blood haemoglobin. Working via a simple transmitter-receiver output, NIRS transmits an 'arced' ray of photons (see Figure 2.0). The subsequent calculation of the absorption and scattering of infrared light on return to the receiver, allows for the measurement of oxygenated (oxy-Hb), deoxygenated haemoglobin (deoxy-Hb) and total haemoglobin (total-Hb). The latter comprises the addition of the two former outputs, providing a proxy for total blood flow.

As NIRS data has been found to correlate strongly with that of fMRI (Strangman et al., 2002), it has been suggested that NIRS can be used as a suitable substitute for fMRI when investigating cortical brain activity associated with cognitive tasks (Cui et al., 2011). The relative changes in oxy-Hb and deoxy-Hb observed in the prefrontal cortex can show the relative changes in cerebral metabolic rate (Tamura et al., 1997; Jackson & Kennedy, 2013). As increased neural activity subsequently increases the requirement for O_2 to be metabolised, CBF consequently increases. The subsequent increase in CBF to match demand results in localised neural activation elevating concentrations of total-Hb and oxy-Hb (Obrig & Villringer, 1997) and, in turn, lowering deoxy-Hb concentrations due to increased cerebral oxygenation (Obrig & Villringer, 2003). This is a consequence of the CBF response to neural activation being greater than that of $CMRO_2$ (Leithner & Royl, 2014) and therefore, deoxy-Hb concentrations occupy a smaller fraction of overall blood flow.

With regards to cerebral haemodynamics and cognitive performance, increases in concentrations of oxy-Hb in the prefrontal cortex have been found to positively correlated

with superior (lower) reaction times during the Stroop task; this may suggest that an increased availability of O_2 could be related to improved cognitive performance (León-Carrion et al., 2008). Moreover, acute consumption of certain nutritional supplements has been observed to significantly increase concentrations of deoxy-Hb during cognitive performance whilst using NIRS. For example, Kennedy et al. (2010) found a single dose of 500 mg resveratrol significantly increased deoxy-Hb concentrations in comparison to placebo, during cognitive performance. Although no cognitive benefits were found alongside such findings, the researchers claimed this increase in deoxy-Hb indexed an enhanced O_2 extraction and utilisation. Interestingly, caffeine has been observed to significantly increase concentrations of deoxy-Hb in conjunction to improved performance on attention tasks (Dodd et al., 2015). Despite no direct correlation being made between the relative increases in deoxy-Hb and cognitive performance, the researchers did highlight that increased deoxy-Hb concentrations were only observed across the post dose task period and not the absorption period; supporting that this haemodynamic response may be indicative of increased neural activation.

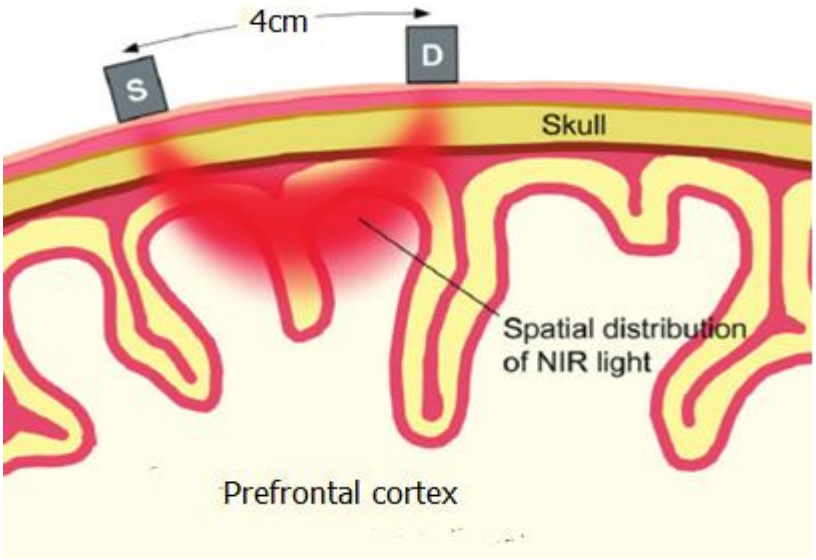

Figure 2.0 Demonstration of the infrared arc ray produced by NIRS. Working via a transmitter (source 'S') to receiver (detector 'D') output, NIRS transmits an 'arced' ray of infrared photons allowing for the absorption and scattering to be calculated on return to the receiver. This permits the continuous monitoring of oxygenated (oxy-Hb) and deoxygenated haemoglobin (deoxy-Hb) concentrations. The continuous wave (CW) NIRS employed for the current thesis, consisted of two pairs of transmitter – receiver outputs. These were separated from one another by 4 cm, placed across the forehead, measuring the hemodynamic response of both the left and right hemispheres of the prefrontal cortex (figure adapted from Phillips (2009)).

2.1.1 Continuous wave (CW) NIRS

All continuous wave (CW) NIRS devices operate by emitting light at a constant frequency and amplitude. The differences in light intensity on return to the receiver can detail the relative changes in concentrations of haemoglobin, through the modified principle of Beer-Lambert law[2] (Obrig & Villringer, 2003). The CW-NIRS device assumes that the degree of scatter and the differential path length (estimated distance travelled by the light) are both homogenous and fixed (Davies et al., 2016). CW-NIRS has been deemed sensitive enough to assess changes in cerebral haemodynamics within the prefrontal cortex in response to neural activity following supplementation with nutritional interventions (Jackson & Kennedy, 2013). To illustrate, caffeine, a well-established vasoconstrictor (Addicott et al., 2009) has been found to cause significant reductions in CBF when monitored by NIRS (Kennedy & Haskell, 2011). In contrast, previous intervention studies have demonstrated increased blood flow from vasodilators such as multivitamins (Kennedy et al., 2016). Of interest to the current thesis, previous research has also shown that resveratrol administration can increase both total-Hb and deoxy-Hb concentrations in the prefrontal cortex when using this method (Kennedy et al., 2010; Wightman et al., 2014). All of the above research supports the utility of CW-NIRS to monitor hemodynamic changes during cognitive tasks following treatment interventions in the current PhD thesis.

The Oxymon system (Artinis Medical Systems B.V.) was employed for the current PhD thesis. This NIRS system emits two nominal wavelengths of light (~765 & ~855 nm), modulated at 50 Hz, via a simple transmitter–receiver opitode headband. The headband consisted of two transmitter- receiver pairs (i.e. two channels), which were separated from each other by 4 cm and covered the full forehead; thus, measuring the hemodynamic response of both the left and right hemispheres (see Figure 2.0). This 4 cm distance between transmitter and receiver provides sufficient spatial resolution for a photo light path to penetrate to a depth of 0.5-2 cm (Fukui et al., 2003); which is sufficient to reach the blood vessels and capillaries of the prefrontal cortex (Haque et al., 1998). Although CW devices have been criticised for being influenced by superficial tissue, this may be due to lower separation differences and, therefore, shallower readings. The 4 cm

[2] The Beer-Lambert law assumes that light attenuation is proportional to the change in the concentrations of tissue chromophores (Kocsis et al., 2006).

separation employed here has been credited to sufficiently reach the cerebral ventricles and provide an accurate measure of cerebral oxygenation (Murkin & Arango, 2009).

As a reference point, each transmitter-receiver pair recorded data from an area of the prefrontal cortex that is inclusive of those corresponding to the international 10-20 system Fp1 and Fp2 electroencephalogram (EEG) positions. All results and outputs from the current device will be detailed as concentration change in micromoles per litre (μmol/L) for oxy-Hb, deoxy-HB, and total-Hb calculated using the modified Beer-Lambert law.

2.1.2 Frequency Domain Near-infrared spectroscopy (FD-NIRS)

Frequency domain (FD) NIRS measures both the specific light intensity attenuation and phase shift. The measurement of the latter allows for the quantification of the specific degree of light scatter in the tissue (Davies et al., 2016). Hence, the values are no longer fixed or assumed and so the FD-NIRS allows for 'absolute' concentrations of oxy-Hb and deoxy-Hb to be calculated (Fantini et al., 1999). It is noteworthy that both CW and FD-NIRS measurements have been found to be in good agreement with one another when monitoring brain oxygenation (Fantini et al., 1999). For example, Davies et al. (2016) compared the abilities of both CW and FD-NIRS in the detection of changes in cerebral tissue saturation in an experimentally induced incremental hypoxia (P_aO_2 at 60 & 40 mmHg respectively). The authors reported no significant differences were observed between saturation changes of either device in both hypoxic conditions.

The OxiplexTS Frequency Domain Near-Infrared Tissue Oximeter (model 99200), with OxiTS software version 3.1 was employed for the current PhD thesis. This NIRS instrument consists of two fiber-optic optodes, comprising light sources/ laser diodes (eight per channel) and a light detector for each. For each of the 8 channels of the laser diodes (modulated at a frequency of 110 MHz), four emit wavelengths of light at 690 nm and the remaining four 830 nm. Working by the multi-distance principle, each of the eight fibres were coupled (two per each wavelength) with each pair located at 2.0-, 2.5-, 3.0-, and 3.5 cm respectively from the detector. A light source is turned on and light passes from one emitter fibre, through the tissue and into the receiver. Once the first light source has been measured, this is turned off and the next light source is activated; this process repeats with each emitter. The FD-NIRS device was calibrated to the manufacturer's specified parameters before the start of each testing session.

The standard formula utilised by the current CW-NIRS is based upon the recommended formula by Duncan et al. (1995), and are valid only for measurements on brain tissue for 17-50 year old humans. Therefore, as the final experimental study employed 50-70 year old humans, the FD-NIRS was utilized over the CW device. Moreover, it is imperative that the CW device remains attached throughout the course of the full testing session, to ensure the detection of any potential treatment-related change in cerebral haemodynamic response is not masked upon the reattachment of the device. This is due to the device only measuring concentration change, which only reflects how haemoglobin concentrations have changed during a recording period. To illustrate, the removal and then the subsequent reattachment of the device post administration of treatment would fail to acknowledge the role of an already heightened treatment-related blood flow response, therefore nullifying any treatment related effects when changed from the baseline measure. Further, given the age of the older participants in the experimental chapter in question, and the extended testing sessions (~2 ¼ hours), it may have been uncomfortable to commit to an extended testing session without access to a comfort break. The measurement of absolute values naturally attenuates the above mentioned methodological constraint and allows for the removal and reattachment of the device without the loss of data and is therefore a more suitable choice in this case.

2.2 Indirect Calorimetry (ICa)

The consumption of nutrients to be metabolised by the body is essential in order to produce energy. The most common form of extracting the chemical energy potential of a substrate is to oxidise it into CO_2 and water (Ferrannini, 1988). Although the pure energy currency of a cell stems from ATP alone, the re-synthesis of ATP is most commonly ascertained by the oxidation of carbohydrates, fat, and protein. Measuring the rate and quantity of these substrates can provide insight into bodily metabolism and fuel oxidation (indicating at what capacity the body is working aerobically). Indirect calorimetry (ICa) is a non-invasive method which provides an accurate estimate of whole body energy expenditure (EE) and fuel substrate utilisation, through the measurement of inhaled O_2 and exhaled CO_2. Although changes in localised CBF may reflect the overall patterns of cerebral metabolic activity, they cannot quantify the extent of cerebral metabolic change, nor the relative change in EE as a whole (Al Naher et al., 2016). Thus, the overall rationale for the utility of ICa in the current thesis is to build upon the monitoring of CBF in the prefrontal cortex and provide insight into cerebral metabolism.

The use of ICa as a proxy for the measurement of cerebral metabolism is small but growing. Studies have found increased overall EE during performance of cognitive tasks (Seematter et al., 2000; Al Naher et al., 2016) and a shift in fuel utilisation during a cognitively demanding game of chess (Troubat et al., 2009). Al-Naher et al. (2016) concluded that ICa was sufficiently sensitive to detect even subtle differences on overall EE during cognitive demand and simple cognitive motor tasks, in healthy participants. The use of ICa during the measurement of cognitive performance following administration with nutritional interventions remains relatively novel however. Here one study found that ICa was capable of observing significant increases in both fat oxidation and total EE during cognitive performance, as a result of administration with a multivitamin supplement in comparison to placebo (Kennedy et al., 2016).

The utilisation of ICa in this thesis will add to the growing number of investigations which utilise it as a proxy for cerebral metabolism. Given that increased O_2 utilisation, EE and local blood-supply are closely related to neural activity and that resveratrol augments localised CBF in the prefrontal cortex, ICa provides an appropriate tool to further explore this concept. For the current PhD thesis, the on-line gas analysis Metalyzer 3B (Cortex, Leipzig, Germany) system was adopted to measure relative changes in pulmonary gases, in conjunction with MetaSoft Studio software. This high-resolution spiroergometric system, provides functional analysis of metabolism at rest and during exercise, breath-by-breath. Measurements of O_2 inhalation and CO_2 excretion were obtained through a face mask connected to the participant covering both the nose and mouth. The standard formula was employed to calculate total EE, carbohydrate and fat oxidation (Frayn, 1983).

2.3 Environmental chamber

In all chapters which employ the use of hypoxia, testing was completed within the normobaric environmental chamber at Northumbria University (Manufacturer: TISS, Model: Environment Simulation for Human Performance). This testing facility is a 20 m^2 laboratory (see Figure 2.1) capable of creating temperatures from −25 to 55ºC (± 0.1ºC), humidity of 10-98% (+ 0.1%) and of a maximum simulated altitude of 5800 m (fraction of inspired oxygen (F_iO_2) = 9.75%). The chamber was equipped with hypoxia silencers, an external compressor and an entrance lobby (from which entry and exit does not interfere with the environment setting of the chamber). The relevant conditions utilised by each study will be detailed in the relevant chapters.

It was hypothesized that the hypoxic environment induced by the chamber would act as a representative, reversible, experimental model of cognitive ageing. The rationale behind the employment of the environmental chamber was to induce the relevant hypoxic environment without the interference of extraneous factors outlined in section 1.6.7, such as environmental conditions (namely humidity and temperature). As air pressure lowers upon the ascent to altitude, temperature also decreases by an average rate of 1^{o}C every 150 m above sea level, in a process known as lapse rate (West et al., 2007; pp 32). As extreme temperatures have been shown to negatively affect cognitive performance (Taylor et al., 2016) the use of an artificial environment allows for control over this factor to neutralise any impact that would occur.

The use of an environmental chamber over other methods of artificially inducing hypoxia may provide a more effective method of blinding the participant to the differing environmental conditions. Indeed, a series of recent experiments have utilised a hypobaric chamber to achieve cognitive deficits; with some authors reporting that participants are unaware of their decline in performance whilst within a hypobaric chamber (Asmaro et al., 2013). Finally, although use of hypoxic gases (via a face mask) provides an alternative and effective method of inducing cognitive deficits (Turner et al., 2015), the additional constrains of wearing several items of restrictive equipment (i.e. neuroimaging) may potentially introduce physical and/or psychological noise into the data set.

Figure 2.1 An image of the environmental chamber at Northumbria University.

2.4 Computerised Cognitive tasks

The Computerised Mental Performance Assessment System (COMPASS) was developed by the Brain, Performance and Nutrition Research Centre at Northumbria University. This software was used to administer all cognitive and mood assessments across all four experimental studies. Responses to cognitive tasks were registered via Cedrus RB-530 response pads, with the exception of word recall and subtractions tasks, which were measured through pen and paper responses and keyboard entries respectively. The below tasks are reported to be sensitive to nutritional interventions (Kennedy & Scholey, 2000; Kennedy & Scholey, 2004; Reay et al., 2006) and (with the exception of the memory tasks) have been found to activate the prefrontal cortex (Drummond et al. 1999; Lawrence et al., 2002; Coull et al., 1996; Kazui et al., 2000; Jansma et al., 2000; León-Carrion et al., 2008). The inclusion of the memory tasks despite their inability to stimulate the prefrontal cortex is based upon the fact that both ageing and hypoxic conditions have been found to decrease aspects of memory performance (see sections 1.5.3 and 1.6.4). The relevant chapter will outline the tasks and the order in which they are presented in the cognitive demand battery, although all task descriptions will be detailed below for cross reference:

Immediate Word Recall – All the words utilized in this task were all proper nouns and between 4-9 letters long (e.g. ‘judge’, ‘beach’, ‘bowl’). Each ‘target’ word list generated for each participant was randomised along with a ‘decoy’ list of words (for the word recognition task) from a depository of words. All individual words were selected on the basis that there was no similarity of spelling between the other words chosen. For example, the word ‘LECTURER’ was removed from either list if the word ‘LECTURE’ was included. For this task, the twelve words of the target word list were individually presented on the screen with a display and interstimulus time of 1000 ms. Once all the words had been shown, the participants were given 60 seconds (an onscreen timer was provided) to recall as many of the words as possible on a separate sheet of paper. Upon completion, participants were instructed to turn over the sheet of paper. The task was scored for the number of correct and incorrect words submitted.

Delayed Word Recall – Participants were required to recall as many words as possible from those originally presented in the first immediate recall task. The participants were given 60 seconds (an onscreen timer was provided) to recall as many of the words as possible on a separate sheet of paper. Upon completion, participants were instructed to

turn over the sheet of paper. The task was scored for the number of correct and incorrect words submitted.

Delayed Word Recognition – This task required participants to respond to individually presented words. Each word was either part of the original presentation of words in the immediate recall task or a decoy. Participants responded to either 'no' (left) or 'yes' (right) to each word to indicate whether that word had been previously presented in the initial word presentation or not. The task was scored for the percentage of correct responses and reaction time (ms) for these correct responses.

Serial subtractions (3s, 7s & 17s) – Participants were presented with the standardised instruction screen, which informed the participant to continuously subtract the Serial number (either 3, 7 or 17) from a given starting number (between 800-999), as quickly and accurately as possible; using the numeric keyboard in front of them to enter each response. Upon giving their first answer, the starting number was then removed from the screen and their response was to be mentally retained. Sequential responses were then entered but were covered by an asterix. Pressing the enter key signalled the end of each response and cleared the three asterisks from the screen. With regards to incorrect responses, any subsequent responses were recorded as correct if they were accurate in relation to the new number. The duration of the Serial subtraction tasks is detailed in the individual chapters. The task was scored for the number of correct entries and the number of errors.

Rapid Visual Information Processing (RVIP) – This task required the participant to monitor a continuous sequence of single digit numbers (1-9) appearing individually on the screen in rapid succession (a rate of 100/min in pseudo-random order). Participants were required to respond to sequences of 3 consecutive even (e.g. 4-8-2) or 3 consecutive odd (e.g. 5-7-1) numbers by pressing the central key on their response pad. The task ran continuous for 2 minutes, with 8 correct target strings being presented in each minute. The task is scored for the percentage of correctly identified sequences, average reaction time for these responses (ms) and the number of false alarms (responding when a correct sequence was not present).

Choice Reaction Time (CRT) – An arrow pointing either LEFT (←) or an arrow pointing RIGHT (→) would appear on the screen individually, at irregular intervals (varying interstimulus interval of between 1 and 3.5 seconds). Participants were required to respond to each appearance of the directional facing arrow, using the corresponding

‘LEFT’ or ‘RIGHT’ buttons on the keypad in front of them, as quickly and as accurately as possible. Forty stimuli were presented in total per repetition of the task (lasting ~2 minutes). The task scored for the percentage of correct responses and the reaction time to the correct responses (in ms). Responses made in less than 150 ms of the presentation of the stimuli were not registered.

Stroop Task – Building on the original Stroop task (Stroop, 1935), four colour names (RED, YELLOW, GREEN, BLUE) would display individually on the screen written in a coloured font. Participants were required to make a response based on the colour font, rather than the written word depicted on the colour-coded response pad. Participants were shown a mixture of both congruent (name of colour and colour of ink the same) or incongruent (name of colour and colour of ink different) stimuli. Forty stimuli were presented in total per repetition of the task (lasting ~1½ minutes). Participants were asked to respond as quickly and as accurately as possible; responses made in less than 150 ms of the presentation of the stimuli were not registered. This task was scored for the percentage of correct responses (irrespective of congruence of the stimuli) and mean reaction time (in ms) for the correct responses.

3Back – A continuous sequence of letters (upper and lower case) appeared individually on the screen (45 in total), with a display time 500 ms and an inter-stimulus interval of 2.5 seconds. Participants were required to respond if the letter on screen was the same as that of the letter of 3 previous by pressing either ‘no’ (left) or ‘yes’ (right) on the response pad in front of them, as quickly and as accurately as possible. This task was scored for percentage accuracy and reaction time (in ms).

Visual analogue scale (VAS) – Each scale was employed to measure an individual mood parameter. Here, participants were initially presented with the adjective (e.g. ‘mental fatigue’) of the corresponding mood, before being presented with a 100 mm line anchored at either side, describing the two extremes of the specific mood being measured (e.g. ‘Not at all’ – ‘Extremely’). Participants were required to click (using a computer mouse) at a point on the scale which represented how they felt at that particular point in time. The individual mood domains measured by individual chapters will be detailed in the relevant method section.

2.5 Ethics

All studies in this thesis received ethical approval from the Northumbria University Psychology Department (within the faculty of Health and Life Sciences) Ethics Committee and were all conducted according to the Declaration of Helsinki (1964).

Chapter 3

The effect of resveratrol consumption on whole body metabolism during continuous high cognitive demand: A double blind, randomised, placebo-controlled investigation.

3.1 Introduction

Dietary polyphenols are found in abundance within plants. Their consumption has been associated with an array of health benefits in humans including reducing the risk of neurodegenerative disorders and CVD (Vauzour, 2017). The bioactivity of polyphenols is primarily attributed to their capacity to interact with various cellular signalling cascades, which regulate survival transcription factors, subsequent gene expression and/or protein synthesis (Spencer, 2010; Kennedy, 2014). Resveratrol (3,4',5-trihydroxy-trans-stilbene) a stilbene phytoalexin, has been found to bolster NO synthesis within human endothelial cells *in vitro*, via its ability to induce an increase in cytoplasmic Ca^{2+} levels (Elíes et al., 2011). Consequently, chronic resveratrol consumption has been found to lower blood pressure (Liu et al., 2015), improve endothelial function (Carrizzo et al., 2013; Wong et al., 2013) and lead to a reduced risked of CVD and its associated risk factors (Bonnefont-Rousselot, 2016). In addition, acute resveratrol administration has been found to promote vascular tone and induce endothelial vasodilation (Wong et al., 2011; Wong et al., 2013).

As eNOS is also the isoform found in the endothelium of cerebral blood vessels (Peterson et al., 2011) it is also responsible for the movement of blood through the cerebral arteries and veins supplying the brain, and, therefore, the modulation of CBF. The capacity of resveratrol to induce NO-dependent vasodilation has consequently sparked interest in its ability to facilitate cognitive enhancement. This, in part, is based on the premise that enhanced neurovascular coupling provides increased access to neural fuel substrates (namely O_2 & glucose), increasing cerebral metabolism through increased aerobic glycolysis. This is supported by a number of human trials which have shown that the administration of O_2 (Moss et al., 1998; Scholey et al., 1998; Chung et al., 2006) and glucose (Kennedy & Scholey, 2000; Scholey et al., 2001) alone, can enhance aspects of cognitive performance.

Kennedy et al. (2010) found acute oral resveratrol consumption (250 & 500 mg) induced dose dependent increases to CBF and superior O_2 extraction (indexed by higher levels of deoxy-Hb) during the absorption period and post-dose (+45-90 mins) cognitive battery. However, despite positive modulation of CBF and fuel utilisation, no cognitive effects were observed from oral resveratrol administration. Two follow-up studies also failed to

demonstrate any robust cognitive effects from acute (Wightman et al., 2014) or chronic (Wightman et al., 2015) resveratrol consumption. It is noteworthy that the above investigations all employed young, healthy participants, who may already be at the peak of their cognitive capacities (Rönnlund et al., 2005). Further, such young, healthy samples experience an elevated CBF response to a concentration higher than demand during heightened neural activity (Raichle, 2010); thus, any additional exaggeration of this CBF response by resveratrol may be surplus to requirement. This is supported further by findings of other vasoactive polyphenols, such as cocoa-flavanols, which have also shown mixed results in the improvement of cognitive performance in young, healthy samples (Socci et al., 2017) even when present in conjunction with increases in CBF (Francis et al., 2006; Decroix et al., 2016). It is unclear therefore, whether augmented CBF is a suitable mechanism to induce acute cognitive enhancement in a young, healthy cohort.

Despite its relative small size, the human brain accounts for approximately 11% of whole cardiac output, equating to ~20% of overall body metabolism at a resting state (Raichle, 2010). This continuous provision of CBF is to supply the large amount of O_2 and glucose required to meet demand. Therefore, increased neural activity is closely related to localised blood supply. However, the body tightly regulates the proportion of the fuel utilised by the brain. As neural fuel substrates are absorbed by the brain via facilitated diffusion (Takata et al., 1997) directly increasing the quantity of fuel (i.e supplementation with O_2 and or glucose) can naturally enhance fuel capacity. However, CBF during sustained neural activity is always greater than the rise in both $CMRO_2$ and ATP consumption (Raichle & Gusnard, 2002; Leithner & Royl, 2014). This may explain why the CBF mechanism of resveratrol thus far has been unsuccessful in improving cognitive performance.

However, the increased levels of O_2 extraction observed during task performance in previous investigations (Kennedy et al., 2010; Wightman et al., 2014) would suggest that resveratrol may also exert direct effects on mitochondrial aerobic respiration and cellular energy production. Indeed, resveratrol has been found to be an activator of the SIRT1-AMPK-PGC-1α pathway (Dolinsky & Dyck, 2014), which is a key regular of mitochondrial biogenesis and EE (Cantó & Auwerx, 2009). Consequently, resveratrol administration has been found to improve mitochondrial functioning, aerobic capacity and EE within rodent models (Baur et al., 2006; Lagouge et al., 2006). Although, measuring changes in the cerebral haemodynamic response only reflects the pattern of cerebral metabolic activity and does not quantify the extent of cerebral metabolic change

or cerebral EE (Al Naher et al., 2016). Consequently, resveratrol mediated increases in CBF and O_2 extraction observed during task performance (Kennedy et al., 2010; Wightman et al., 2014) does not necessarily detail whether this is facilitating cerebral metabolism and fuel utilisation directly.

ICa is a non-invasive technique which measures the inhalation and excretion of pulmonary gases, providing an accurate estimate of body EE. It is postulated that this method can provide a proxy of cerebral metabolism and examine the changes in EE and fuel utilisation during cognitive demand. Al Naher et al. (2016) found that ICa sufficiently detected subtle differences in cognitive demand between tasks with identical response requirements. In addition, Kennedy et al. (2016) utilised ICa to assess the metabolic consequences of acute and chronic supplementation of multivitamins and minerals in healthy women. The researchers found that acute supplementation with higher doses of multi-vitamins and minerals were capable of increasing fat oxidation and overall EE; which was further consolidated after 8 weeks supplementation. The lower dose of the vitamin and mineral supplement was found to modulate CBF after an acute dose. Here, significant increases in both total and oxy-Hb were also found throughout the post-dose task battery, 60 minutes post administration. Although no significant influences on cognition or mood parameters were found, the above supports the use of ICa to explore the potential benefits of resveratrol further.

Indeed, as the measurement of pulmonary gases that inform ICa are based upon the efficacy of O_2 transport and mitochondrial content/function, ICa should also be sensitive to nutritional supplements that increase blood flow, as both also provide important indication of an efficient cardiovascular system (Dolinsky & Dyck, 2014). Moreover, by examining the metabolic consequences of resveratrol during increased neural demand, this could provide further support for the capacity of resveratrol to modulate the hypothesized additional neural substrates that come concomitant with relative increases in CBF. The current chapter therefore aimed to examine of the ability of resveratrol to influence whole body metabolism (as a proxy for cerebral metabolism) and fuel utilisation during high neural demand.

To the best of this researcher's knowledge, no investigation to date has employed the novelty of ICa to examine the whole body metabolic profile of resveratrol administration during neural demand. It is hypothesized that an acute dose of resveratrol will amplify fuel utilization and whole-body EE during increasing neural demand. Given the novelty

of the current paradigm, the hypothesis for fuel utilisation will be left fairly open. It is noteworthy however, that metabolic rate and fuel utilisation during cognitive performance are dependent upon the immediate fuel available to the body. While, Troubat et al. (2009) found that the body switches from an initial higher carbohydrate (CHO) oxidation after an acute response to a cognitive demanding game of chess, to a more oxidative fuel supply (i.e. increased fat oxidation) when in a fasted state.

3.2 Method

3.2.1 Participants

Twenty-seven (15 male, 12 female) healthy participants were recruited for the current investigation (see Table 3.0 for demographics of the current sample). All participants self-reported themselves to be in good health and to have good or corrected vision. Participants were unable to take part if they had any food intolerances or sensitivities, or had any gastrointestinal, hepatic or renal diagnoses; this was to ensure no extraneous factors interfered with the absorption of the treatments. Participants reported themselves to have not suffered any neurological trauma (such as a head injury) or possess any neuropathologies. No participant reported themselves to be taking any herbal or pharmaceutical treatments (with the exception of the contraceptive pill & topical acne medication), whilst only consuming low to moderate amounts of caffeine (<6 cups of coffee / tea or its caffeine equivalent daily). Due to the requirements of the tasks, participants were excluded if they had dyscalculia (or any relevant learning difficulty). Participants were required to fast for 12 hours (except for water) and avoid strenuous exercise prior to each testing session. Each participant confirmed to still be in line with the study inclusion / exclusion criteria at the start of every testing session. They received £60 for their participation upon completion of the study.

Table 3.0 The mean and standard deviation (SD) of demographics for participants.

Parameters	Mean	(SD)
Age (years)	22.15	3.64
Stature (m)	1.74	0.10
Mass (kg)	69.30	14.03
BMI (kg/m^2)	22.71	2.39
Average Weekly Physical Activity (hours per week)	3.33	1.14
Average Daily Fruit and Vegetable intake (UK portions)	5.37	3.90

3.2.2 Cognitive Task Battery

Based on previous literature suggesting that more substantial cognitive demand is required in young, healthy samples in order to hinder ceiling ability, the current study adopted a demanding battery of tasks. In terms of CBF, neural fuel utilisation is also sensitive to increases in both time and difficulty of the task during cognitive performance

(Kennedy & Scholey, 2000; Fairclough & Houston, 2004). The cognitive battery for this chapter employed the Serial 3, 7 and a less utilized Serial 17 subtraction task. The use of Serial subtraction tasks has been reported previously to be cognitively demanding by participants and demonstrated to increase both glucose utilisation and heart rate beyond that of peripheral mechanisms (Scholey et al., 2001; Kennedy & Scholey, 2004; Kennedy et al., 2016). To avoid any task-time bias, the order of the tasks was counterbalanced in a Latin square. Each task lasted 5 minutes, thus each repetition lasted for 15 minutes in total. All tasks in each repetition of the battery were completed in a continuous fashion, in order to induce maximal cognitive demand and fatigue to the participants. Two visual analogue scales (VAS) were employed pre and post completion of all the Serial subtraction batteries, measuring self-reported 'Mental Tiredness' and 'Concentration'.

3.2.3 Treatments

Active treatments of resveratrol were acquired from megaresveratrol.net (Danbury, CT, USA). The integrity of the extract used was 99.73%, as verified by the manufactures statement. Treatments were administered on separate days, with each testing session being conducted no more than 48 hours before, and no more than 14 days after the previous session. During the three study visits, participants received three single-dose treatments in an order dictated by random allocation to a (Latin Square) counterbalanced order. Two capsules were consumed on each testing day either:

1) ×2 placebo capsules (CABOT MP5 fumed silica).
2) ×1 250 mg dose of resveratrol with ×1 placebo.
3) ×2 250 mg resveratrol capsules (equating to a 500 mg dose).

All treatments were administered within the same vegetarian soft-gel capsules. All treatments were counterbalanced and were prepared by the lead investigator, then coded by a third party who had no further involvement in any aspect of the study. No member of the investigational team was aware of the contents of the capsules until completion of the statistical analysis. This was the procedure for all subsequent studies of this thesis.

3.2.4 Indirect Calorimetry (ICa)

For the current investigation, inhaled and expired pulmonary air was measured using an on-line analysis system (Metalyzer 3B, Cortex, Leipzig, Germany). The standard formula was employed to calculate EE, CHO and fat oxidation (Frayn, 1983). The gas analysis system was attached to the participants via a facemask, which covered both the nose and mouth, connected by falconia tubing.

3.2.5 Procedure

Participants were required to attend the Brain, Performance and Nutrition Research Centre at Northumbria University on four separate occasions. The first of these visits to the laboratory was to screen the participant against the inclusion / exclusion criteria and familiarise them with the cognitive demand battery. Here, the participants completed 2 full repetitions of the task battery to achieve an appropriate level of competency for the tasks. The following three testing visits were identical to each other: participants arrived at 8 am, fully fasted (consuming nothing but water) for 12 hours prior to the session; this was to restrict any influence from prandial factors. Participants were also required to refrain from engaging in any strenuous exercise that morning. Upon arrival, each participant was asked to confirm they were still compliant with the inclusion criteria before testing began. Following confirmation, participants were connected to the gas analysis equipment via a facemask and mouthpiece. The mask was adjusted to ensure there were no gaps, and was fitted to the participant's comfort. Participants then remained at rest for 10 minutes, watching a nature documentary (BBC's Planet earth). After, participants completed a pre-dose baseline measurement of the Serial subtraction cognitive demand battery and mood scales. Upon completion, participants were then disconnected from the gas analysis equipment and then administrated with their treatment for the day, before continuing to rest for a further 35 minutes.

Participants were then reconnected to the gas analysis device and completed a post-dose rest period for 10 minutes whilst continuing to watch the documentary. This measure was included in response to the blood flow effects of resveratrol being observed at thirty-five to forty-five minutes, post administration at rest (Kennedy et al., 2010; Wightman et al., 2014), while also coinciding with pharmacokinetic data which has detected resveratrol in plasma metabolites from approximately 30 minutes (Walle, et al., 2004). After establishing a post-dose rest measure, the participant then completed the first of the post-dose mood scales and cognitive demand battery. The participants were then required to complete the battery and VAS scales at further 2-hour and 3-hour post administration time points. This was due to the half-life of resveratrol being noted up to 3 hours (Almeida et al., 2009). ICa measures were taken throughout all task performances (Figure 3.0).

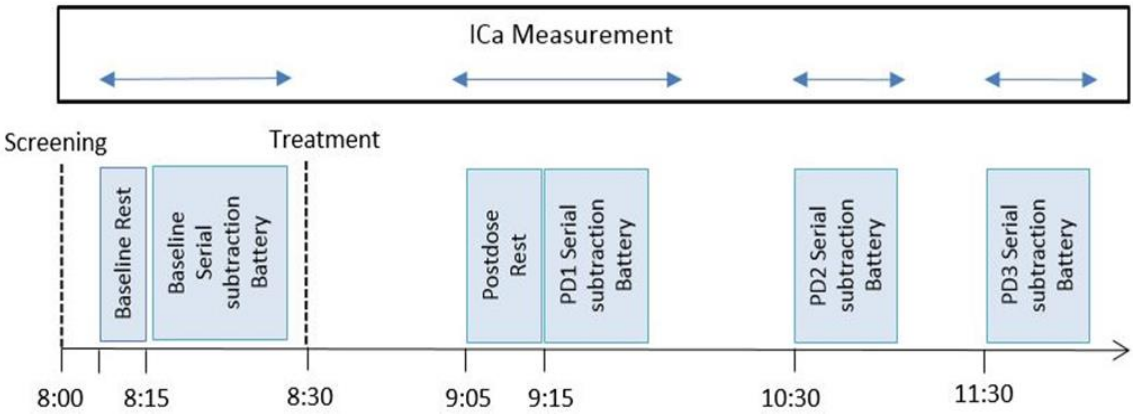

Figure 3.0 Timeline for study 1 testing day

3.3 Statistical approach

All ICa analysis was completed within Minitab 17 Statistical Software (State College, PA: Minitab, Inc.), while all behavioural data was analysed in SPSS version 22 for windows (IBM SPSS Statistics Armonk, NY).

3.3.1 Main ICa analysis

Raw data was extracted and averaged across 1-minute epochs for each task within the 3 individual post dose repetitions. Each of the five, 1-minute epochs were first cleaned before being condensed and averaged to represent a 5-minute average time point. Here data was first graphed to highlight potential extreme values or large inconsistent shifts in overall patterns. This cleaning process led to the removal of two full data sets and a further set was not included due to data catchment errors (missing baseline performance). The final sample for ICa analysis was N=24[3] (post-dose rest analysis data N=23).

All remaining ICa data during cognitive demand was converted to change from baseline, utilising the relative baseline task performance from each task. Analysis consisted of 3 × 3 × 3 within-subjects' ANOVAs assessing Treatment (250 mg resveratrol, 500 mg resveratrol & placebo) × Task (Serial 3, Serial 7 & Serial 17 subtractions) × Repetition (×3). Subsequently, planned comparisons were conducted upon the emergence of significant interaction effects, utilising the mean square (MS) error values from the initial ANOVA. Only comparisons to placebo and not between active treatments were

[3] The novelty of the study meant there was no directly comparable data for calculation of an appropriate sample size. Therefore, a sample size was based upon the power analysis of the 3-prior resveratrol / NIRS studies and was also used throughout the following studies of this thesis.

conducted. With regards to the analysis of the post dose rest measure, an overall average was created for the 10-minute period, before a series of one-way ANOVAs were conducted to assess difference between the two resveratrol treatments against placebo.

3.3.2 Behavioural analysis

Raw cognitive data was cleaned before being converted into a change from baseline measure (N=26). Raw baseline scores for each task were then analysed to assess if there was any difference between treatment baseline scores. Following this, nine within-subjects' ANOVAs investigating Treatment (500 mg resveratrol, 250 mg resveratrol & placebo) × Repetition (×3) × Task (3s, 7s & 17s) were conducted, followed, where appropriate, by Bonferroni corrected post-hoc pairwise comparisons. Only significant treatment related results will be reported from the cognitive analysis.

Concerning mood analysis, scores for 'concentration' and 'mental fatigue' were converted to change from baseline (the score submitted following completion of the baseline battery of cognitive tasks was used as a baseline). Two within-subjects' ANOVAs investigating Treatment (500 mg resveratrol, 250 mg resveratrol & placebo) × Repetition (×3) × Time (pre-completion & post-completion of the cognitive battery) were conducted. Again, this was followed up with Bonferroni corrected post-hoc pairwise comparisons on the presentation of a significant F test. Only significant treatment related results will be reported, with the exception of any main effects of time; this was reported to provide support for the claim that the cognitive battery was 'cognitively demanding'.

3.4 Results

3.4.1 ICa data for task performance

Total energy expenditure (EE)

The repeated measures ANOVA revealed no significant of treatment or treatment related interaction on EE during cognitive performance.

Carbohydrate (CHO) oxidation

The within-subjects' measures ANOVA revealed no significant of treatment or treatment related interaction on CHO oxidation during cognitive performance.

Fat oxidation

The ANOVA revealed no significant of treatment or treatment related interaction on fat oxidation during cognitive performance.

Respiratory exchange ratio (RER)

The within-subjects' ANOVA demonstrated a significant treatment × task × repetition interaction [$F(8, 184) = 1.13$, $p = .039$] indicating a significant treatment related change in fuel utilisation during task performance. Follow up planned comparisons demonstrated that administration of 250 mg resveratrol evinced a significant increase in RER during Serial 3 ($p = .002$) and an increase nearing significance[4] during Serial 7 ($p = .085$) and Serial 17 ($p = .068$) subtraction performance during repetition 1. Moreover, administration with 250 mg resveratrol significantly increased RER during Serial 3 ($p = .001$) and 7 ($p = .001$) task performance during repetition 3 in comparison to placebo. However, the same dose was found to induce a significant reduction in RER during Serial 17 task performance ($p < .001$) at the final repetition in comparison to placebo. In addition, administration with 500 mg resveratrol evinced an increase in RER across all tasks and repetitions in comparison to placebo. These were found to be significant during Serial 3 and 7 subtraction performance at repetition 1 (S3: $p = .029$; S7: $p = .018$) and 3 (S3: $p = .003$; S7: $p = .001$), whilst trending an increase in RER during Serial 3 ($p = .061$) and 17 ($p = .066$) subtraction performance during repetition 2 relative to placebo (Figure 3.1).

[4] Trending or nearing significance is highlighted when the p value is between >.05 and ≤.09. This was the case for the rest of the thesis.

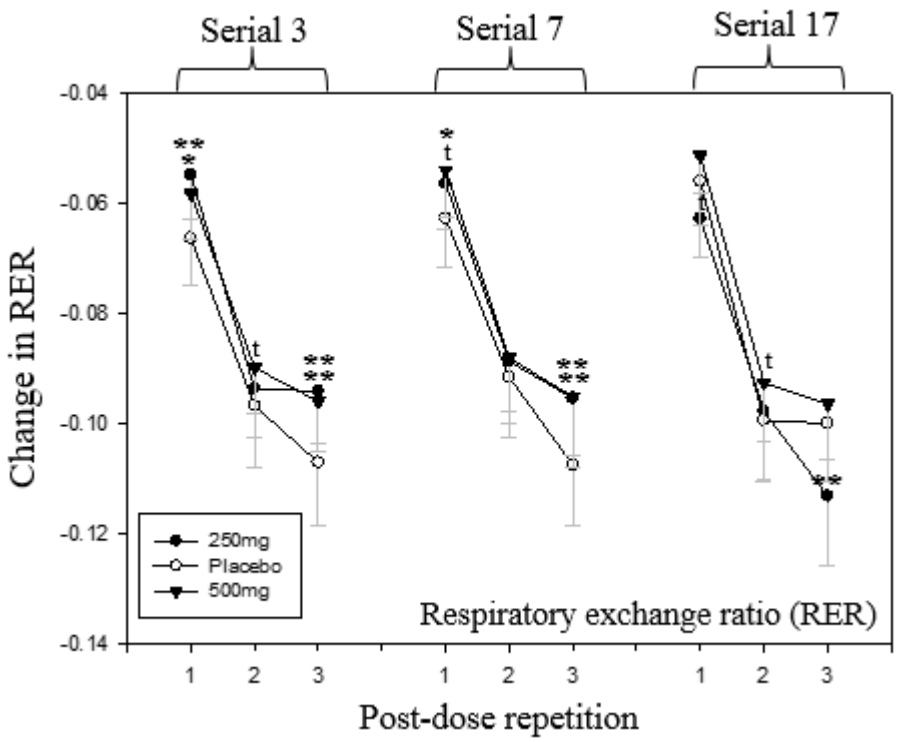

Figure 3.1 The acute effects of 250 mg and 500 mg resveratrol on respiratory exchange ratio (RER). Graph depicts mean changes in change from baseline RER (with SEM error bars), during the three repetitions of the post-dose tasks in 24 healthy adults following 500 mg resveratrol, 250 mg resveratrol or placebo. *p <.05, **p <.01 and t – trend (nearing significance).

The proportion (%) of carbohydrate oxidation to overall energy expenditure.

The omnibus ANOVA revealed a significant treatment × task × rep [F (8, 184) = 2.14, p = .034] interaction in percentage change in CHO oxidation during post-dose performance. With regards to follow up planned comparisons, administration of 250 mg resveratrol demonstrated a significantly higher percentage CHO of total EE during Serial 3 subtractions at repetition 1 (p =.006) in comparison to placebo. Similarly, the same dose showed significant increases in CHO of total EE during Serial 3 (p =.004) and 7 (p =.004) subtractions but evinced a significant decreased during Serial 17 (p =.001) subtraction performance during the final repetition in comparison to placebo. The 500 mg dose of resveratrol consistently increased the percentage CHO of total EE across all tasks at all 3 repetitions, relative to placebo. These increases were found to be significant during Serial 3s and 7s at repetitions 1 (S3: p =.044; S7: p =.020) and 3 (S3: p =.007; S7: p =.001) and trending during repetition 2 for Serial 3 (p =.077) and 17 (p =.082) subtractions (Figure 3.2).

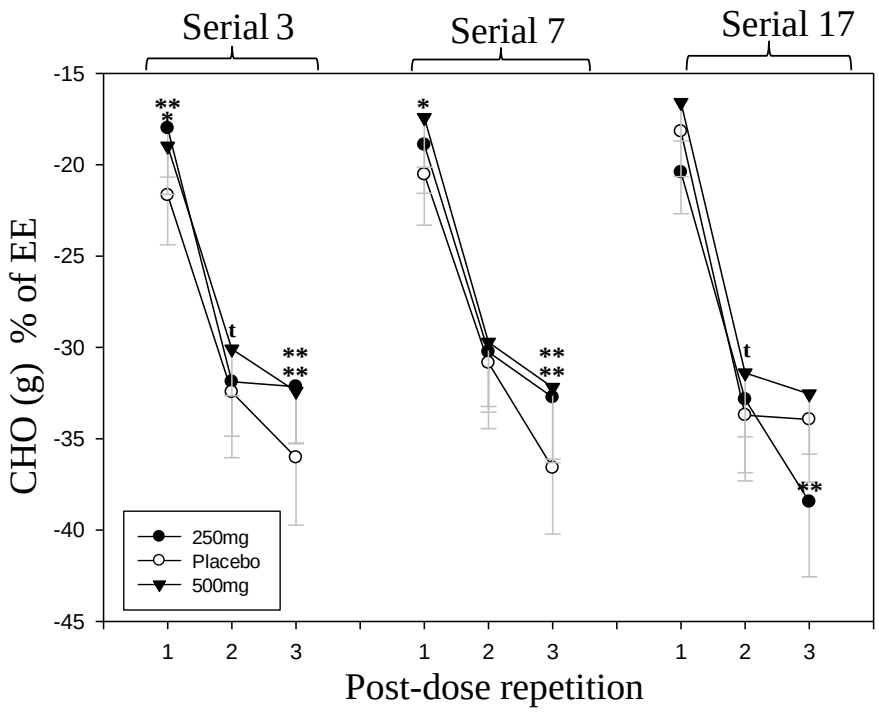

Figure 3.2 The acute effects of 250 mg and 500 mg resveratrol on the proportion of carbohydrate oxidation (g) making up overall energy expenditure (% EE). Graph depicts mean changes in the percentage of carbohydrate oxidation (with SEM error bars), during the three repetitions (rep) of the post-dose tasks in 24 healthy adults following 500 mg resveratrol, 250 mg resveratrol or placebo. *p <.05, **p <.01 and t – trend (nearing significance).

3.4.2 ICa Post dose rest

The series of one-way ANOVAs revealed no significant treatment related differences during the 10-minute rest period post administration for any of the metabolic outputs (EE, CHO oxidation, Fat oxidation or RER).

3.4.3 Behavioural Data

Baseline analysis – Prior to any analysis, a series of one-way ANOVAs were conducted on baseline scores for each cognitive task outcome and the two mood sub-measures. No significant differences were found between any of the baseline scores for any of the mood or cognitive outcomes ($p > .10$).

3.4.3.1 Mood Analysis

No significant treatment related or treatment interaction effects were found on either self-reported concentration or mental tiredness. However, a significant main effect of time was found for mental tiredness [$F(1, 25) = 10.711$, $p = .003$], where by post completion of the cognitive battery ($M = -1.31$) lead to significantly higher self-reported mental tiredness in comparison to scores reported prior to completion ($M = -7.84$). Mood data is reported in full in Appendix 1.0.

3.4.3.2 Cognitive Performance

No significant cognitive results of treatment or treatment related interactions were found in the current study. Cognitive performance data is reported in full in Appendix 1.1.

3.5 Discussion

The aim of the current investigation was to build upon the small, but growing literature surrounding the efficacy of resveratrol as a cognitive enhancer in healthy, young humans (Kennedy et al., 2010; Wightman et al., 2014; Wightman et al., 2015). Previous investigations have demonstrated resveratrol-mediated increases in CBF and O_2 extraction during cognitive performance. However, such modulation has failed to result in any subsequent improvements to performance. It remains unclear whether augmented CBF and O_2 extraction is capable of cognitive improvements in young, healthy humans. The current study employed ICa to provide a proxy for cerebral metabolism and neural fuel utilisation in order to elucidate the metabolic profile of resveratrol under high cognitive demand[5].

The current study observed a significant (treatment × task × repetition) interaction effect for change in RER across post dose task performance. RER provides insight into predominant fuel oxidation, with higher RER values suggesting a greater utilisation ratio of CHO to fat (Simonson & DeFronzo, 1990). Follow up analysis revealed that administration of both doses of resveratrol induced significant increases in RER; although only the 500 mg resveratrol demonstrated consistently higher RER across all tasks and repetitions in comparison to placebo. In addition to increases in RER, a single dose of 500 mg resveratrol was found to subsequently increase the proportion of CHO oxidation generating overall EE during Serial 3 and 7 subtraction performance, 45 minutes and 3 hours post administration. Together these results highlight that resveratrol can induce a subtle, yet significant, increase in the oxidation of glucose during cognitive performance. However, no cognitive benefits of resveratrol were observed in the current study.

In the absence of a somatic control, it is difficult to distinguish whether the aforementioned metabolic effects of resveratrol were influenced further by the varying levels of difficulty of the individual Serial subtraction tasks. As the artefact of typing rates varies for the Serial subtraction tasks (i.e. higher number of attempts during Serial 3s to Serial 7 etc.), this muddies the water of current paradigm, making it uncertain if the resveratrol-mediated changes in metabolism was task dependant. It should be noted however, that there was no significant increase in the total number of responses attempted between any of the three treatment conditions for any of the individual Serial subtraction

[5] The concept of the cognitive task battery being 'cognitive demanding' is supported by the significant increase in self-reported mental tiredness registered by the participants following the completion the battery compared to before undertaking it.

tasks (see Appendix 1.1). This suggests that the shift in fuel oxidation of the 500 mg resveratrol was not mediated by additional somatic movements. In sum, a single dose of resveratrol can increase oxidation of CHO during high neural demand. Such findings clarify that acute oral resveratrol administration can increase the uptake / utilisation of available fuel substrates in the young, healthy populations; although, as anticipated, this increase in metabolic activity has not been found in conjunction with cognitive enhancement in a cohort with a hypothesized high neurocognitive ability.

In the absence of significant modulation to cognitive performance by any treatment, it is unclear whether the resveratrol-mediated modulation of metabolism observed here has been beneficial. Currently, only two investigations have observed significant modulation of RER in conjunction with improved cognitive performance. However, these studies have revealed opposing results; reporting a significant increase (Delistraty et al. 1991) and a significant decrease (Troubat et al., 2009) in RER during task performance respectively. However, as CHO oxidation produces a (15%) greater ATP yield in comparison to fat, higher CHO oxidation has been suggested to represent an O_2-saving strategy, to economize O_2 when performing energy-demanding tasks in environments/events of lower O_2 (Schippers et al., 2012). This may suggest the effects of resveratrol are more beneficial in populations that suffer a reduction in O_2 metabolism.

The lack of cognitive improvements observed in the current study coincide with those in previous investigations (Kennedy et al., 2010; Wightman et al., 2014; Wightman et al., 2015) and suggests that the young, healthy sample employed here are likely unable to benefit resveratrol administration. As the CBF response to sustained neural activity is always greater than that of the rise in both $CMRO_2$ and ATP consumption (Raichle & Gusnard, 2002; Leithner & Royl, 2014), any further modulation of this process may be redundant. In addition, this population may be in the peak of their cognitive capacities (Rönnlund et al., 2005) leaving little scope for cognitive improvement by resveratrol. In contrast, the natural ageing process is associated with significant reductions in cognitive performance (Peters, 2006; Glisky, 2007; Salthouse, 2010; Harada et al., 2013). This, in part, is due to the diminishing efficacy of the human vasculature, which results in the supply and $CMRO_2$ becoming increasingly compromised with age. It is possible therefore, that the vaso-metabolic effects of resveratrol will be of increased utility to naturally ageing populations who suffer a reduction in maximal O_2 uptake and O_2 metabolism.

Chapter 4

The cerebral hemodynamic response and cognitive effects of acute resveratrol administration in young, healthy adults in hypoxic and normoxic conditions: a model for the ageing brain

4.1 Introduction

The results of Chapter 3 demonstrated that a single 500 mg dose of resveratrol was capable of significantly shifting fuel utilisation towards higher glucose oxidation during cognitive demand. However, as this increase in metabolism was not found in conjunction with any improvements in cognitive functioning, it was proposed that resveratrol may be unable to provide consistent cognitive enhancement to young, healthy samples due to their already heightened neurocognitive ability. It was suggested therefore, that the CBF and metabolic effects of resveratrol may be of increased utility in cohorts that suffer a reduction in cognitive performance and /or cerebral metabolism, such as natural ageing populations.

As the brain cannot store its own energy source, it requires a continuous provision of CBF to provide the large quantity of neural fuel substrates to meet demand. Consequently, a tightly regulated relationship between CBF and cognitive functioning exists, whereby even a small comparative decline to blood flow can negatively impact cognitive performance (Duschek & Schandry, 2007; Poels et al., 2008). Given that the natural ageing process leads to an observable reduction in rCBF (Martin et al., 1991; Kalaria, 2009; De Vis et al., 2015) and a subsequent decrease in $CMRO_2$ (Aanerud et al., 2012; Chen et al., 2011), it is perhaps of no surprise that the age-related deterioration of the cerebral vasculature has been shown to be a significant contributor to cognitive decline (Celsis et al., 1997). It is noteworthy however, that the rate of reduction in CBF is not uniform across the brain, with subcortical regions remaining relatively unchanged (Chen et al., 2011) while areas such as the prefrontal cortex appear to be more notably affected (Lu et al., 2010).

The ability of resveratrol to promote an increase in both CBF and O_2 extraction within the prefrontal cortex (Kennedy et al., 2010; Wightman et al., 2014; Wightman et al., 2015) may present a viable method with which to attenuate age-related reductions in rCBF and, in turn, cognitive performance. Other vasoactive polyphenols such as cocoa-flavanols, have already been found to induce increases in cerebral perfusion in older cohorts (Sorond et al., 2008; Sorond et al., 2008; Sorond et al., 2010; Brickman et al., 2014; Lamport et

al., 2014). Further, a small but growing number of controlled intervention studies have demonstrated improvements to aspects of cognitive functioning and cardiovascular measures following chronic cocoa-flavanol administration in older adults (Mastroiacovo et al., 2015; Desideri et al., 2012; Brickman et al., 2014). Although evidence regarding the efficacy of acute doses and the simultaneous measurement of both CBF and cognition is lacking, the growing cocoa-flavanol research in elderly samples may lend support to the argument that resveratrol has the capacity to increase cognitive performance in naturally ageing cohorts, via augmented CBF.

However, age-related cognitive decline is, of course, multifactorial. Individual variability in biological, psychological, and lifestyle factors, are all likely to contribute to differing levels of cognitive performance in ageing populations (Harada et al., 2013). Indeed, the brain undergoes a series of other physiological changes in conjunction with observed reductions to rCBF. For example, varying levels of regional atrophy, increased neuroinflammation and declining gluco-regulation have all been shown to contribute to declining cognitive functioning with age (Peters, 2006; Cevenini et al., 2013; Lamport et al., 2009). Research has also found 'cognitively intact' humans up to 100 years of age (Perls, 2004); demonstrating the diversity of individual variability that can take place within 'normal' ageing. The challenge here, therefore, consists of identifying any potential cognitive benefits that may be the direct result from resveratrol-mediated attenuation of reductions in rCBF and $CMRO_2$.

A diminishing efficacy of the human vasculature during ageing alters the diffusion of O_2 at the capillary level. Thus, ageing is commonly characterized by a decrease in O_2 supply (Valli et al., 2015). Consequently, the use of hypoxia has been suggested as an experimental model sufficient for studying ageing processes (Cataldi & Di Giulio, 2009). As the brain has a particular susceptibility to a reduction in O_2 supply (Larson et al., 2014), the current study therefore proposes that the disruption of O_2 availability in young, healthy samples, may serve to induce cognitive impairments which will resemble those experienced as part of the ageing process. In support, a series of reviews have revealed that exposure to acute (altitude induced) hypoxia can induce observable, yet reversible, reductions in cognitive performance in humans (Petrassi et al., 2012; McMorris et al., 2017). Indeed, poorer attention, reaction time and error rates have all been found during exposure to altitude induced hypoxia (Davranche et al., 2016; Petrassi et al., 2012). This is particularly interesting given that these specific cognitive domain reductions are also

observed in natural ageing populations (Glisky, 2007; Harada et al., 2013), offering a direct comparison between populations.

There is some ambiguity regarding the level at which cognitive deficits begin to occur during hypoxia. Despite no consistent cognitive deficits having been reported below 3000 m altitude (Bahrke & Shukitt-Hale, 1993) many investigations have adopted varying durations of exposure to the hypoxic conditions. Instant exposure to hypoxia ensures minimal adaptations to the body, suggesting that cognitive deficits may be more apparent within the initial stages of exposure. Therefore, the use of mild hypoxia (< 3000 m stimulated altitude) is still warranted if it is employed in a short and immediate bout, to enable maximal deficits and minimal adaptation to take place (Petrassi et al., 2012). Given the novelty of the current paradigm, it is proposed that this concept should be explored initially at a mild hypoxic level (equivalent to 16% F_iO_2) to assess if even small reductions in cerebral oxygenation can disrupt cognitive performance.

The current study aimed to ascertain whether the use of acute hypoxia could be employed to model the cognitive impairments experienced during the natural ageing process. In addition, the current chapter aimed to assess whether oral resveratrol administration was capable of attenuating the subsequent reductions in cognitive performance, evinced by exposure to the proposed hypoxic model (in a young, healthy cohort). It is anticipated that the previously observed increases to oxy-Hb and deoxy-Hb in the prefrontal cortex (Kennedy et al., 2010; Wightman et al., 2014; Wightman et al., 2015) induced by resveratrol, will be replicated here irrespective of the environmental condition. Although hypoxia can induce either hypocapnia mediated vasoconstriction (Raichle & Plum, 1972) or hypercapnia mediated vasodilation (Vavilala et al., 2002), this is not anticipated to influence the outcome of the current study. Given that resveratrol can bolster NO production and improve NO bioavailability (Xia et al., 2010), this should allow for a greater exaggeration of CBF during exposure to acute hypoxia irrespective of hypoxia induced vasodilation or vasoconstriction. Consequently, as a result of the increases to CBF, it is predicted that such effects will subsequently lead to increased cognitive performance within the hypoxic condition.

4.2 Method

4.2.1 Participants

Twenty-four adults (8 males, 16 females; mean age = 22, range = 18-34) with good or corrected vision were recruited for the current study. All participants reported themselves to be in good health and did not suffer from any food intolerances / allergies or digestive problems, which may interfere with the supplementation and absorption of the treatment. No participants reported themselves to be using any social drugs (including tobacco), medication (excluding the contraceptive pill), or supplementing with any herbal / food extracts; they also confirmed to consume <6 cups of caffeinated tea / coffee a day (or the 450 mg caffeine equivalent). Participants were also screened for any neurological or neuro-developmental disorders and were unable to take part if they had suffered a head injury. Given the nature of the current study, participants also confirmed that they had not lived at an altitude of 2000 m or above in the last 3 months; this was to avoid any advantageous adaptations (such as exaggerated erythropoiesis). Moreover, as the cognitive tasks utilised here have not been validated on non-native English speakers, all participants reported to have English as their 1st language.

Participants arrived at the laboratory on testing days fully fasted from liquids (except for water), food and having had no alcohol for least 24 hours, whilst confirming that they were still in compliance with the exclusion criteria. A fasted state was considered a more appropriate protocol, given the unknown effects of individual foodstuffs on resveratrol consumption.

4.2.2 Treatments

During the four testing study visits, participants received a single-dose treatment in compliance with a randomised allocation to a (Latin Square) counterbalanced order. Treatments were administered on separate days, with each testing session being conducted no more than 48 hours before, and no more than 14 days after, the previous session. Two capsules were consumed by participants on each testing day being either:

1) ×2 placebo capsules (CABOT MP5 fumed silica).
2) ×2 250 mg resveratrol capsules (equating to a 500 mg dose).

Again, all treatments were prepared by the lead investigator, before being coded by a third party who had no further involvement in any aspect of the study. No member of the

investigation team was aware of the contents of the capsules until the completion of the statistical analysis.

4.2.3 Near-Infrared spectroscopy (NIRS)

The functional NIRS employed for this study was Oxymon system (Artinis Medical Systems B.V.). The NIRS headband was worn for the full duration of all four of the testing sessions. Markers were inserted throughout the recording of the NIRS data to time stamp specific epochs corresponding to individual cognitive tasks. Data collected during the memory tasks was excluded from the analysis due to artefacts caused by the physical movement associated with the paper and pencil responses.

4.2.4 Environmental chamber

All testing for the current study was completed within the environmental chamber at Northumbria University. An environment with 16% atmospheric F_iO_2 (2134 m above sea level), 40% humidity and a temperature of 23^oC was simulated for the hypoxic condition. The normoxic condition adopted the same humidity and temperature but employed an F_iO_2 level imitating sea level (20.93% available O_2). All baseline measures were conducted at normoxia and the relevant O_2 status was set / initiated at the beginning of the absorption period. For testing sessions in the normoxic condition the O_2 status remained unchanged.

4.2.5 Cognitive tasks

A cognitive battery of tasks was delivered via COMPASS and consisted of seven tasks. With the exception of the memory tasks (which were omitted from the NIRS analysis), all other tasks have shown in previous neuroimaging studies to activate the prefrontal cortex (Drummond et al. 1999; Lawrence et al., 2002; Coull et al., 1996; Kazui et al., 2000). The battery lasted approximately 12 minutes and was employed to tax the participant sufficiently to increase demand for neural substrates. The cognitive battery comprised (in order of appearance): Immediate word recall, Serial subtractions (3 & 7), RVIP, 3back, Delayed word recall and Delayed word recognition.

4.2.6 Procedure

Participants were required to attend the laboratory on five separate occasions. The first of these visits was a familiarisation session, which involved briefing the participant and

receiving their informed consent before screening them against the exclusion criteria. Training on the individual tasks in the cognitive battery was then provided to ensure the participants were competent and confident to complete all tasks on the battery without assistance; here, participants completed two full repetitions of the cognitive battery. The remaining 4 visits were testing sessions, in which participants arrived at the testing facility at 8 am, fully fasted for 12 hours (except for water). Upon arrival, participants were screened to ensure that they still complied with the exclusion criteria, before taking a brief rest. Participants were then led to the environmental chamber and connected to the NIRS device; this was worn throughout the full testing procedure. A baseline measurement of the cognitive battery was then completed and followed immediately by a 10-minute rest (while watching a non-stimulating video). The treatment for the day was then consumed. At this point, the chamber was then set to the appropriate O_2 level (blind to the participant) and the participant remained at rest for a further 45 minutes (continuing with the video) to allow for adequate absorption. The participant was then required to complete 3 full series of the cognitive battery consecutively (Figure 4.0).

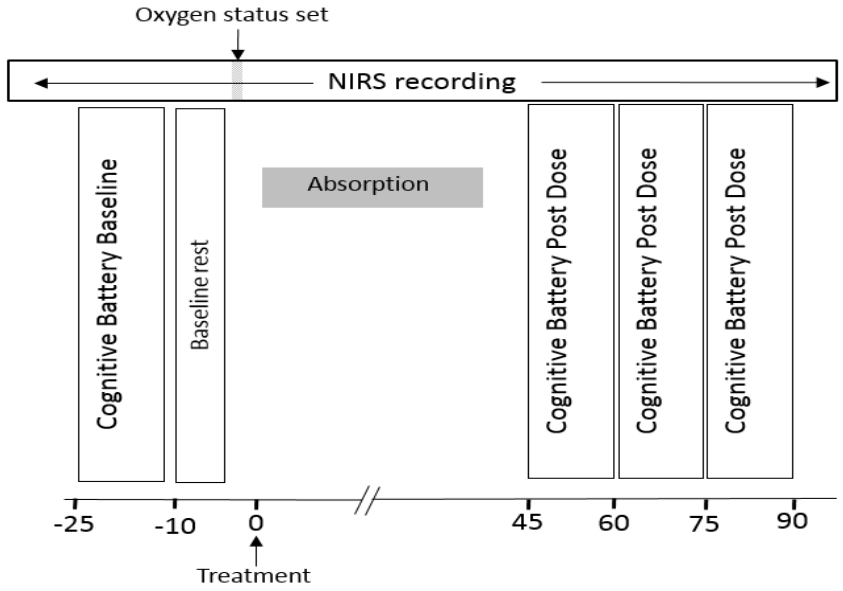

Figure 4.0 A timeline of the second experimental study testing day. A baseline measure of the cognitive tasks was first established. After completing a 10-minute rest period, treatment was administered before participants rested for a 45-minute absorption period. Participants then completed 3 repetitions of the cognitive battery. Near-Infrared spectroscopy (NIRS) was worn for the full session and data collected throughout. All NIRS data was baseline adjusted from the first minute of the absorption period.

4.3 Statistical Approach

All behavioural data was analysed using SPSS version 22 for windows (IBM SPSS Statistics Armonk, NY), while all NIRS analysis was analysed using Minitab 17 (State College, PA: Minitab, Inc.).

4.3.1 Main NIRS analysis

All raw data was first graphed (prior to any analysis) to determine the existence of potential outliers (prior to any analysis or un-blinding of treatments). In the first instance, a ±2.5 standard deviation of the mean was applied to the data to provide a rough guide to identify outliers, yet the removal of data was left to the researcher's discretion. Although an arbitrary measure, this allowed for a certain amount of flexibility to permit for individual differences in natural CBF levels. This data clearing method was applied to all NIRS data in all studies within this thesis; with the means and standard deviations calculated for each individual study. After the removal of errors and outliers, the final sample for NIRS analysis was N=21. In the first instance, NIRS data was analysed with hemisphere (left & right) as a factor, although, no effect of hemisphere was found here or in any other chapter in this thesis. Therefore, the data from both the left and right hemispheres were combined and averaged for analysis. This was also done for the two remaining studies in this thesis.

All NIRS data was converted to change from baseline (the baseline being the first minute of the absorption period) and averaged into 2-minute epochs across the remaining absorption and subsequent post dose task period. Due to the artefacts created by the movement associated with pen and paper responses within the NIRS data, memory task performance was removed from the analysis. The initial analysis of the averaged NIRS data was performed via within-subjects' ANOVAs with treatment (500 mg × Placebo) analysed against O_2 status (hypoxia × normoxia) and Epoch (comprising the thirty-seven 2-minute epochs, covering both the absorption and post task period). Upon the emergence of a significant interaction effect, subsequent planned comparisons were conducted, comparing resveratrol to placebo at each epoch, within the relevant environmental condition; such t-tests were calculated using the MS error values from the initial ANOVA.

4.3.2 Behavioural data analysis

All raw cognitive data was inspected for potential outliers and for missing data points prior to analysis. Participants with missing data were removed from behavioural analysis for the relevant task. One participant was excluded due to a data catchment error (missing several complete data sets), resulting in a sample of N=23 for analysis[6]. Cognitive performance data was analysed as change from pre-dose baseline performance for all task sub-measures in the cognitive battery. This comprised a total of 14 within subjects' ANOVAs investigating Treatment (500 mg resveratrol & placebo) × Repetition (×3) × O_2 status (hypoxia & normoxia), followed, where appropriate, by Bonferroni corrected post-hoc pairwise comparisons between resveratrol and placebo (within the relevant O_2 status only).

4.3.3 The analysis of overall cognitive domains across treatments and hypoxic conditions

In accordance with the aims of the current study, subsequent analysis was carried out to determine the success of the hypoxic model. ANOVAs were conducted for the 3 NIRS data outcomes (oxy-Hb, deoxy-Hb & total-Hb) comparing only the placebo conditions to isolate the influence of the O_2 status itself.

Regarding cognitive performance, Z scores were compiled through change from baselines scores and combined for the complete sets of behavioural data (N=19), to represent the cognitive domains assessed in the cognitive battery. Mean scores were calculated for each task outcome measure (regardless of repetition). Each individual change from baseline score was then subtracted from the mean overall score and then divided by the standard deviation to produce a Z score. Scores for each task outcome were compiled for the following cognitive domains: **accuracy** (correct responses from: Serial subtractions tasks, RVIP & 3Back), **speed of processing** (correct reaction time scores from: 3back, RVIP & word recognition) **errors** (incorrect responses from: Serial subtractions tasks, RVIP & 3Back) and **secondary memory** (correct recall responses from: both recall tasks & delayed word recognition). These were then analysed in a within subjects' ANOVA (Rep × Treatment × O_2 status) to ascertain any overall effects of hypoxia on the cognitive domains. Only main effects of O_2 status and treatment or subsequent interaction with each other or repetition from the initial ANOVA will be reported.

[6] Removal of participants from individual tasks was done due to either missing baseline data and / or data catchment errors (namely due to data responses failing to register for specific tasks). See appendix 2.0 for the sample size of individual tasks.

4.4 Results

4.4.1 Near-Infrared Spectroscopy (NIRS) data

Deoxygenated haemoglobin (deoxy-Hb):

The ANOVA revealed a significant main effect of hypoxia/normoxia [F (1, 660) = 44.67, p<.001] along with a complementary interaction with epoch [F (33, 660) = 32.56, p<.001], indicating a significant increase in concentrations of deoxy-Hb in the hypoxia condition in comparison to the normoxia condition. The ANOVA also revealed a significant treatment × hypoxia × epoch interaction [F (33, 660) = 1.75, p = .006] demonstrating that administration of 500 mg resveratrol evinced significantly higher concentrations of deoxy-Hb, in comparison to placebo, during the absorption period within hypoxia. As can be seen from figure 4.1, planned comparisons showed that this was significant during epochs 5-23 (5-9, 11, 13, 15-17, 20-22 (p <.05); 12-13, 18-19 (p <.01) with the exception of the epochs 10 and 14 (trending at p= .070 p =.056 respectively). Within normoxia, the resveratrol/normoxia condition evinced a significant reduction during the absorption period in comparison to the placebo/normoxia condition, at epochs 18, 20, 22 (p <.05), and trending at epochs 17, 19, 21 (at p =.062, p =.077 & p =.059 respectively).

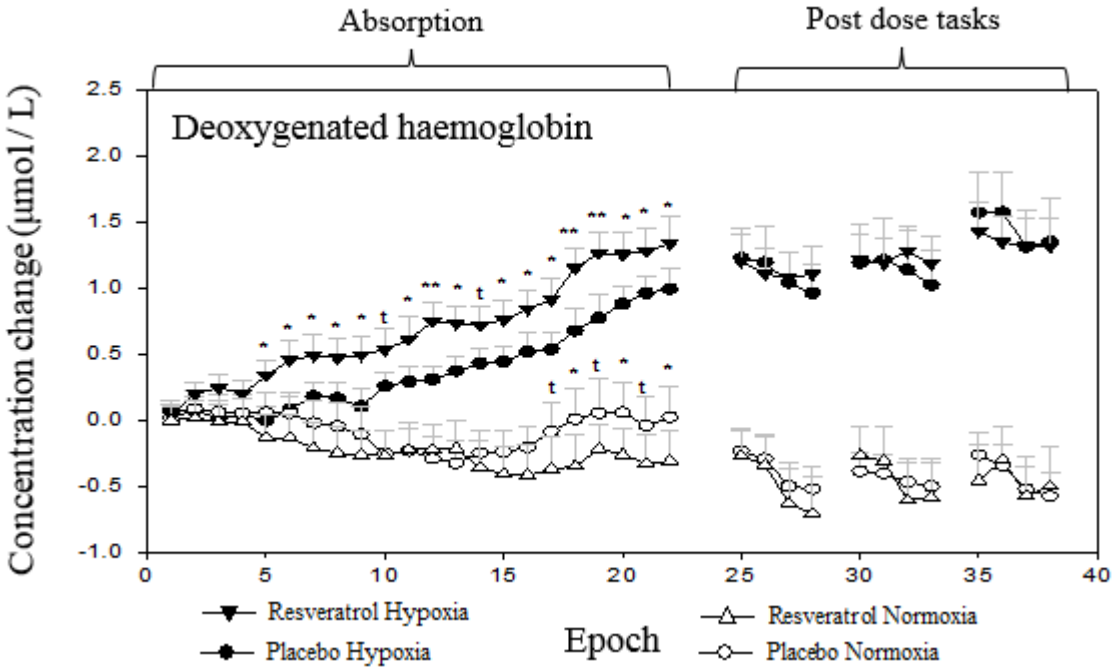

Figure 4.1 - The acute cerebral haemodynamic effects following administration of 500 mg resveratrol and placebo in the prefrontal cortex in hypoxic and normoxic conditions. Figure displays average changes to deoxygenated haemoglobin concentrations (with SEM error bars), throughout the absorption and post dose tasks. **p<.01, *p<.05 and t = trend.

Oxygenated haemoglobin (oxy-Hb):

The ANOVA revealed no significant main effect of treatment nor subsequent treatment related interaction with epoch and or hypoxia for changes in concentrations of oxy-Hb.

Total haemoglobin (total-Hb):

The ANOVA revealed no significant main effect of treatment nor subsequent treatment related interaction with epoch and or hypoxia for changes in concentrations of total-Hb.

4.4.1.1 Cerebral haemodynamic response to hypoxia

Oxygenated haemoglobin (oxy-Hb):

The initial ANOVA revealed a significant hypoxia × epoch interaction [$F(33, 660) = 2.45$, $p < .001$] indicating significantly lower concentrations of oxy-Hb within the placebo/hypoxia condition in comparison to the placebo/normoxia condition. Planned comparisons revealed a reduction (nearing significance) at epoch 2 ($p = .078$) and 20 ($p = .90$), whilst revealing significant reductions at epochs 5, 19, 21 ($p < .05$) and 22 ($p < .01$) in oxy-Hb concentrations within the placebo/hypoxia condition, during the absorption period, in comparison to the placebo/normoxia condition. A similar pattern was found during the post-dose task period where a significant reduction in oxy-Hb concentrations was found within the placebo/hypoxia condition at epochs 23 ($p < .01$), 24-26 ($p < .05$), 28 ($p < .01$), 31-33 ($p < .01$) and trending reductions at 29 ($p = .089$) and 34 ($p = .063$), in comparison to the placebo/normoxia condition.

Deoxygenated haemoglobin (deoxy-Hb):

The ANOVA revealed a significant main effect of hypoxia/normoxia [$F(1, 660) = 18.19$, $p < .001$] along with a complementary interaction with epoch [$F(33, 660) = 17.13$, $p < .001$], indicating a significant increase in concentrations of deoxy-Hb in the hypoxia condition, in comparison to normoxia. Follow up planned comparisons showed a significant increase in deoxy-Hb concentrations in the placebo/hypoxia condition in comparison to the placebo/normoxia condition, starting at epoch 10 which then continued to the end of the testing session ($p < .01$) (Figure 4.5).

Total haemoglobin (total-Hb):

The ANOVA revealed no significant main effect of treatment nor subsequent interaction with epoch for changes in concentrations of total-Hb.

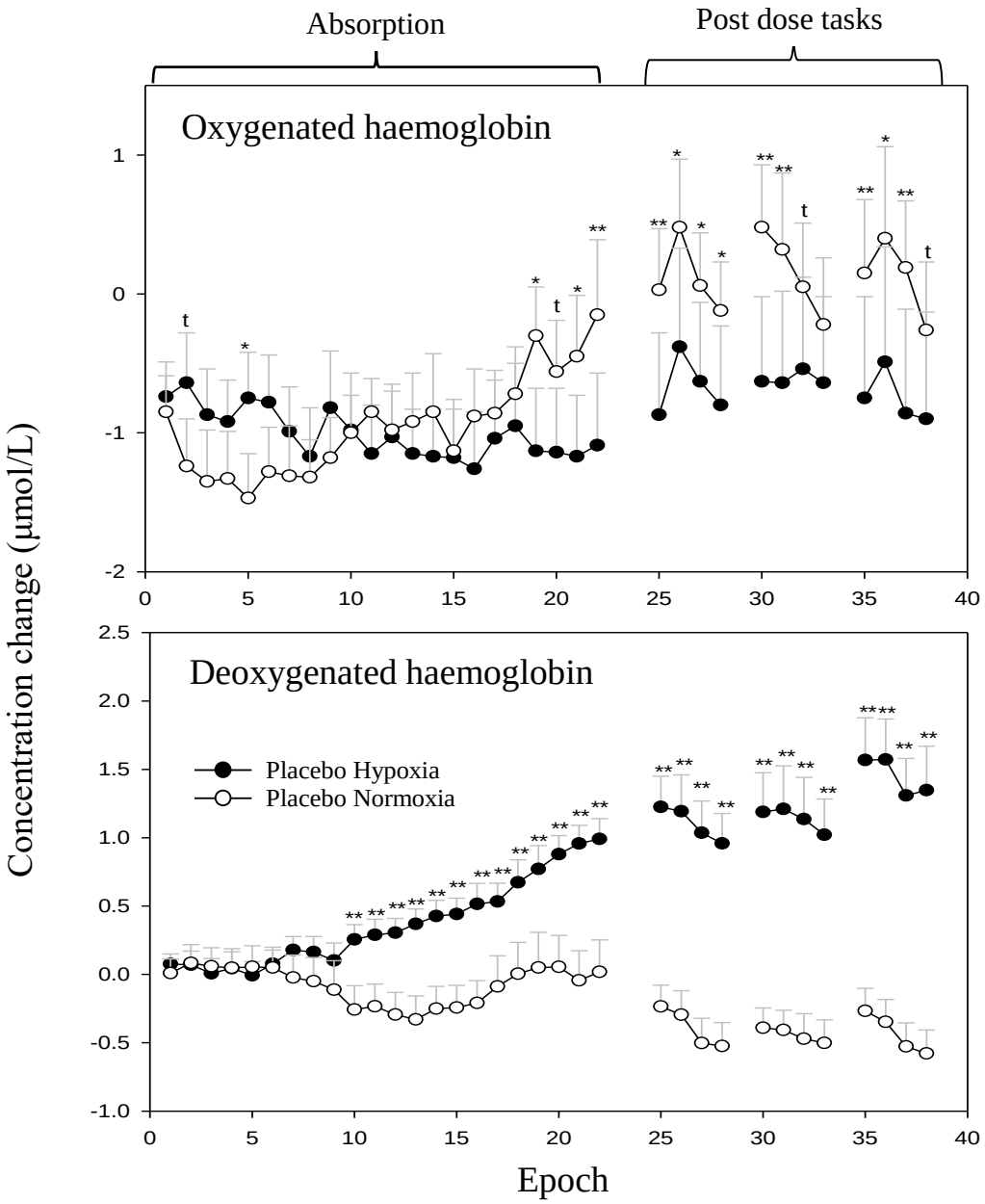

Figure 4.2 - The acute cerebral hemodynamic effects following administration of placebo within the prefrontal cortex in hypoxic and normoxic conditions. Figure displays average changes to oxygenated and deoxygenated haemoglobin concentrations (with SEM error bars), throughout the absorption and post dose tasks after administration with placebo, across both hypoxia and normoxia. **p<.01, *p<.05 and t = trend (nearing significance).

4.4.2 Behavioural data

Baseline analysis – Prior to any analysis, a series of one-way ANOVAs was conducted on all baseline scores for each cognitive outcome sub-measure. The results showed that there was a significant difference between baseline scores for Serial 3 errors [F (3, 88) = 3.159, p =.029], revealing that the resveratrol hypoxia condition had a significantly higher baseline in comparison to the placebo hypoxia (p =.004), placebo normoxia (p= .047) and resveratrol normoxia (p = .034) conditions. All other baseline scores for the rest of the cognitive outcome sub-measures showed to be statistically non-significant from one another (p >.10).

Serial 3 subtractions:

A significant main effect of treatment was found for the number of incorrect responses entered during performance of the Serial 3 subtraction task [F (1, 22) =4.813, p= .039]. This revealed that administration with resveratrol (M= .058) significantly reduced the number of errors made in comparison to placebo (M= 1.26) (Figure 4.2).

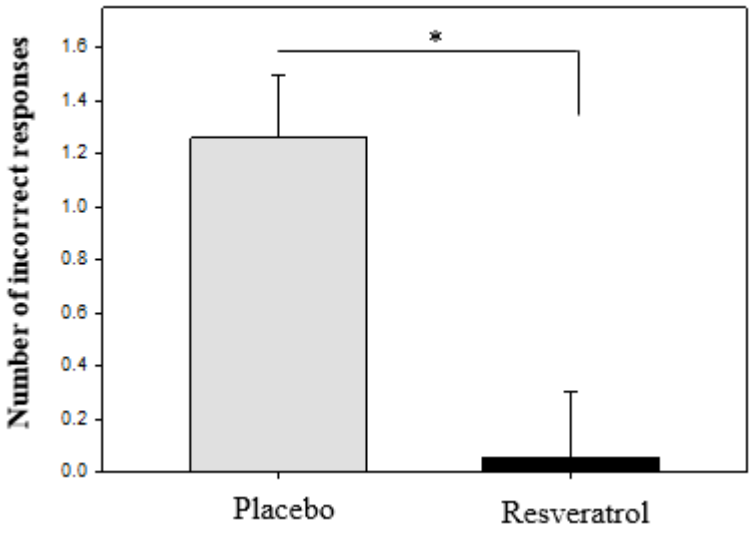

Figure 4.3 The main effect of treatment for 500 mg resveratrol and placebo administration on number of error responses on the Serial 3 subtraction task. Figure displays change from baseline means (with SEM error bars), of number of Serial subtraction incorrect responses after administration with 500 mg resveratrol or placebo irrespective of the environmental condition (*p<.05).

Serial 7 subtractions:

A significant main effect of treatment was found for the number of incorrect responses made on the Serial 7 subtraction task [F (1, 22) =5.447, p= .029]. This showed that administration of resveratrol (M= -.230) significantly reduced the number of errors made in comparison to placebo (M= .710) (Figure 4.3).

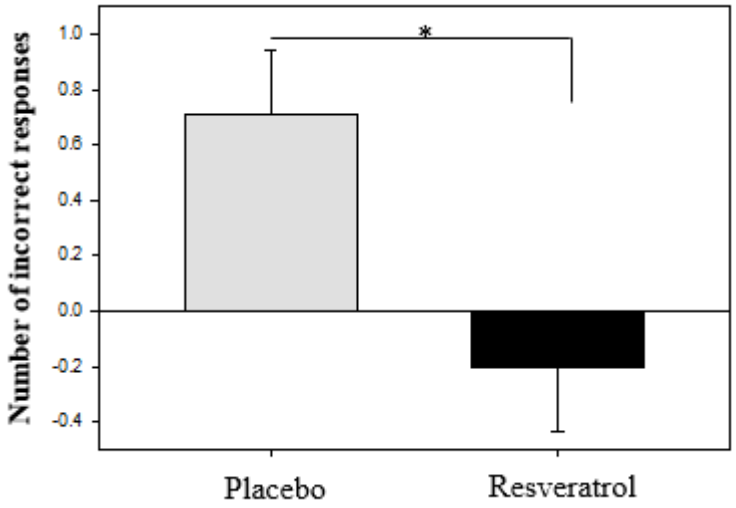

Figure 4.4 The main effect of treatment for 500 mg resveratrol and placebo administration on number of error responses on the Serial 7 subtraction task. Figure displays change from baseline means (with SEM error bars), of number of Serial subtraction incorrect responses after administration with 500 mg resveratrol or placebo irrespective of the environmental condition (*p<.05).

4.4.2.1 Overall cognitive domain performance

Accuracy – A significant treatment × rep interaction was found for overall accuracy performance across the tasks [F (6, 108) =4.441, p= .019]. Further investigation into this interaction via Bonferroni corrected post-hoc t tests revealed a significant reduction (p =.017) in accuracy in the placebo condition in repetition 2 (M = -.137) in comparison to the placebo condition during repetition 1 (M =.06), while trending (p = .059) a reduction in comparison to the resveratrol condition during rep 2 (M =.093).

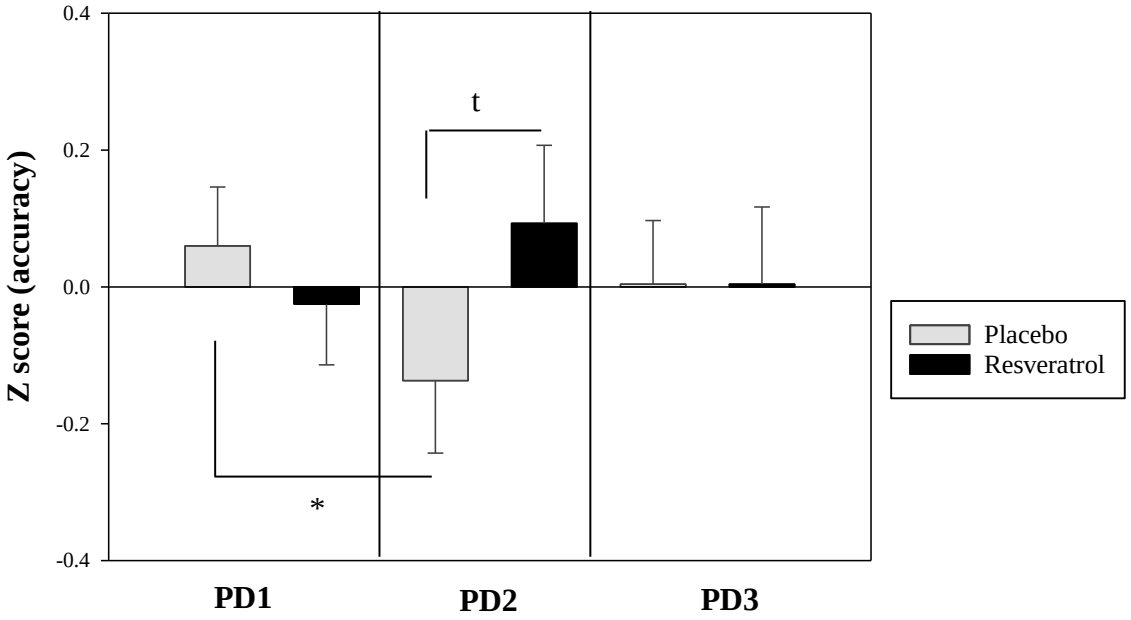

Figure 4.5 The treatment × repetition interaction effect of treatment for 500 mg resveratrol and placebo administration on overall task accuracy. Figure displays change from baseline Z score means (with SEM error bars), on overall accuracy (% / number correct) across Serial 3 & 7 subtraction, RVIP and 3back tasks irrespective of the environmental condition (*p<.05, t= trend).

Table 4.0 The effects of 500 mg resveratrol and placebo on Serial subtraction performance in both hypoxic and normoxic conditions. Table displays baseline and change from baseline scores (*with SEM values in brackets*) for significant results across the 3 post-dose battery repetitions after placebo and 500 mg resveratrol for 23 healthy, young adults. Table also displays ANOVA F and P values for main effects of treatment (T), oxygen status (OS) the interaction between the two (T*OS), repetition (T*R) all three (T*OS*R). *<.05, **<.01 and t= trend (nearing significance).

Measure	Treatment	N	Task Battery				ANOVA		
			Baseline	1	2	3	Effect	F	P
3's Correct (number)	Placebo Hypoxia	23	48.04 (*2.95*)	.09 (*1.15*)	.48 (*1.59*)	-2.13 (*1.84*)	T	.858	.364
	Placebo Normoxia	23	44.87 (*3.04*)	1.22 (*1.83*)	.35 (*1.50*)	1.43 (*1.96*)	OS	2.585	.122
	Resveratrol Hypoxia	23	45.78 (*2.52*)	-1.00 (*1.45*)	2.17 (*1.35*)	.22 (*1.54*)	T*R	1.431	.250
	Resveratrol Normoxia	23	44.70 (*3.05*)	2.78 (*1.32*)	3.39 (*1.91*)	1.09 (*1.87*)	T*OS	.029	.866
							T*OS*R	2.145	.129
3's Incorrect (number)	Placebo Hypoxia	23	1.09 (*.29*)	.91 (*.50*)	.96 (*.42*)	2.61 (*.73*)	T	4.813	.039*
	Placebo Normoxia	23	1.65 (*.50*)	.22 (*.59*)	1.52 (*.59*)	1.35 (*.59*)	OS	2.261	.147
	Resveratrol Hypoxia	23	2.87 (*.42*)	-.26 (*.38*)	.48 (*.67*)	1.00 (*.62*)	T*R	.489	.617
	Resveratrol Normoxia	23	1.57 (*.47*)	-.91 (*.62*)	-1.00 (*.50*)	1.04 (*.65*)	T*OS	.067	.798
							T*OS*R	3.321	.062t
7's Correct (number)	Placebo Hypoxia	23	26.43 (*1.97*)	1.61 (*1.10*)	1.35 (*1.24*)	3.00 (*1.14*)	T	1.355	.257
	Placebo Normoxia	23	25.61 (*1.97*)	1.26 (*1.41*)	.22 (*1.42*)	1.17 (*1.44*)	OS	.732	.401
	Resveratrol Hypoxia	23	26.04 (*1.97*)	.00 (*1.23*)	3.17 (*1.32*)	.87 (*1.84*)	T*R	2.284	.114
	Resveratrol Normoxia	23	25.48 (*2.30*)	3.57 (*.93*)	3.65 (*1.04*)	4.57 (*1.01*)	T*OS	3.266	.084t
							T*OS*R	1.526	.229

7's Incorrect (number)	Placebo Hypoxia	23	2.17 *(.54)*	.43 *(.73)*	1.83 *(.47)*	.65 *(.63)*	T	5.447	.029*
	Placebo Normoxia	23	2.39 *(.43)*	.04 *(.46)*	.52 *(.53)*	.78 *(.55)*	OS	.773	.389
	Resveratrol Hypoxia	23	2.65 *(.66)*	-.17 *(.66)*	.00 *(.62)*	.04 *(.73)*	T*R	.933	.401
	Resveratrol Normoxia	23	2.91 *(.44)*	-.65 *(.45)*	-.17 *(.47)*	-.26 *(.42)*	T*OS	.066	.799
							T*OS*R	.940	.398

4.4.2.2 Cognitive performance in hypoxia

There was no significant main effects of hypoxia or hypoxia related interactions on cognitive performance in the current study.

4.4.2.3 Overall cognitive domain performance in hypoxia

There was no significant main effects of hypoxia or hypoxia related interactions on overall cognitive domains in the current study.

Cognitive results for individual tasks in this chapter are reported in full in Appendix 2.0, while results for overall cognitive domains are reported in Appendix 2.1.

4.5 Discussion

The aims of current study were two-fold: The first was to assess the efficacy of employing mild hypoxia as a representative, experimental model of cognitive ageing. Here, it was hypothesized that cognitive performance would decrease within the hypoxia condition as a result of a reduction in O_2 supply. Secondly, the present study also aimed to evaluate the efficacy of resveratrol to induce increases in CBF, and, in turn, attenuate any reductions in cognitive performance induced by the hypoxic condition.

The results here demonstrated significantly lower oxy-Hb concentrations during the final stages of the absorption period and across the full post dose task period in the hypoxia condition, confirming that the hypoxic level employed was capable of reducing cerebral oxygenation. Despite this, the hypoxia condition failed to induce any significant reductions in cognitive performance. Additionally, significantly higher concentrations of deoxy-Hb were present across both the absorption and post task period from exposure to hypoxia; which may signify an increase in O_2 extraction too offset the hypoxic condition. Although, it is noteworthy the anticipated increase in CBF (as indexed by higher concentrations of total-Hb) was not observed here. The lack of an adaptive CBF response, in the face of no clear cognitive deficits, would suggest that the hypoxic level employed here was simply not sufficient to disrupt the high neurocognitive efficacy of the young, healthy sample.

Indeed, it remains unclear whether mild hypoxia can deteriorate cognitive performance in healthy cohorts (Bartholomew et al., 1999) as most investigations only document consistent domain specific cognitive deficits at >3000 m above sea level (Bahrke & Shukitt-Hale, 1993). Ambiguity lies in the use of different tasks and the varying durations of exposure employed by previous investigations, making it difficult to ascertain what level of hypoxia would be appropriate to initially model cognitive ageing. Although reductions in mental performance have been observed at levels as low as 1524 m above sea level (Denison et al., 1966) most decreases to performance at these lower levels of hypoxia are most commonly found when measured during physical exertion (Bahrke & Shukitt-Hale, 1993). The increase in demand for O_2 brought about by physical exertion, exacerbates the reduced access to O_2 which may exaggerate the cognitive deficits reported at mild levels of hypoxia. The results of the current study would certainly support the notion that mild hypoxia is insufficient to disrupt cognitive performance in young, healthy

individuals. It is therefore proposed that a more disruptive hypoxic level is required to model the effects of the ageing brain.

With regards to treatment related differences, the results showed that a single 500 mg dose of resveratrol was able to amplify the hypoxia-induced increases in O_2 utilization across the absorption period, as indexed by significantly higher deoxy-Hb concentrations in comparison to placebo. However, in contrast to predictions, no significant modulation of CBF by resveratrol was found during the post dose task performance period during hypoxia. The findings here suggest that resveratrol was unable to promote an adaptive cerebral hemodynamic response of hypoxia during task performance. Alternately, this may simply highlight that the young cohort of participants did not require any additional modification to neural fuel access after consuming resveratrol. Although, to add further ambiguity, resveratrol also showed no effect on cerebral hemodynamics across the post dose task period in the normoxia condition relative to placebo, which is at odds with previous investigations (Kennedy et al., 2010; Wightman et al., 2014; Wightman et al., 2015).

In an attempt to explain these anomalous findings, it is noteworthy that ascent to moderate-high altitude has been found to induce a wide range of adverse psychological changes. This includes increased feelings of anxiety, depression, aggression and fatigue alongside reduced feelings of vigour and happiness (Shukitt-Hale & Lieberman, 1996; Shukitt-Hale et al., 1998; Bardwell et al., 2005). Further, despite the chamber being equipped with silencers to blunt the noise of air regulation, a certain and constant level of noise is continually present due to the regulation of air in both hypoxic and normoxic conditions, which may have been disconcerting to the participants. Moreover, as the O_2 status was blind to the participant, even the perceived reduction in O_2 may have further exacerbated an anxiogenic effect. Interestingly, anxiety has been found to produce local and global changes in CBF that are unrelated to regional neuronal activation and, therefore, may confound the interpretation of neuroimaging data (Giardino et al., 2007). Consequently, the anxiogenic environment of the chamber may have caused such a level of noise in the neuroimaging data that this may have dissipated the well-established CBF effects of resveratrol in normoxia.

With regards to cognitive performance, a significant effect was observed on the analysis of overall cognitive domains in the form of a treatment × rep interaction for task accuracy. However, upon further analysis, no consistent pattern revealed itself between the two

treatments, with the effect being likely exaggerated by the large difference within treatments across the post dose repetitions. However, in contrast to previous investigations, this is perhaps the first study to demonstrate that acute resveratrol administration can enhance cognitive performance in young, healthy cohorts. A significant reduction in the number of errors made on both subtraction tasks was found after administration of resveratrol alone, relative to placebo. It is also noteworthy, that the baseline differences for the Serial 3 subtraction task errors sub-measure showed that the resveratrol hypoxia baseline was significantly higher to all other scores, which may have influenced this result. Moreover, to contextualise the magnitude of the cognitive enhancement observed here, this was a single reduction in the error response rate across both Serial subtraction tasks; representing a single improvement across a variety of cognitive tasks / domains.

In the absence of a resveratrol-mediated increase in CBF during task performance, it is unclear what mechanism may have contributed towards this improvement in reduced errors on the Serial subtraction tasks. It is conceivable, that the cognitive benefits of resveratrol observed here may be the result of mood enhancement or more specifically anxiolytic effects; whereby resveratrol may have attenuated the aforementioned anxiogenic nature of the testing environment. Indeed, administration of resveratrol (15 mg/kg per day for 16 days) has been found to exert antidepressant and antianxiety effects in subclinical hypothyroidism rats (Ge et al., 2016). In terms of mechanisms, resveratrol has been found to inhibit MOA activity in a dose dependent manner, whilst increasing serotonin and noradrenaline levels, following 7 days supplementation in rodents (Xu et al., 2010).

Although evidence in humans is lacking, berry polyphenols have been identified as inhibitors of MOA-A activity in human trials (Watson et al., 2015). In addition, a single dose of 300 mg EGCG has been shown to significantly improve self-reported calmness and stress (Scholey et al., 2012) while a 500 mg dose of cocoa polyphenols has been found to significantly improve self-rated calmness and contentedness following 30 days of supplementation (Pace et al., 2013). Although the latter study does not detail anxiolytic effects specifically, it is postulated that polyphenols may provide a benefit in situations where a calm or content temperament is advantageous, i.e. under conditions of high stress or anxiety (Pace et al., 2013). Of course, in the absence of any mood measures, it is not possible to state categorically that resveratrol has induced an anxiolytic effect or indeed, that the chamber was deemed anxiogenic by the participants. It is important therefore that

further investigation into the hypoxia model of cognitive ageing should be done so with a corresponding measurement of mood states such as self-reported anxiety.

The current study has revealed that a mild hypoxic environment can reduce cerebral oxygenation in the prefrontal cortex, but this was not disruptive enough to induce cognitive deficits. It is proposed that a higher level of hypoxia is required to model the decreases in $CMRO_2$ observed in ageing populations and, in turn, age-related cognitive deficits. In addition, this is perhaps the first investigation to observe cognitive improvements, albeit tentative, following a single dose of resveratrol in young, healthy individuals. However, in the absence of significant modulation to CBF, it is unclear what mechanism may have contributed to this effect.

Chapter 5

The cerebral hemodynamic response and cognitive effects of acute resveratrol administration in young, healthy adults in moderate hypoxic and normoxic conditions.

5.1 Introduction

The results of the preceding chapter revealed that a mild hypoxia could decrease cerebral oxygenation within the prefrontal cortex but not significantly reduce cognitive performance on any individual task outcome or overall cognitive domain. It is noteworthy that deficits in cognitive performance at rest have been found more consistently at altitudes of >3000 m above sea level (Bahrke & Shukitt-Hale, 1993; Petrassi et al., 2012). Indeed, it has been proposed that the 'critical zone' for cognitive reductions is between 4000-5000 m above sea level (12.7-11.2% atmospheric O_2) (Nelson, 1982). This is supported further by a series of reviews reporting significant reductions in cognitive functioning at greater levels of hypoxia (Asmaro et al., 2013; Aquino Lemos et al., 2012; Bjursten et al., 2010; Petrassi et al., 2012).

Contrary to predictions, the previous chapter also showed that acute administration of resveratrol was unable to modulate CBF during task performance in either the hypoxia or normoxia condition. It was proposed that the perceived reduction in O_2 and the enclosed nature of the environmental chamber, might have contributed towards potential anxiogenic effects, and consequently, noise within the neuroimaging data. It is noteworthy that exposure for more than 1 hour at moderate-high altitude can provoke a negative impact on mood states in healthy individuals (Li et al., 2000); which seems to be further exacerbated with an increasing level of altitude (Shukitt, & Banderet, 1987). As the same chamber will be employed again in the current study, in addition to an increasing hypoxic level, it may be prudent to measure self-reported mood across the testing sessions. Interestingly, self-reported reductions in mood have been restored when supplemented with additional O_2 during exposure to hypoxia (Legg et al., 2016). This potentially supports the reciprocal role of decreasing O_2 and deterioration of mood parameters, but perhaps offers a further therapeutic role for vasodilators that can mediated CBF effects in hypoxia.

In a recent study, Davranche et al. (2016) exposed 11 healthy males to 4350 m altitude for 4 days, measuring cognitive performance and CBF (via NIRS) at initial ascent (3-5

hours exposure), then after 2 and 4 days' exposure respectively. Upon initial exposure to hypoxia, the researchers observed poorer reaction times and an increased number of errors. Interestingly however, the researchers also found that increased CBF within the prefrontal cortex significantly correlated with better reaction times, implying that the greater the CBF response, the more superior attenuation of the hypoxia induced cognitive deficit. Such results provide support for further exploration into the efficacy of resveratrol as a cognitive enhancer at a higher level of hypoxia. Indeed, as an inverse relationship exists between hypoxia and maximal O_2 uptake (Babbar & Agarwal, 2012) hypoxia will naturally reduce $CMRO_2$, however, as an increased CBF response has been found to correlate with improved cognitive functioning in hypoxia; the capacity of resveratrol to enhance CBF should function to attenuate the reductions in O_2 supply and consequently cognitive performance.

The current study aims to build upon the results of the previous chapter and employ a more severe level of hypoxia ($F_iO_2 = 12.7\%$), to model age-related cognitive impairment. This higher level of hypoxia is hypothesized to sufficiently compromise O_2 delivery to induce relative decreases in cognitive performance and negatively impact self-reported mood in comparison to normoxia. It is also hypothesized that resveratrol will induce significant increases in cerebral perfusion, irrespective of O_2 status. However, the increase in CBF within the hypoxic condition will function to attenuate the hypoxia-induced reductions to behavioural performance.

5.2 Method

5.2.1 Participants

The current study sample comprised 24 healthy adults aged between 19-33 years (16 female, mean age = 23, SD = 4.20, right handed = 23) with good or corrected vision. No participant reported to suffer from colour blindness. The current study recruited participants based upon the same inclusion / exclusion criteria as the previous chapter (see section 4.2.1 for details).

5.2.2 Cognitive battery

The current study maintained the continuous task design employed by the previous investigations in this thesis. The tasks, in order of appearance in the cognitive battery were as follows: Immediate word recall, Serial 3s, Serial 7s, Choice Reaction Time (CRT), RVIP, Delayed word recall, Stroop task and Word Recognition. The inclusion of the CRT and Stroop tasks was in an attempt to better match the tasks used by other studies that have observed cognitive deficits at hypoxia (Leifflen et al., 1997; Asmaro et al., 2013; Petrassi et al., 2011; Petrassi et al., 2012). Five mood VAS scales were also employed upon completion of each repetition of the task battery at both baseline and post dose repetitions. The current study measured self-reported: 'Difficulty', 'Mental-fatigue', 'Anxiety', 'Friendliness' and 'Aggression'. Refer to section 2.4 for VAS task description.

5.2.3 Treatments

During the four testing study visits, participants received a single-dose treatment in compliance with a previously randomised allocation to a (Latin Square) counterbalanced order. Treatments were administered on separate days, with each testing session being conducted no more than 48 hours before, and no more than 14 days after, the previous session. Two capsules were consumed by participants on each testing day being either:

1) ×2 placebo capsules (CABOT MP5 fumed silica).
2) ×2 250 mg resveratrol capsules (equating to a 500 mg dose).

Again, all treatments were prepared by the lead investigator, before being coded by a third party who had no further involvement in any aspect of the study. No member of the investigational team was aware of the contents of the capsules until the statistical analysis of the current study was complete.

5.2.4 Environmental Chamber

The environmental conditions remained constant with those of the previous chapter, with the exception that the O_2 status of the hypoxic condition was increased from 16% F_iO_2 (2134 m) to 12.7% F_iO_2 (4000 m). This was in response to previous studies which have found more consistent cognitive deficits at this simulated altitude. Furthermore, in contrast to the previous chapter, the initiation of the O_2 status was set at the start of the testing session, rather than at the beginning of the absorption period. The rationale behind this change was twofold: firstly, the chamber required 2 hours to achieve the above detailed hypoxic level, making it impossible to achieve the required environmental condition prior to engaging in the post dose repetitions if the level was set at the end of the absorption period as with the previous chapter. Secondly, it could be argued that allowing the participants to stay inside the chamber, while the change in O_2 status progressively occurs, could aid in the adaptation to the reduced availability of O_2. This is perhaps supported by the progressive increase in deoxy-Hb concentrations during the latter stages of the absorption period in Chapter 4 and the lack of hypoxia related effects on cognitive performance. Despite the participants being immediately exposure to hypoxia, this was not predicted to influence baseline performance; analysis was conducted to verify this claim (see section 5.3.2).

5.2.5 Near-Infrared spectroscopy (NIRS)

CBF was monitored in the prefrontal cortex by NIRS throughout all four testing sessions. The same procedure and system was adopted as the previous chapter (refer to section 2.1 for further information on this technique and the system used).

5.2.6 Procedure

Participants were required to attend the laboratory on five occasions. The first visit was a screening / training session, where participants declared themselves to be in line with the inclusion criteria and provided informed consent. Participants received training on the individual tasks in the cognitive battery to ensure they were competent and confident to complete all tasks on the battery without assistance; participants were required to complete two full repetitions of the cognitive battery. For the 4 subsequent testing visits, participants arrived at the environmental chamber at 8 am, fully fasted for 12 hours (except for water). After confirmation of their continued compliance to the inclusion

criteria, participants were then connected to the NIRS device; this was worn throughout the full testing procedure. After a brief rest, participants completed a baseline measurement of the cognitive battery before watching a non-stimulating video (Grand designs) for 10-minutes. Upon completion of the baseline rest measure, the treatment for the day was administered and the participant remained at rest for a further 45 minutes (continuing with the video), to allow for absorption of the treatment. After, the participant then completed 3 full consecutive repetitions of the cognitive battery (Figure 5.0). On completion of the final session, participants were fully debriefed. Participants were offered £65 upon completion of the study.

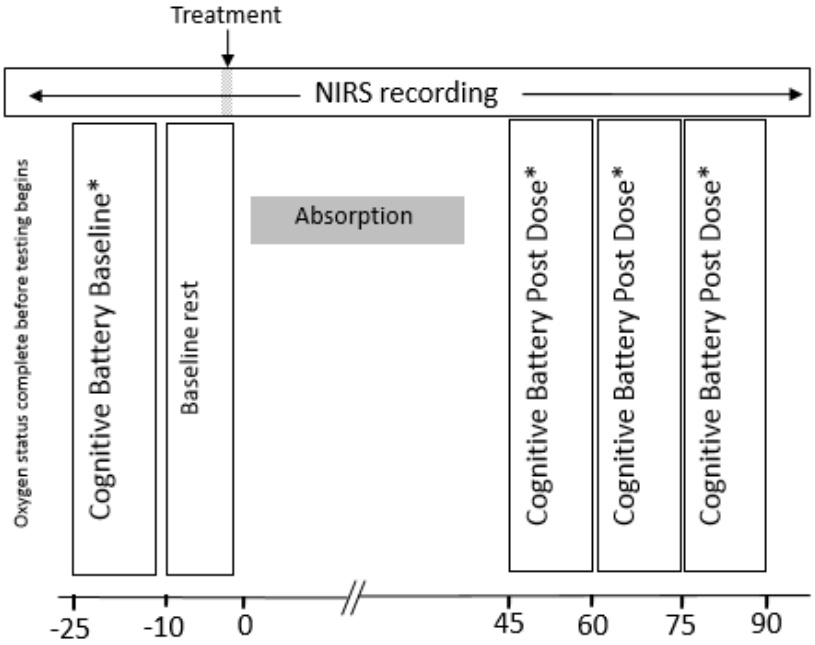

Figure 5.0 A timeline of the third experimental study testing day.

5.3 Statistical approach

All behavioural data was analysed using SPSS version 22 for windows (IBM SPSS Statistics Armonk, NY), while all NIRS analysis was analysed using Minitab 17 (State College, PA: Minitab, Inc.).

5.3.1 NIRS analysis

The current study adopted the same analysis plan and approach to data cleaning as Chapter 4 (see section 4.3.1). After data cleaning, the final sample for NIRS data was N=19.

5.3.2 Behavioural data analysis

The same approach to data cleaning and statistical analysis, as carried out in Chapter 4, was adopted for the cognitive data here (see Chapter 4.3.2 for details). No participants were removed from the data set as a result of the data cleaning (N = 24). A series of ANOVAs and independent t-tests were carried out on each of the baseline task and mood outcome sub-measures to assess for any potential differences between baselines, and to evaluate if the immediate exposure to hypoxia at baseline had had any influence on behavioural performance (Lefferts, 2015).

With regards to mood, all VAS data for 'Difficulty', 'Mental-fatigue', 'Anxiety', 'Friendliness' and 'Aggression' were converted to change from baseline (utilising the score submitted after completion of the baseline repetition of the cognitive tasks, as a baseline measure) before individually being analysed in a 2 (treatment) × 3 (repetition) × 2 (O_2 status) repeated measures ANOVA. Upon the emergence of a significant F test, these were follow up with Bonferroni corrected post-hoc analysis. In addition, to examine the claims made in the previous chapter regarding the potential negative impact of the chamber on mood, analysis was carried on all mood scores submitted prior to undertaking baseline mood outcome task (with the exception of difficulty) for each day via a 4 (day) × 2 (O_2 status) ANOVA.

5.3.3 The analysis of overall cognitive domains across treatments and hypoxic conditions

In accordance with the preceding chapter, Z scores were compiled and combined for the complete sets of behavioural data (N=23) to represent the cognitive domains assessed in the cognitive battery: **accuracy** (correct responses from: Serial subtractions tasks, CRT RVIP, & Stroop), **speed of processing** (correct reaction time scores from: CRT, RVIP & Stroop) **errors** (incorrect responses from: Serial subtractions tasks & RVIP) and **secondary memory** (correct recall responses from: both recall tasks & delayed word recognition). These were then analysed in a within subjects' ANOVA (Rep × Treatment × O_2 status) to ascertain any overall effects of hypoxia on the cognitive domains. Only main effects of O_2 status and treatment or subsequent interaction with each other or repetition from the initial ANOVA will be reported.

5.4 Results

5.4.1 NIRS data

Oxygenated haemoglobin (oxy-Hb):

The initial ANOVA demonstrated a significant treatment × hypoxia/normoxia × epoch interaction [F (36, 612) = 1.49, p = .035] indicating higher concentrations of oxy-Hb during the hypoxia conditions in comparison to the normoxia conditions. In addition, the resveratrol/normoxia condition showed a significant increase in oxy-Hb concentrations in comparison to the placebo/normoxia condition. This indicates a higher cerebral oxygenation across the post dose task period following resveratrol in comparison to placebo during the normoxia condition. With reference to planned comparisons, the placebo/hypoxia condition showed an increase (nearing significance) in concentrations of oxy-Hb within the absorption period at epoch 8 (p =.075) and 18 (p =.066) in comparison to the resveratrol/hypoxia condition. No significant differences were found between treatments during hypoxia across task performance. During normoxia, planned comparisons revealed an increase in oxy-Hb index after placebo consumption, in comparison to resveratrol, by a significant increase at epoch 5 (p = .039) and trending increases at 19 (p = .077) and 21 (p = .065) within the absorption period. However, this shifted during performance of the post dose tasks, revealing a significant increase of oxy-Hb concentrations after resveratrol, at all time points 23-37 (p <.01) in comparison to placebo (Figure 5.1).

Total haemoglobin (total-Hb):

The within subjects ANOVA revealed a significant treatment × epoch [F (36, 612) = 2.98, p <.001] and a treatment × hypoxia × epoch [F (36, 612) = 0.74, p <.001] interaction. Planned comparisons revealed an increase (nearing significance) in concentrations of total-Hb within the absorption period at epoch 8 (p =.075) within the placebo/hypoxia condition relative to the resveratrol/hypoxia condition. In addition, a significant increase in total-Hb concentrations was found at epochs 31 (p =.025) and 37 (p =.035), whilst trending an increase at epoch 32 (p =.081) and 35 (p =.080) in the placebo/hypoxia condition, in comparison to the resveratrol/hypoxia condition, during the post dose task period. With regards to normoxia, resveratrol induced significantly higher total-Hb concentrations across all epochs (23-37) of the post dose task period [all p <.01; except for epoch 25-26 (p <.05)] in comparison to placebo (Figure 5.2).

Deoxygenated haemoglobin (deoxy-Hb):

The ANOVA revealed no significant main effect of treatment nor subsequent related interaction with epoch or hypoxia for changes in concentrations of deoxy-Hb.

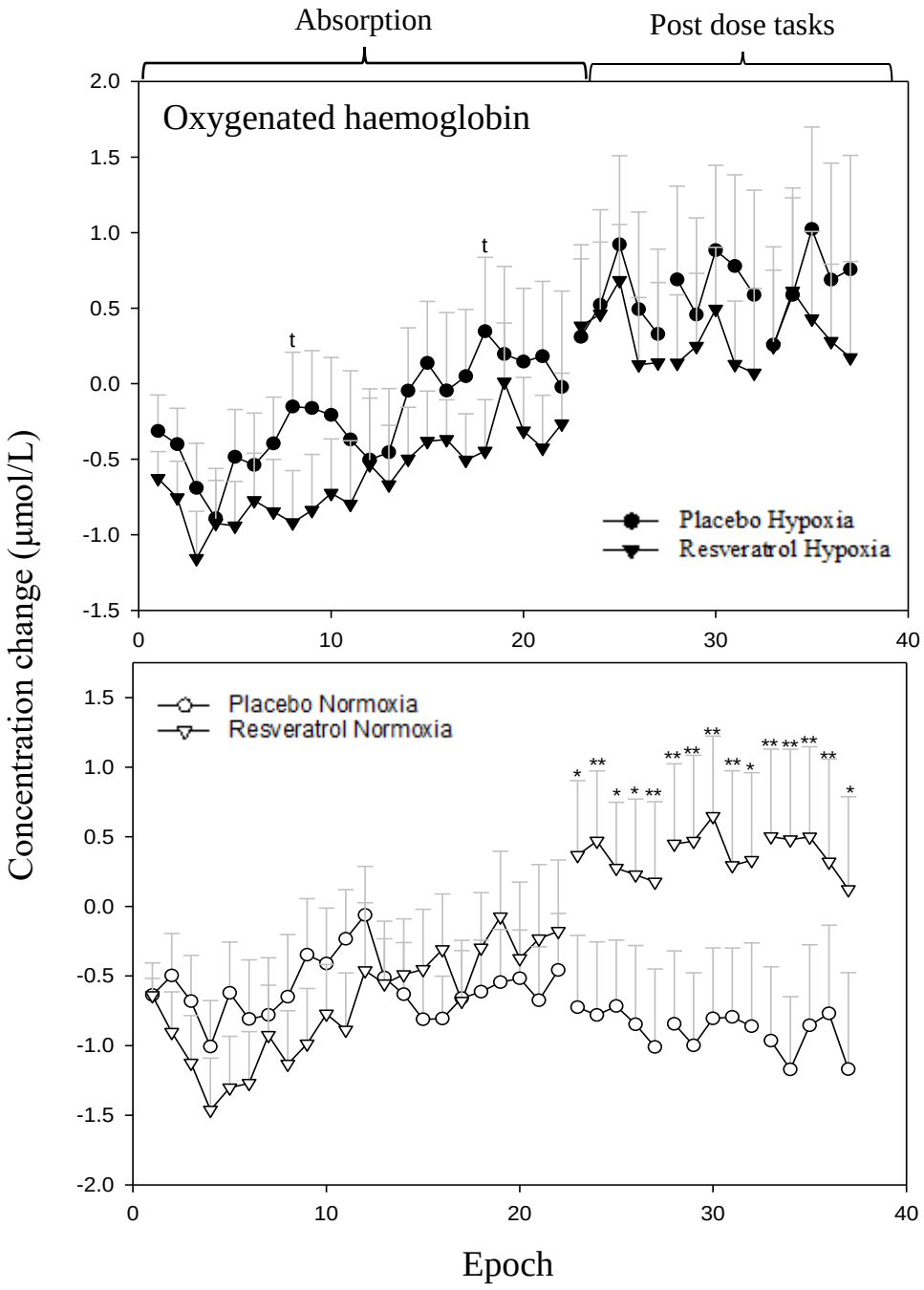

Figure 5.1 - The acute cerebral hemodynamic effects to oxygenated haemoglobin following administration of 500mg resveratrol and placebo in the prefrontal cortex within the hypoxic and normoxic conditions. Figure displays average changes in oxygenated haemoglobin concentrations (with SEM error bars), throughout the absorption and post dose tasks. **p<.01, *p<.05 and t = trend (nearing significance).

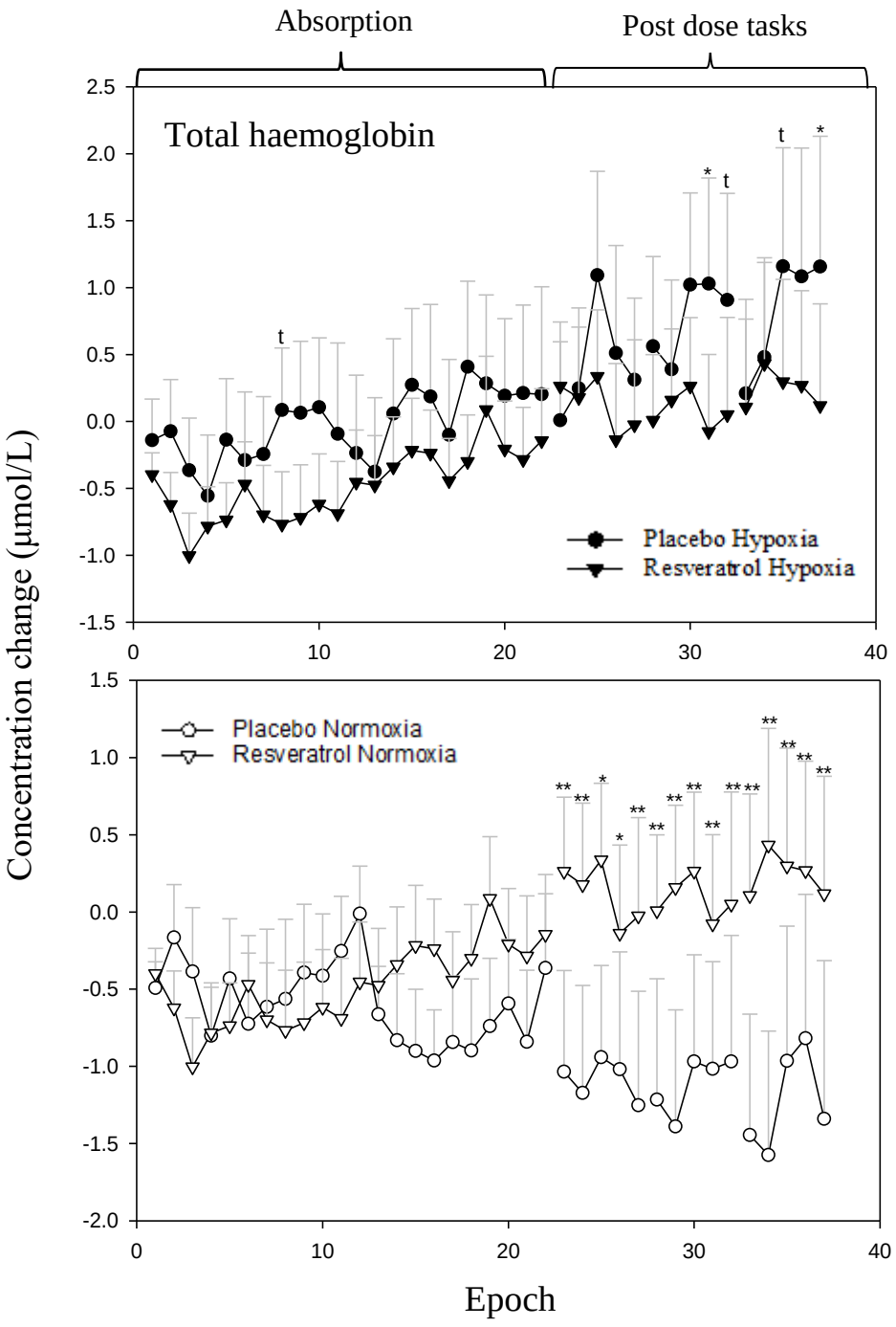

Figure 5.2 - The acute cerebral hemodynamic effects of total haemoglobin following administration of 500 mg resveratrol and placebo in the prefrontal cortex within the hypoxic and normoxic conditions. Figure displays average changes in total haemoglobin concentrations (with SEM error bars), throughout the absorption and post dose tasks. **p<.01, *p<.05 and t = trend (nearing significance).

5.4.1.1 Cerebral haemodynamic response to hypoxia

Oxygenated haemoglobin (oxy-Hb):

The initial AVOVA revealed a significant hypoxia × epoch interaction [F (36, 648) = 1.77, p = .004] indicating significantly higher concentrations of oxy-Hb within hypoxia in comparison to normoxia. Planned comparisons demonstrated a significant increase at epochs 15 and 18 ($p < .05$), whilst trending an increase at 16 ($p = .089$) and 21 ($p = .055$) respectively, during the absorption period in hypoxia, in comparison to normoxia. Moreover, a significant increase in oxy-Hb concentrations within the placebo/hypoxia condition was observed across the entire the post-dose task period (epochs 23-37, $p<.01$; epoch 23 $p<.05$) in comparison to the placebo/normoxia condition.

Total haemoglobin (total-Hb):

The within subjects ANOVA revealed a significant hypoxia × epoch F (33, 660) = 1.80, $p < .001$] interaction effect indicating higher concentrations of total-Hb in the placebo/hypoxia condition in comparison to the placebo/normoxia condition. Post hoc comparisons revealed a trending increase in total-Hb concentrations within the placebo/hypoxia condition in comparison to placebo/normoxia at epochs 19 ($p =.056$) and 21 ($p =.050$) and significant increases at epochs 15-16 and 18 ($p <.05$), across the absorption period. This increase in total-Hb concentrations continued within the placebo/hypoxia condition across all epochs of the post dose task period, demonstrating a trending increase at epoch 23 ($p =.052$) and a significant increase in all subsequent epochs (24-37 $p<.01$), in comparison to the placebo/normoxia condition (Figure 5.6).

Deoxygenated haemoglobin (deoxy-Hb):

The ANOVA revealed no significant main effect of hypoxia or subsequent interaction with epoch for changes in concentrations of deoxy-Hb.

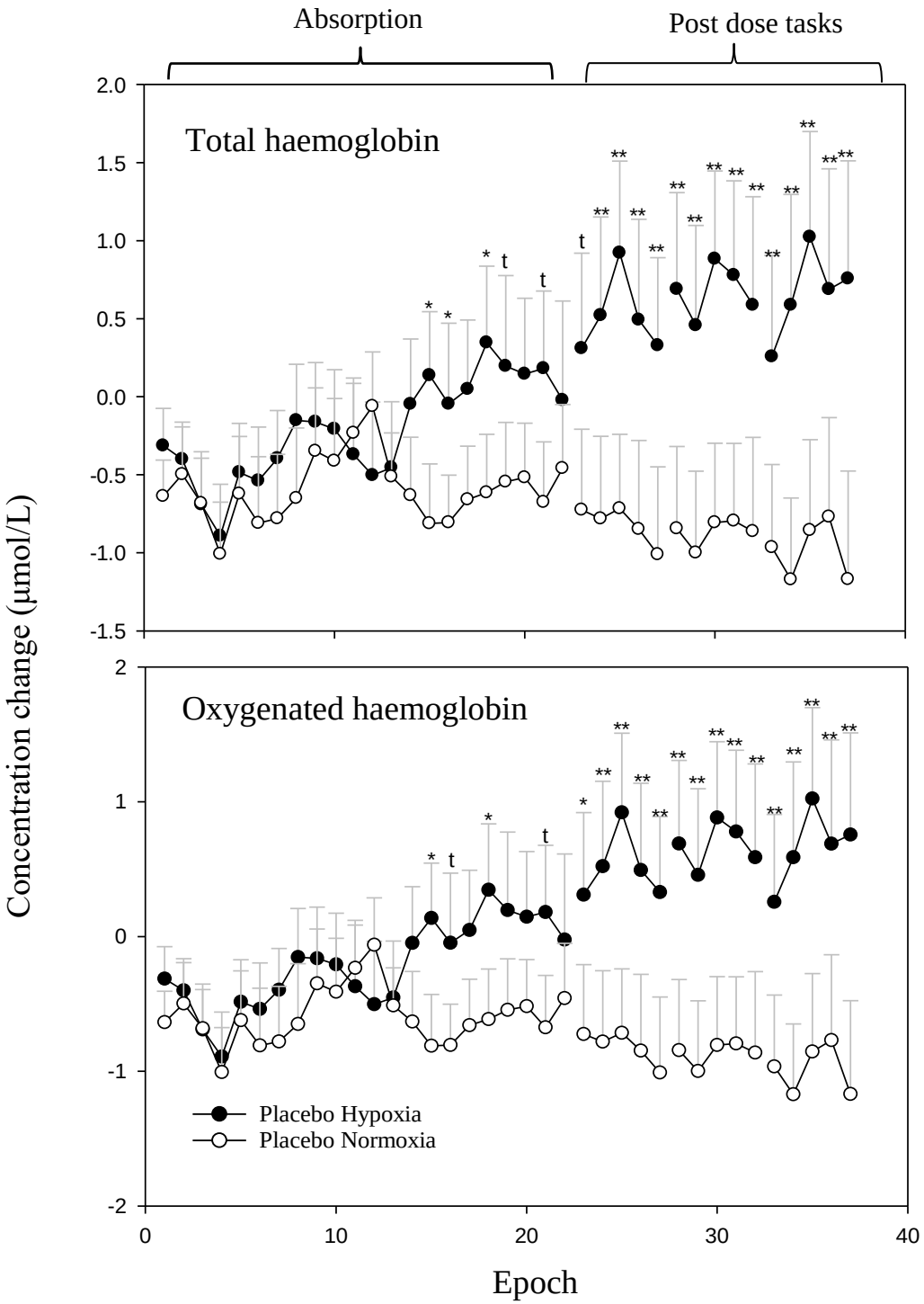

Figure 5.3 - The acute cerebral hemodynamic effects following administration of placebo in the prefrontal cortex in hypoxic and normoxic conditions. Figure displays average changes to total and oxygenated haemoglobin concentrations (with SEM error bars), throughout the absorption and post dose tasks. **p<.01, *p<.05 and t = trend (nearing significance).

5.4.2 Behavioural data

Assessment of baseline – A series of ANOVAs and independent t-tests were conducted for each task outcome, to comparing differences between the baselines and to ensure there was no effect of hypoxia on the baseline scores. All differences between baselines were statistically non-significant (p >.10).

Serial 3 subtractions – A significant treatment × hypoxia / normoxia × repetition interaction [F (2, 46) = 3.467, p =.040] was found for correct responses entered during the Serial 3 subtraction task (Figure 5.4). However, follow up post-hoc t tests revealed no significant differences between either treatment (during hypoxia & normoxia respectively) at any of the three post-dose repetitions. However, within treatments, the resveratrol normoxia condition showed to score significantly higher on the number of correct entries on the Serial 3 subtraction task in comparison to the resveratrol hypoxia condition across all 3 repetitions of the post dose tasks (p =.012; p =.011; p =.047 respectively). Moreover, the number of correct responses in the placebo normoxia condition at repetition 3, revealed to be significantly higher than that of repetition 1 (p =.005) and 2 (p =.019).

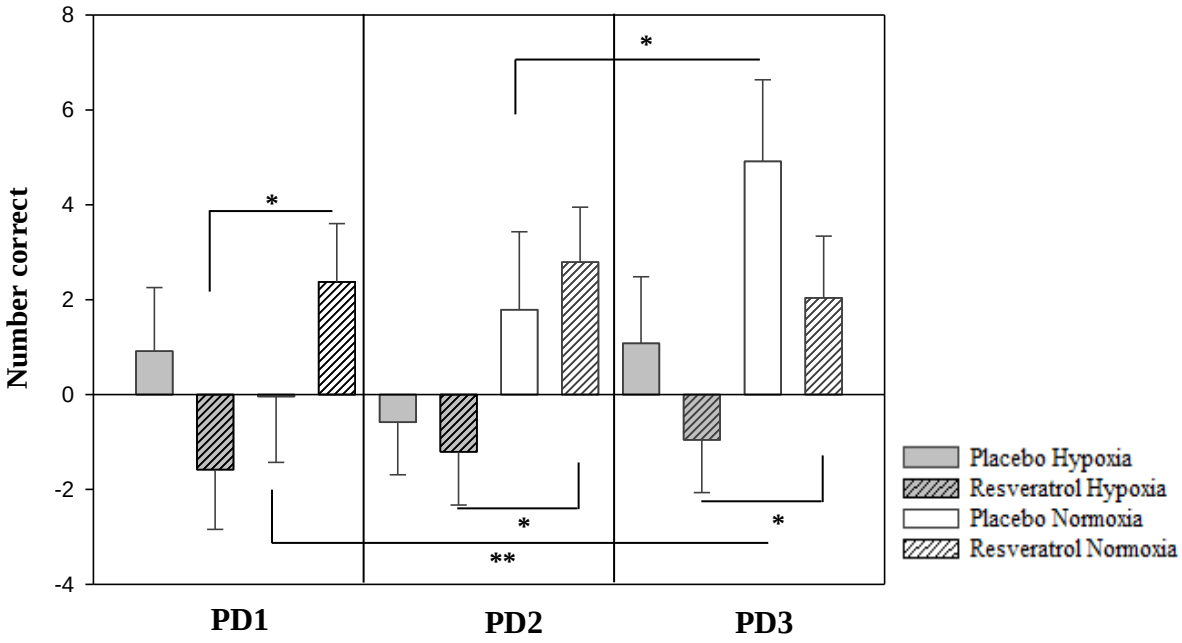

Figure 5.4 The treatment × hypoxia / normoxia × repetition interaction effect of 500 mg resveratrol and placebo administration on the number of correct responses on the Serial 3 subtraction task. Figure displays change from baseline means (with SEM error bars), of number of Serial subtraction correct responses after 500 mg resveratrol or placebo, in hypoxia and normoxia, across the three post dose repetitions.

CRT – A significant main effect of treatment was found for correct reaction time [F (1, 23) = 4.827, p =.038], revealing that supplementation with resveratrol (M= 13.64) increased reaction time in comparison to placebo (M= -6.93) (Figure 5.5).

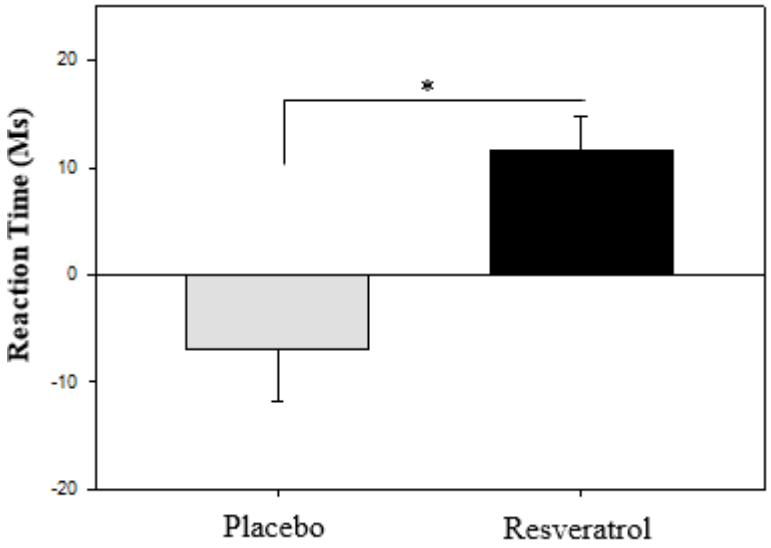

Figure 5.5 The main effect of treatment for 500 mg resveratrol and placebo administration on reaction time on the choice reaction time task. Figure displays change from baseline means (with SEM error bars), of correct reaction time on the choice reaction time task after administration of 500 mg resveratrol or placebo (*p<.05).

RVIP – A significant treatment × hypoxia / normoxia × repetition interaction for RVIP correct reaction time was observed [F (2, 44) = 5.406, p =.016]. Follow up post-hoc student t tests revealed no significant differences between either of the treatments or at any of the three post-dose repetitions (Figure 5.6). Although a trending improvement (decrease) in reaction time was found in the resveratrol normoxia in comparison to the placebo normoxia condition (p =.065) during the first repetition, no other difference between treatment were found across the environmental condition or repetition. While, a similar improvement was observed for the resveratrol hypoxia condition in contrast to the placebo hypoxia condition (p =.074) during the second repetition. Within treatments, the placebo hypoxia condition during repetition 2 showed to have a significantly worse (higher) reaction time in comparison to the same condition during repetition 1 (p =.009) and 3 (p =.004).

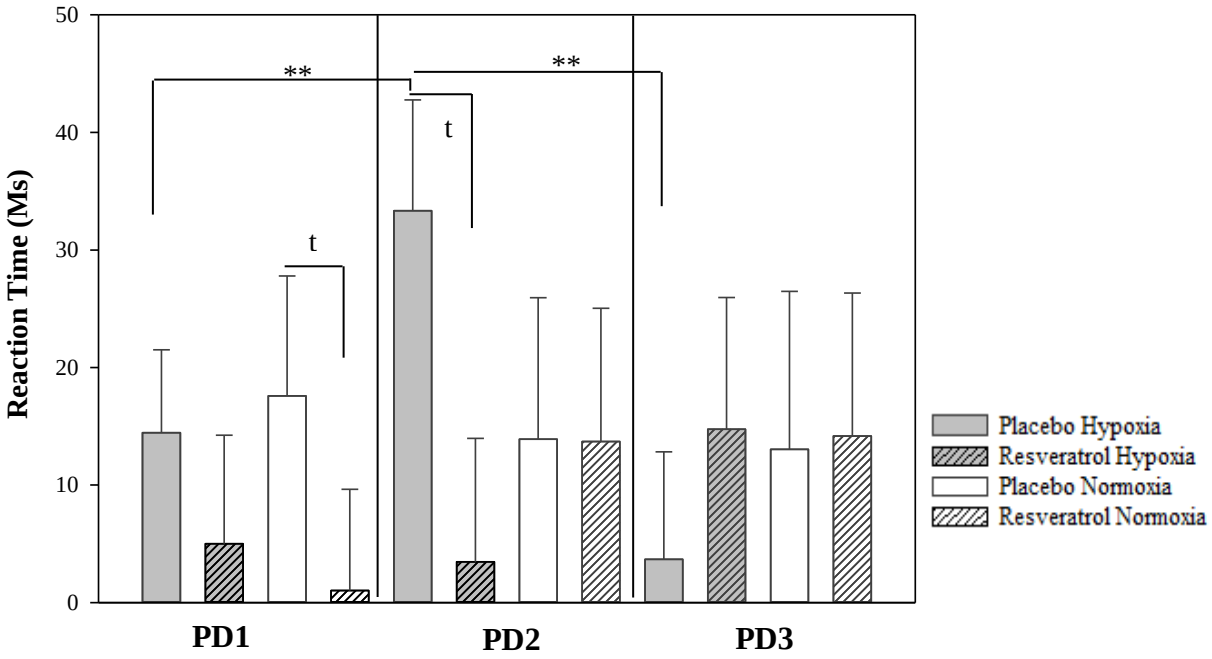

Figure 5.6 The treatment × hypoxia/ normoxia × repetition interaction effect of 500 mg resveratrol and placebo administration on RVIP reaction time. Figure displays change from baseline means (with SEM error bars), of reaction time (ms) from the correct responses on the RVIP task. This was following 500 mg resveratrol or placebo, in hypoxia and normoxia, across the three post dose repetitions.

5.4.2.1 Overall cognitive domain performance

No significant treatment or subsequent treatment related interaction with repetition was found on any of overall cognitive domains.

5.4.3 Mood data

No significant treatment or subsequent treatment related interaction with repetition was observed for any of the self-reported mood parameters.

Table 5.0 The effects of 500 mg resveratrol against placebo on cognitive performance in both hypoxic and normoxic conditions. Table displays baseline and change from baseline scores (*with SEM values in brackets*) for the 3 post-dose battery repetitions after placebo and 500 mg resveratrol for 24 healthy, young adults. Table also displays ANOVA F and P values for main effects of treatment (T), oxygen status (OS) the interaction between the two (T*OS), repetition (T*R) and all three (T*OS*R). *<.05, **<.01 and T= trend.

Measure	Treatment	N	Task Battery				ANOVA		
			Baseline	1	2	3	Effect	F	P
3's Correct (number)	Placebo Hypoxia	24	48.29 (*3.37*)	.92 *(1.34)*	-.58 *(1.11)*	1.08 *(1.40)*	T	.603	.445
	Placebo Normoxia	24	46.88 (*3.87*)	-.04 *(1.39)*	1.79 *(1.64)*	4.92 *(1.72)*	OS	5.872	.024*
	Resveratrol Hypoxia	24	49.12 (*4.10*)	-1.58 *(1.26)*	-1.21 *(1.12)*	-.96 *(1.11)*	T*R	2.344	.107
	Resveratrol Normoxia	24	48.25 (*3.76*)	2.38 *(1.23)*	2.79 *(1.16)*	2.04 *(1.30)*	T*OS	.628	.436
							T*OS*R	3.467	.040*
3's Incorrect (number)	Placebo Hypoxia	24	2.00 (*.48*)	.71 *(.54)*	.83 *(.45)*	1.04 *(.70)*	T	.015	.904
	Placebo Normoxia	24	1.63 (*.31*)	.13 *(.48)*	-.21 *(.40)*	.00 *(.47)*	OS	11.797	.002**
	Resveratrol Hypoxia	24	1.63 (*.39*)	.71 *(.41)*	1.00 *(.47)*	1.17 *(.42)*	T*R	1.108	.328
	Resveratrol Normoxia	24	2.46 (*.45*)	-.83 *(.53)*	-.04 *(.44)*	.25 *(.52)*	T*OS	.102	.753
							T*OS*R	.624	.540
Choice Reaction Time % Correct	Placebo Hypoxia	24	96.46 (*1.01*)	-.10 *(.68)*	.83 *(.78)*	.42 *(.84)*	T	.326	.573
	Placebo Normoxia	24	96.46 (*.94*)	1.67 *(.76)*	1.67 *(.78)*	.42 *(.78)*	OS	1.424	.245
	Resveratrol Hypoxia	24	97.08 (*.65*)	-.31 *(.63)*	-.10 *(.57)*	.94 *(.69)*	T*R	.861	.429
	Resveratrol Normoxia	24	97.08 (*.76*)	.83 *(.36)*	.94 *(.49)*	.10 *(.65)*	T*OS	.309	.583
							T*OS*R	.342	.712
	Placebo Hypoxia	24	428.05 (*17.22*)	-15.02 *(10.65)*	-13.44 *(8.68)*	-1.72 *(14.64)*	T	4.827	.038*

Choice Reaction Time % Correct RT (msecs)	Placebo Normoxia	24	430.02 (*19.63*)	-14.80 *(15.29)*	.17 *(12.21)*	-1.56 *(9.51)*	OS	.189	.668
	Resveratrol Hypoxia	24	409.23 (*12.44*)	9.51 *(6.57)*	11.06 *(6.91)*	9.77 *(7.10)*	T*R	.530	.602
	Resveratrol Normoxia	24	422.21 (*10.70*)	8.69 *(7.98)*	9.25 *(7.19)*	17.60 *(9.70)*	T*OS	.024	.861
							T*OS*R	1.581	.243
RVIP % Correct	Placebo Hypoxia	23	72.56 (*12.44*)	.82 *(3.30)*	-.82 *(3.13)*	-6.79 *(3.13)*	T	.108	.746
	Placebo Normoxia	23	72.28 (*3.85*)	1.09 *(2.74)*	-.54 *(3.31)*	1.63 *(3.95)*	OS	4.221	.052t
	Resveratrol Hypoxia	23	78.26 (*2.99*)	-1.90 *(2.79)*	-5.98 *(3.32)*	-8.42 *(3.44)*	T*R	1.325	.276
	Resveratrol Normoxia	23	70.38 (*3.67*)	6.52 *(2.68)*	-.27 *(2.41)*	1.63 *(2.92)*	T*OS	.550	.466
							T*OS*R	.831	.442
RVIP Correct RT (msecs)	Placebo Hypoxia	23	463.38 (*12.98*)	14.44 *(7.07)*	33.34 *(9.43)*	3.68 *(9.15)*	T	1.079	.310
	Placebo Normoxia	23	471.36 (*12.72*)	17.58 *(10.21)*	13.89 *(12.06)*	13.03 *(13.45)*	OS	.000	.983
	Resveratrol Hypoxia	23	468.77 (*10.87*)	5.00 *(9.23)*	3.47 *(10.50)*	14.74 *(11.22)*	T*R	2.781	.073t
	Resveratrol Normoxia	23	469.85 (*11.49*)	1.03 *(8.61)*	13.69 *(11.35)*	14.17 *(12.17)*	T*OS	.109	.745
							T*OS*R	5.406	.016*
RVIP Errors (number)	Placebo Hypoxia	23	1.48 (*.63*)	-.83 *(.55)*	-.52 *(.53)*	-.48 *(.59)*	T	.150	.871
	Placebo Normoxia	23	.87 (*.30*)	.39 *(.23)*	.17 *(.20)*	.35 *(.36)*	OS	2.225	.437
	Resveratrol Hypoxia	23	1.17 (*.31*)	-.30 *(.24)*	-.13 *(.22)*	.04 *(.24)*	T*R	.058	.398
	Resveratrol Normoxia	23	1.13 (*.28*)	-.30 *(.30)*	-.04 *(.29)*	-.04 *(.33)*	T*OS	1.720	.203
							T*OS*R	1.333	.274
	Placebo Hypoxia	24	5.94	-2.54	-2.54	-1.44	T	.106	.748

Delayed Word Recall Correct (number)			(*.09*)	(*.48*)	(*.61*)	(*.60*)	OS	4.787	.039*
	Placebo Normoxia	24	5.78 (*.45*)	-1.17 (*.42*)	-.65 (*.47*)	-.98 (*.54*)	T*R	1.946	.410
	Resveratrol Hypoxia	24	6.06 (*.46*)	-1.21 (*.55*)	-2.75 (*.45*)	-2.02 (*.62*)	T*OS	1.192	.286
	Resveratrol Normoxia	24	6.17 (*.55*)	-1.56 (*.38*)	-1.23 (*.47*)	-1.40 (*.40*)	T*OS*R	1.946	.154
Delayed Word Recall Incorrect (number)	Placebo Hypoxia	24	.33 (*.12*)	.42 *(.22)*	.62 *(.24)*	.46 *(.21)*	T	.874	.359
	Placebo Normoxia	24	.50 (*.14*)	-.08 *(.24)*	.42 *(.31)*	.25 *(.30)*	OS	.008	.929
	Resveratrol Hypoxia	24	.75 *(.23*)	-.29 *(.20)*	.13 *(.27)*	.21 *(.23)*	T*R	.850	.410
	Resveratrol Normoxia	24	.21 (*.09*)	.13 *(.09)*	.33 *(.17)*	.42 *(.16)*	T*OS	2.552	.124
							T*OS*R	.952	.394

5.4.4 Cognitive performance in hypoxia

Serial 3 subtractions – A significant main effect of hypoxia / normoxia was found [F (1, 23) = 5.872, p =.024] demonstrating a significant reduction in the number of correct responses on the Serial 3 subtraction task within the hypoxia condition (M= -.389) in comparison to the normoxia condition (M= 2.312) (Figure 5.7).

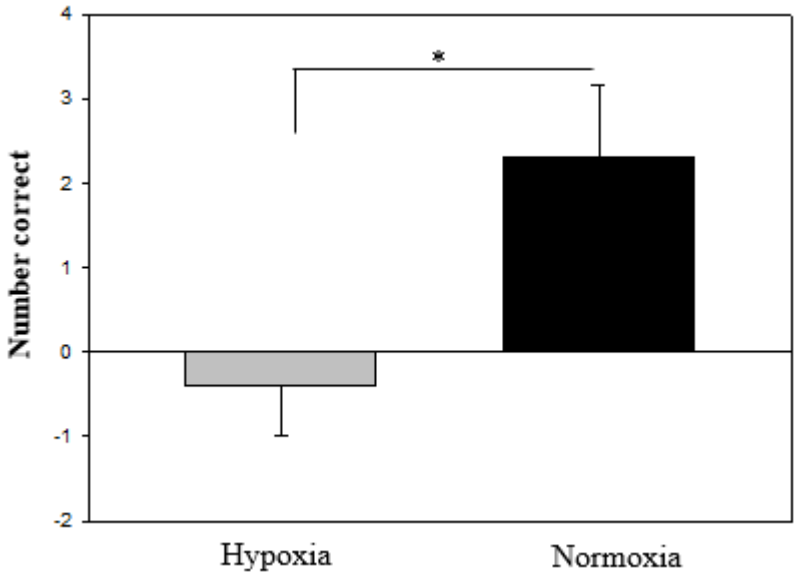

Figure 5.7 The main effect of hypoxia/normoxia on the number of correct responses on Serial three subtraction tasks. Figure displays change from baseline means (with SEM error bars), on correct Serial subtraction responses in hypoxia and normoxia (*p<.05).

A significant main effect was also found of hypoxia/normoxia [F (1, 23) = 11.797, p =.002] demonstrating a significant increase in the number of errors entered within the hypoxia condition (M= .910) in comparison to the normoxia condition (M= -.118) (Figure 5.7).

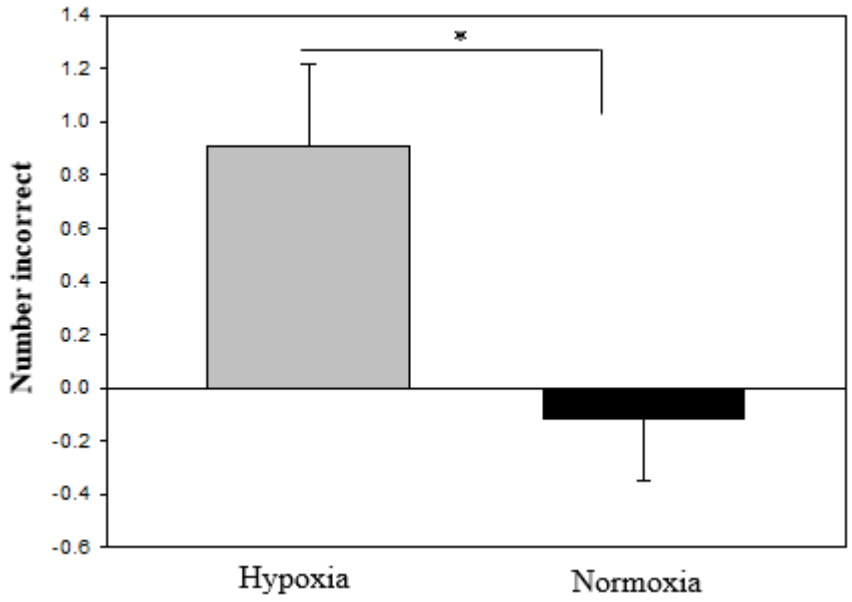

Figure 5.8 The main effect of hypoxia/normoxia on the number of error responses on Serial three subtraction tasks. Figure displays change from baseline means (with SEM error bars), of incorrect responses on the Serial subtraction task whilst in hypoxia and normoxia (*p<.05).

Delayed word recall – A significant main effect of hypoxia/normoxia was found on the number of correctly recalled words on the delayed word recall task [F (1, 23) = 4.787, p =.039]. This demonstrated a significant reduction in the number of correct responses within the hypoxic condition (M= -.2.083) in comparison to the normoxic condition (M= -1.163) (Figure 5.9).

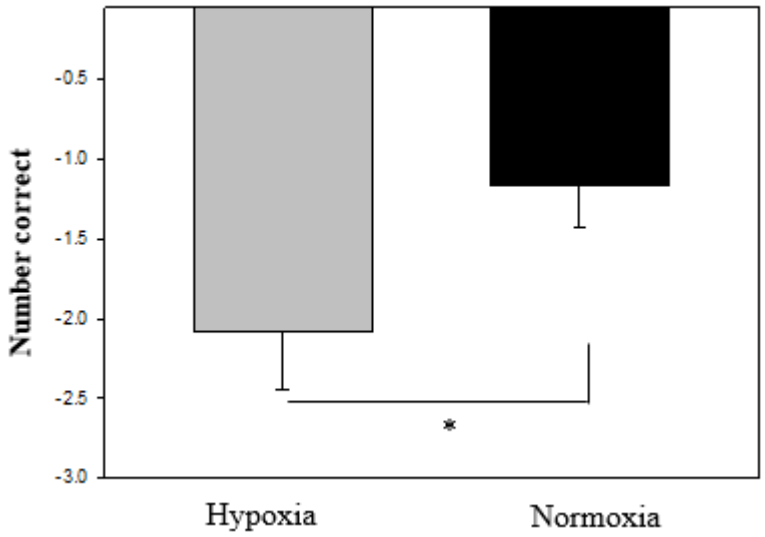

Figure 5.9 The main effect of hypoxia / normoxia on overall number of words correctly recalled on the delayed word recall task. Figure displays change from baseline means (with SEM error bars), of correct words recalled in hypoxia and normoxia (*p<.05).

5.4.4.1 Overall cognitive domain performance in hypoxia

Accuracy – A significant main effect of hypoxia/normoxia was found for overall accuracy performance across tasks [F (1, 22) =7.324, p= .013] which showed lower accuracy within the hypoxic condition (M= -.041) in comparison to the normoxic condition (M= .041) (Figure 5.10).

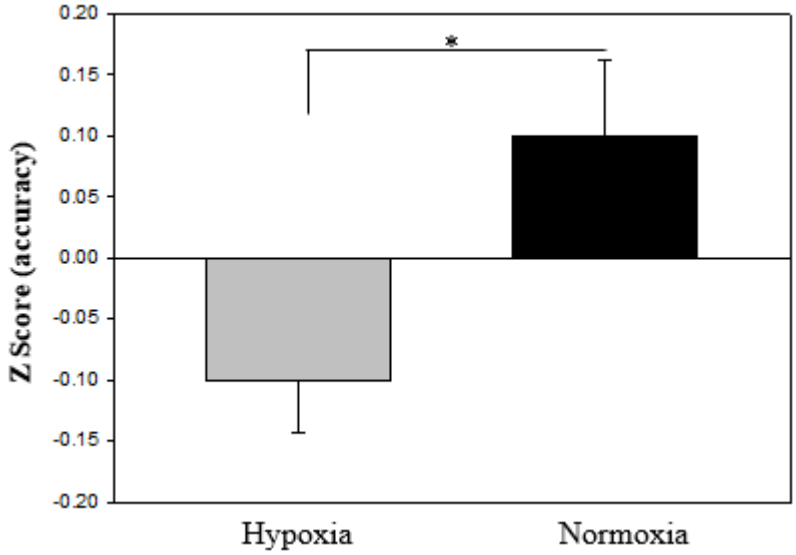

Figure 5.10 The main effect of hypoxia / normoxia on overall accuracy (number of correct responses) across task performance. Figure displays change from baseline Z score means (with SEM error bars), on overall accuracy (% / number correct) across Serial 3 & 7, CRT, RVIP and Stroop tasks in both hypoxia and normoxia (*p<.05).

5.4.5 Mood in hypoxia

Mental fatigue – A significant main effect of hypoxia/normoxia was found on self-reported levels of mental fatigue [F (1, 23) = 25.326, p <.001] revealing that hypoxia (M = 12.37) significantly increased mental fatigue in comparison to normoxia (M = 2.65) (Figure 5.11).

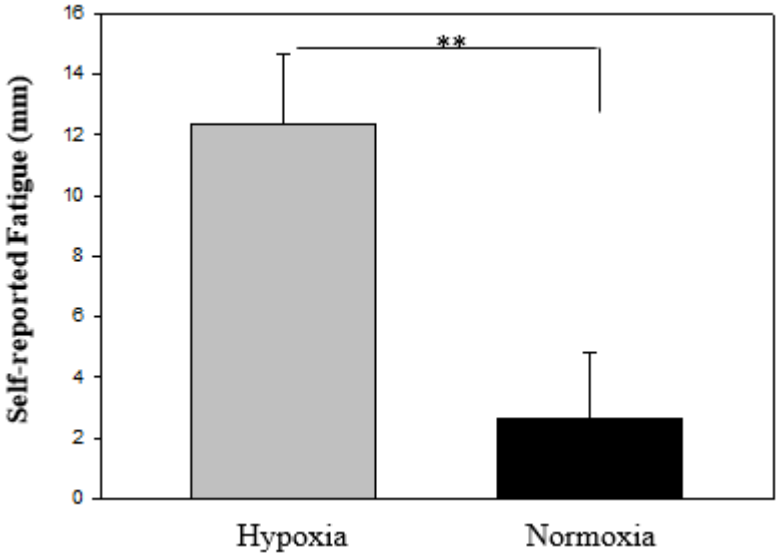

Figure 5.11 The main effect of hypoxia / normoxia self-reported levels of mental fatigue. Figure displays change from baseline means (with SEM error bars), of self-reported mental fatigue (mm) in normoxia and hypoxia (**p<.01).

5.4.5.1 The effect of the environmental chamber on mood

No significant main effect of day, hypoxia / normoxia nor subsequent interaction effect was found for any of the self-reported mood parameters (p >.80).

Cognitive results for all individual tasks (and their subsequent outcomes measures) in this chapter are reported in full in Appendix 3.0, while results for overall cognitive domains are reported in Appendix 3.2. Mood data can be found reported in full in Appendix 3.1.

5.5 Discussion

The current study aimed to build upon the results of the preceding chapter by employing a more disruptive level of hypoxia (12.7% F_iO_2) to model the role of age-related decline in cognitive performance. It was hypothesized that this higher level of hypoxia would induce significant decline on several individual tasks and overall cognitive domains. Moreover, it was also predicted that a single 500 mg dose of resveratrol would be capable of modulating CBF, irrespective of the environmental condition and that this would be exaggerated further within hypoxia; serving to attenuate any hypoxia induced cognitive deficits.

In contrast to Chapter 4, the results here showed that the hypoxia condition significantly increased CBF in the prefrontal cortex, as indexed by significantly higher concentrations of total-Hb, relative to the normoxia condition. Consequently, significantly increased concentrations of oxy-Hb were observed across both the absorption and post dose task performance during hypoxia relative to normoxia. This is perhaps unsurprising, as the brain will continually combat reductions to cerebral oxygenation with increased cerebral perfusion to avoid potential damage to the CNS (Reis et al., 1994; Harris et al., 2013). Despite this adaptive response, as anticipated, the current study did find that exposure to hypoxia significantly reduced cognitive performance across several task outcomes.

Indeed, significant reductions were observed in both the number of correct and incorrect entries on the Serial 3 subtraction task during hypoxia in comparison to normoxia. This was further complemented by a significant reduction in the number of correctly identified words on the delayed word recall task in the hypoxia condition relative to the normoxia condition. Moreover, on analysis of the composite cognitive Z scores, overall accuracy was found to significantly decrease across task performance. Such decreases in cognitive performance are consistent with previous research into both hypoxia (Petrassi et al., 2012; McMorris et al., 2017) and ageing populations (Harada et al., 2013); providing firm evidence of the overall success of hypoxia as a representative, experimental model of the cognitive ageing process.

Interestingly, participants also reported to experience significantly higher levels of mental fatigue within hypoxia in comparison to normoxia. This provides further confirmation that exposure to a reduced O_2 supply can also impact mood, although, contrary to the suggestions of the previous chapter, no significant main effect or relevant interaction with O_2 status was found for self-reported ratings of anxiety. However, these findings may

actually support claims for the aforementioned anxiogenic nature of the testing environment, as this may infer that the nature of the environment itself / the perceived nature of the reduction in O_2 was anxiety inducing, rather the reduction in O_2 itself. Although, additional analysis of the baseline mood scores showed that there was no significant main effect of day, O_2 status or subsequent interaction between the two factors for any of the mood measures. It is reasonable to assume that the increased exposure to the chamber over the 4 testing sessions would naturally reduce any negative effect on mood, yet this was not supported by the results here.

With regards to treatment related effects on cognition, a significant treatment × hypoxia / normoxia × repetition interaction effect was found for both Serial 3 correct responses and RVIP correct reaction time. However, the series of follow up student t-tests provided no interpretable pattern between the treatments and hypoxia / normoxia conditions across the 3 post-dose repetitions. A single significant main effect on reaction time on the CRT task which revealed resveratrol increased (worsened) reaction time relative to placebo; however, as this represents 1 / 17 task outcomes, this is likely to be a type I error. Moreover, on further observation of the difference between the two treatments, the resveratrol condition showed an increased reaction time of less than 20 ms in comparison to the placebo condition; thus, it would be difficult to argue that such a small decrease could indeed impact cognitive performance.

The results here demonstrated that a single 500 mg dose was capable of inducing CBF changes in the prefrontal cortex within the normoxic condition, in line with previous investigations (Kennedy et al., 2010; Wightman et al., 2014; Wightman et al., 2015). This was indexed by significant increases in both total-Hb and oxy-Hb concentrations in comparison to placebo, across the post dose task period. However, contrary to the initial hypothesis, resveratrol was again unable to significantly increase CBF during hypoxia when compared to placebo. The lack of resveratrol-mediated modulation of CBF during hypoxia is somewhat unanticipated. Of course, it is noteworthy that the insult of hypoxia is global across the brain and therefore the measurement of oxygenation of the prefrontal cortex here may not be representative of the oxygenation status of additional brain regions.

However, as the vasodilatory response to acute hypoxia is NO mediated, it is possible that further stimulation of NO synthesis is not possible by any exogenous means, resveratrol or otherwise. In support, a small but growing body of research has shown that

administration of other nutritional NO-dependent vasodilators have also demonstrated poor success in increasing cerebral oxygenation in hypoxic conditions. In a randomized, double-blind, crossover investigation, Lefferts et al. (2015) found that acute supplementation of dietary nitrates was unable to promote increased neurovascular coupling or cognitive performance in moderate-high hypoxia (11.6% O_2). Additionally, Masschelein et al. (2012) found that 6 days of nitrate loading prior to each testing session improved arterial and muscle oxygenation status, but not cerebral oxygenation during exercise in high hypoxia (11% O_2). Querido and Sheel (2007) note that, as the autoregulation of the CBF response operates to protect the CNS from hypoxic damage, it is only interrupted once capillary O_2 saturation (S_aO_2) reaches <60%, therefore any attempt to exaggerate this response above this level may be redundant (Masschelein et al., 2012). As <60% capillary O_2 saturation could not have been achieved by the hypoxic level employed in the current study, it is clear that the CBF effects of resveratrol here may have been unable to modulate the heightened CBF response further.

It appears therefore, that the use of the hypoxic model employed here may have been a doubled edged sword. Despite clear reductions in cognitive performance in the current chapter, 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 hypothesized to increase cognitive performance. Moreover, 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 the model was intended to mimic. As naturally ageing cohorts typically experience a reduction in both peripheral and cerebral blood flow, evaluating the capacity of resveratrol to indirectly enhance an already heightened vasodilatory response is not necessarily directly comparable. Therefore, it is clear research directly into ageing cohorts is still warranted.

Chapter 6

The cerebral hemodynamic response and cognitive effects of acute resveratrol administration in a healthy, 50-70yrs cohort: A double blind, crossover investigation.

6.1 Introduction

The hypothesis underpinning this thesis is that acute administration of resveratrol will be capable of exerting CBF and cognitive benefits in naturally ageing adults. As natural ageing results in reductions to rCBF and $CMRO_2$, it is proposed that the CBF effects of resveratrol will provide an increased benefit to an ageing/ older cohort and subsequently improve cognitive performance via attenuation of the reduction in $CMRO_2$. This hypothesis has hitherto been examined through the ability of resveratrol to mitigate cognitive reductions induced by hypoxia. However, the results of the previous two chapters have revealed that a single dose of resveratrol was unable to significantly increase CBF and/or subsequently attenuate cognitive reductions induced by hypoxia (Chapter 5 only).

Despite the inauspicious performance of resveratrol thus far, research directly into naturally ageing adults is still warranted. Not only is resveratrol unable to increase blood flow further due to a tight regulation of CBF in hypoxia, examining the ability of resveratrol to enhance an already heightened vasodilatory response does not fully represent the reduction in both cerebral and peripheral blood flow experienced by naturally ageing samples. Therefore, further study is needed to evaluate the efficacy of resveratrol to increase a vasodilatory response in an ageing cohort that naturally suffer a reduction in blood flow capacity. In support, despite a lack of success in amplifying the CBF response in hypoxia, acute nitrate administration has been observed to increase cerebral perfusion alongside cognitive enhancement in older adults (Presley et al., 2011; Kelly et al., 2013). Resveratrol has shown greater success in inducing an increased vasodilatory response within individuals who directly suffer a reduction in eNOS expression, such as those with endothelial dysfunction (Wong et al., 2011; Wong et al., 2013). Additionally, a small but growing body of evidence has demonstrated that administration of other vasoactive polyphenols, such as fruit flavanones and cocoa-flavanols, can increase cerebral perfusion (Lamport et al., 2016; Sorond et al., 2008; Lamport et al., 2015) and cognitive performance (Kean et al., 2015; Mastroiacovo et al., 2015; Brickman et al. 2014) in healthy, older adults.

To date, only one study has examined the efficacy of an acute dose of resveratrol in improving cognitive functioning in naturally ageing populations. Scholey et al. (2014) examined the behavioural effects of a resveratrol-enriched wine, within a cohort of older adults (mean age = 70.44 years). The results showed that wine infused with 200 mg of resveratrol evinced a significant improvement in Serial 7 subtraction performance at post dose repetitions two and four in comparison to wine alone. These findings should be interpreted with caution however; with the study failing to compare the results to a dealcoholized control. Moreover, in the absence of monitoring blood flow parameters, it is difficult to attribute these results to increases in CBF. In a recent double blind, placebo controlled investigation, Evans et al. (2017) examined the efficacy of smaller (75 mg) and more frequent administrations (twice daily for 14 weeks) of resveratrol on cerebral responsiveness in a cohort of postmenopausal women (mean age = 61.5); a population acknowledged to suffer a reduction in CBF. The authors reported that the resveratrol group demonstrated significantly augmented cerebral responsiveness during the cognitive test battery (comprising memory and interference tasks), which correlated with an overall increase in cognitive performance. The results of this study provide support for the ability of resveratrol to directly increase cerebral perfusion in cohorts who suffer a reduction in CBF and the subsequent capacity to improve cognitive performance.

Significant age-related decline in cognition has been estimated to begin at approximately 50 years (Aartsen et al., 2002; Rönnlund, et al., 2005), coinciding with initial reductions observed in rCBF and $CMRO_2$ (Lu et al., 2010; De Vis et al., 2015; Aanerud et al., 2012). It is proposed, therefore, that healthy individuals aged >50 years will possess a small but significant decline in rCBF and cognitive performance and that this will be sensitive enough for the vasodilatory effects of resveratrol to be beneficial to cognitive functioning. However, cognitive ageing is thought to accelerate from >70 years (Filley & Cullum, 1994), with individuals beyond this age showing a greater risk of pathological and neurodegenerative decline (Howieson, 2015). Additionally, given the extremes of the ageing process, substantial differences within cognitive functioning can be observed in individuals over this age (Letenneur et al., 2007). Consequently, it may be more prudent to examine the efficacy of resveratrol as a cognitive enhancer between the ages of 50-70 years; thus, reducing the impact of other unforeseen variables in those older than this threshold. With this in mind, this final chapter aimed to investigate whether a single 500 mg dose of resveratrol was capable of increasing CBF, and consequently improving cognitive performance in healthy, ageing adults aged 50-70 years.

6.2 Method

6.2.1 Participants

The current study recruited 24 healthy older adults, aged 50-69 years (18 female, mean age = 59.36, SD 6.00, 22 right handed), who reported themselves to be free of any diagnosed developmental or neurological disorders or who had suffered head trauma. Participants reported themselves to be in good health, free from any food allergies, intolerances or digestive problems and possessed good or corrected vision. All participants abstained from social drugs (including tobacco), herbal / food supplements, and were not taking part in any other intervention study during the full course of their involvement in the study. Female participants taking hormonal replacement for menopause were still eligible to participate. In addition, participants who were pregnant (or seeking to be) smoked, or consumed excessive caffeine (<six cups of coffee or equivalent / daily) were excluded from the current study. Due to the requirements of the tasks, participants were also excluded if English was not their first language, if they suffered from colour blindness, or a relevant learning difficulty.

6.2.2 Standardised diet

Prior to each testing day, participants were required to follow a standardised diet, which required the participants to abstain from polyphenol containing foods and beverages for 24 hours. This included the avoidance of all alcoholic beverages, fruit (fresh, dried, tinned or otherwise), vegetables (including processed products with vegetables) or sauces that included any of the above (e.g. tomato based sauces). Moreover, participants were required to avoid all cocoa containing products including chocolate (white, milk or dark), chocolate confectionery, biscuits and baked goods whilst also abstaining from all soy containing products (e.g. soy beans), nuts or nut based food items. Finally, this diet included avoiding liquorice, honey or herbs, including their extracts, and foods or beverages containing these ingredients (including herbal teas). In addition to the standardised diet, participants were required to fast for 2 hours (except water), abstain from caffeinated foods and beverages for 18 hours prior to arrival and also refraining from taking oral antihistamines and over the counter medications (such as paracetamol & ibuprofen) for 48 hours before testing commenced. The rationale behind implementing these dietary restrictions was to remove the need for these older participants to fast for an extended period (i.e. 12 hrs) while still avoiding the interference from the consumption of other phenolics, caffeine and/or alcohol.

6.2.3 Treatments

The individual treatments were administered on the two separate testing days, with each testing session being conducted no more than 48 hours before, and no more than 14 days after, the previous session. During the two study visits participants received a single-dose treatment in a counterbalanced (Latin square) order, dictated by random allocation:

1) ×2 capsules containing 250 mg resveratrol (equating to a 500 mg dose).
2) ×2 capsules both containing placebo

The treatments were administered in identical, size 0 vegetable capsules, which were prepared by the lead researcher and coded by a third party who had no further involvement in any aspect of the study. Again, no member of the investigational team was aware of the contents of the capsules until the analysis was completed.

6.2.4 Cognitive battery

For consistency and direct comparison to the hypoxic model, the cognitive tasks remained the same as Chapter 5 (see section 5.2.3 for details).

6.2.5 Frequency Domain Near-Infrared Spectroscopy (FD-NIRS)

The OxiplexTS Frequency Domain Near-Infrared Tissue Oximeter (model 99200), with OxiTS software version 3.1, was employed for the current study. Refer to section 2.2.2 for full specification of this device. To maintain a light noise-free operation, all testing took place in a darkened room, with minimal artificial or natural light. Additionally, upon placement of the two NIRS optodes to the participant's forehead, an adhesive bandage was also applied to prevent the interference of any remaining penetrating light. Markers were inserted throughout the recording of the NIRS data to time stamp specific epochs corresponding to individual cognitive tasks. Data collected during the memory tasks was excluded from the analysis due to artefacts caused by the physical movement associated with the paper and pencil responses.

6.2.6 Procedure

For consistency across the thesis, the same testing paradigm employed in Chapters 4 and 5 was also adopted for this final study, with the exception that a different NIRS device was utilized. Participants were required to attend the laboratory on three occasions. The initial visit was a screening / training session, where participants declared themselves to be in line with the inclusion criteria after providing informed consent. Participants were

then trained on the tasks of the cognitive demand battery (this comprised 3 repetitions of shortened version of each task and then 2 full repetitions of the cognitive battery) before the compliance requirements for the following visits were explained. The following two visits were testing sessions, where participants arrived at the testing facility at 1 pm, having adhered to the standardised diet for 24 hours, and fasted for 2 hours (except for water). After confirmation of their continued compliance to the inclusion criteria, participants were connected to the NIRS device. After a brief rest, participants completed a baseline measure of the cognitive battery before watching a non-stimulating video for 10 minutes. Upon completion of the baseline rest measure, the treatment for the day was administered and the participant remained at rest for a further 45 minutes (continuing to watch the same non-stimulating video), to allow for absorption. The participant then completed 3 consecutive repetitions of the cognitive battery. On the final testing visit participants were thanked for their time and fully debriefed. The participants received £30 compensation upon completion of the study.

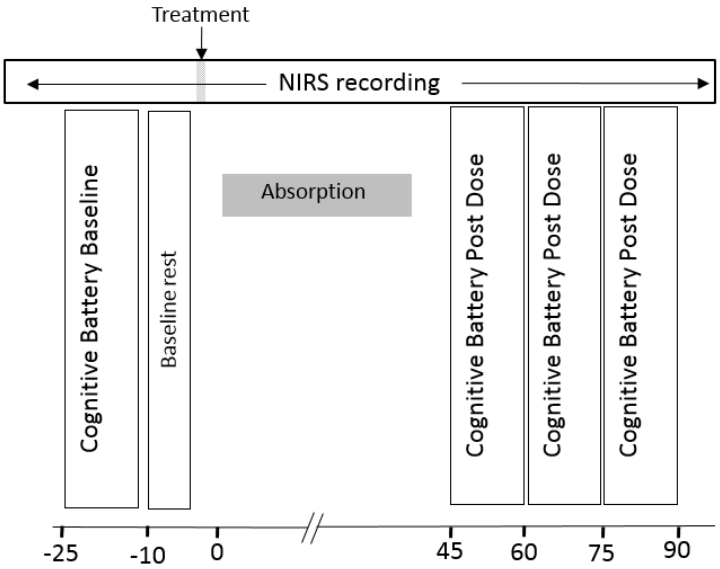

Figure 6.0 A timeline of the fourth experimental study testing day.

6.3 Statistical analysis

All behavioural data was analysed using SPSS version 22 for Windows (IBM SPSS Statistics Armonk, NY), and all NIRS analysis was analysed using Minitab 17 (State College, PA: Minitab, Inc.).

6.3.1 Main NIRS analysis

Prior to any analysis, raw data was graphed and cleaned for the existence of potential outliers / artefacts with the data set. This led to the removal of 5 participants (N=19). Consistent with the previous investigations in this thesis both left and right hemispheres were combined and averaged for analysis. All subsequent NIRS data was converted to change from baseline (utilising the first minute of the absorption period as the baseline) and averaged into 2-minute epochs across the remaining 44-minutes of the absorption and ~40-minute post-dose task periods. Again, due to the artefacts in the data created by the gross movements involved in completing the pen and paper memory tasks, both immediate and delayed word recall tasks were removed from the NIRS analysis. The averaged NIRS data was analysed via within-subjects' ANOVAs, analysing Treatment (resveratrol × placebo) against Epoch (×37 two-minute averaged time points across both the absorption and post task period). Upon significance of the initial F test, subsequent planned comparisons were conducted, using t tests calculated with the MS error values from the initial ANOVAs.

6.3.2 Behavioural analysis

No data was removed during the cleaning process, resulting in a final sample of N=24. Post dose cognitive data and its task sub-measures were then converted to change from baseline performance. For each task sub measure, a Treatment (resveratrol × placebo) by Repetition (×3) repeated measures ANOVA was conducted, followed up by Bonferroni corrected post-hoc pairwise comparisons (on the emergence of significant interaction effects). To provide an indication of the relative cognitive ability of the older cohort, differences in cognitive task performance between the young sample used in Chapter 5 and the older cohort employed here were analysed. A series of independent sample t-tests were conducted on each task outcome for baseline scores from visit 1 for both studies.

6.4 Results

6.4.1 NIRS Data

The ANOVA revealed no significant main effect of treatment nor subsequent interaction with epoch for changes in concentrations of oxy-Hb, deoxy-Hb or total-Hb.

6.4.2 Behavioural data

Baseline analysis – Prior to any analysis, a series of independent sample t-tests were carried out on all baseline scores for each cognitive sub-measure. All other differences between baseline scores showed to be statistically non-significant (p >.094).

RVIP correct – A significant main effect of treatment was found for correct responses on the RVIP task [F (1, 23) = 9.514, p= .005], demonstrating that administration of resveratrol (M= -4.080) led to a lower percentage of correct responses in comparison to administration with placebo (M= 5.12) (Figure 6.1).

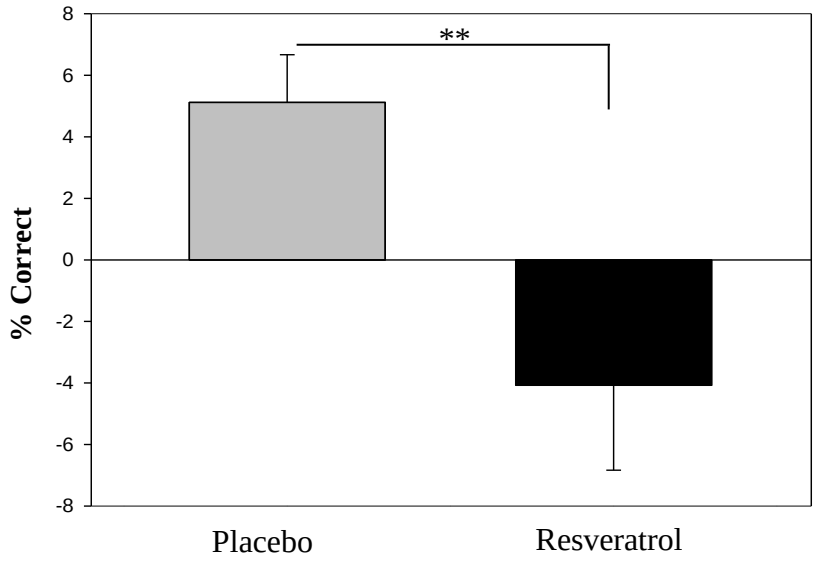

Figure 6.1 The main effect of 500 mg resveratrol and placebo administration on percentage correct responses on the RVIP task. Figure displays change from baseline means (with SEM error bars), of percentage correct responses after 500 mg resveratrol and placebo on the RVIP task (**p<.01).

RVIP reaction time (RT) – A significant main effect of treatment was also found for correct RT on the RVIP task [F (1, 23) = 6.991, p= .015], showing that administration of resveratrol (M= 26.19) led to slower correct responses in comparison to placebo (M= -14.20) (Figure 6.2).

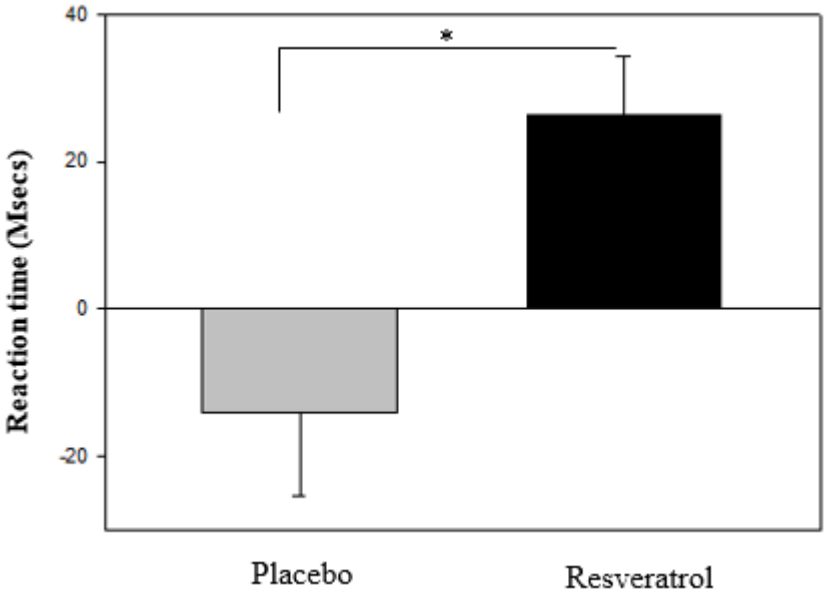

Figure 6.2 The main effect of 500 mg resveratrol and placebo administration on RVIP correct response time. Figure displays change from baseline means (with SEM error bars), of time (in ms) taken to register a correct response following 500 mg resveratrol or placebo on the RVIP task (*p<.05).

No other significant treatment related effects were found for cognitive performance. See Appendix 4.0 for the full reporting of cognitive results.

Table 6.0 The effects of 500 mg resveratrol and placebo on cognitive performance. Table displays baseline and change from baseline scores (*with SEM values in brackets*) for significant treatment related results across the three post-dose battery repetitions after placebo and 500 mg resveratrol for 24 healthy, older adults. Table also displays ANOVA F and P values for main effects of treatment (T) and repetition (R) and an interaction between the two (T*R) with *<.05, **<.01.

Measure	Treatment	N	Task Battery				ANOVA		
			Baseline	1	2	3	Effect	F	P
RVIP % Correct	Placebo	24	56.77 (*5.16*)	.52 (*2.68*)	6.51 (*2.42*)	8.33 (*2.37*)	R	1.579	.217
							T	9.514	.005**
	Resveratrol	24	63.28 (*5.00*)	-2.86 (*3.19*)	-6.77 (*3.49*)	-2.60 (*2.98*)	T*R	3.026	.058t
RVIP Correct RT (msecs)	Placebo	24	529.73 (*14.81*)	-28.32 (*23.46*)	-5.73 (*10.40*)	-8.55 (*10.67*)	R	.026	.935
							T	6.991	.015*
	Resveratrol	24	494.05 (*14.72*)	38.72 (*13.69*)	20.59 (*9.59*)	19.23 (*12.42*)	T*R	1.352	.265

6.4.3 Analysis comparing the cognitive performance of the young and older cohorts

Only significant differences between cohorts are reported here. See Appendix 4.1 for the full reporting of the comparisons between the young (18-35 years) and older (50-70 years) cohorts.

Serial 3: The t test revealed the young cohort (M = 41.17) scored a significantly higher number of correct entries on the Serial 3 subtraction task [t (46) = 4.329, p <.001] in comparison to the older cohort (M = 23.83).

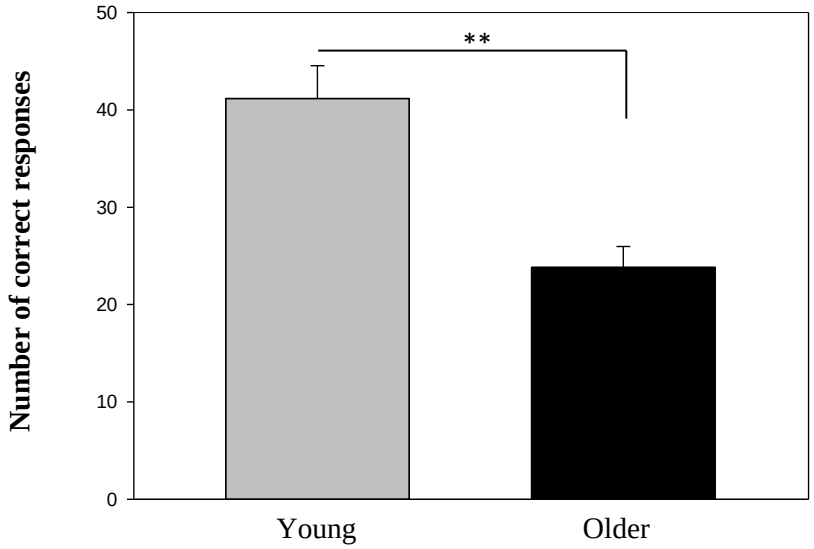

Figure 6.3 The difference between average correct responses entered on the Serial 3 subtraction task for both the young and older cohort. Figure displays the baseline means (with SEM error bars) of the correct number of entries for the Serial 3 subtraction task for the young cohort of Chapter 5 and older cohort of Chapter 6 at baseline of visit 1 (**p<.01).

Serial 7: The t test revealed the young cohort (M = 27.17) scored a significantly higher number of correct entries on the Serial 7 subtraction task [t (46) = 2.086, p = .044] in comparison to the older cohort (M = 18.04).

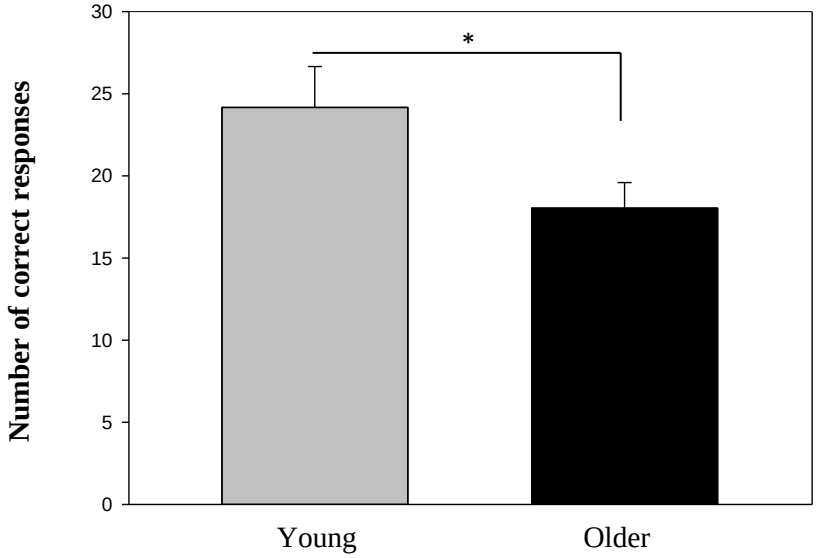

Figure 6.4 The difference between average correct responses entered on the Serial 7 subtraction task for both young and older cohorts. Figure displays the baseline means (with SEM error bars) of the correct number of entries for the Serial 7 subtraction task for the young cohort of Chapter 5 and older cohort of Chapter 6 at baseline of visit 1 (*p<.05).

CRT: The t test revealed the young cohort (M = 441.47) responded significantly quicker for correct responses on the choice reaction time task [t (46) = 2.102, p = .041] in comparison to the older cohort (M = 501.57).

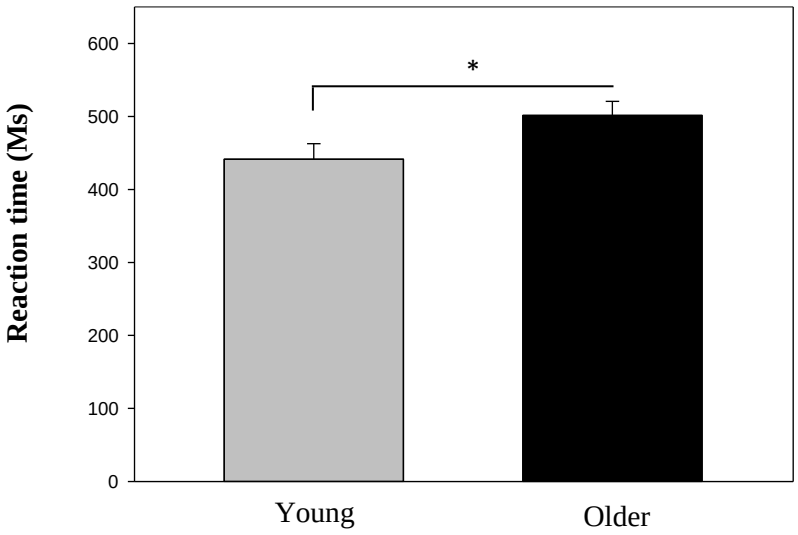

Figure 6.5 The difference between average reaction time to correct responses entered on the CRT task for both young and older cohorts. Figure displays the baseline means (with SEM error bars) for reaction time (in ms) for the young cohort of Chapter 5 and older cohort of Chapter 6 at baseline of visit 1 for the CRT task (*p<.05).

RVIP: The t test revealed the young cohort (M = 448.58) responded significantly quicker to responses on the RVIP task [t (46) = 2.047, p = .046] in comparison to the older cohort (M = 504.67).

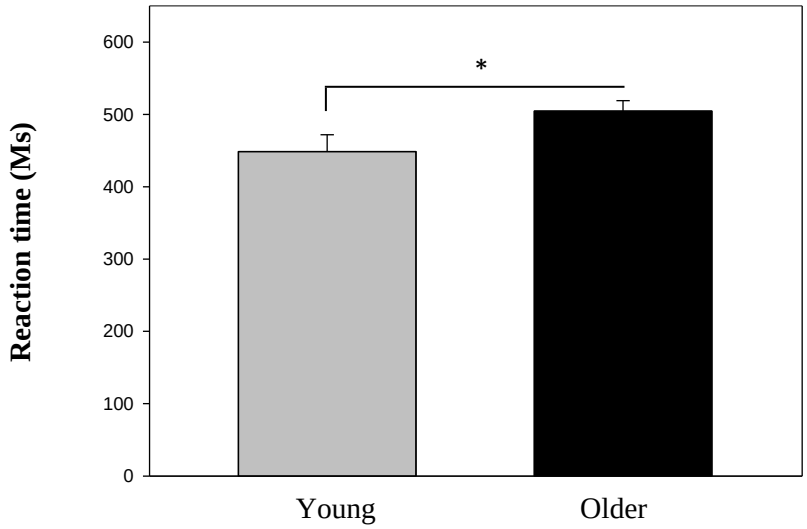

Figure 6.6 The difference between average reaction time to correct responses entered on the RVIP task for both young and older cohorts. Figure displays the baseline means (with SEM error bars) for reaction time (in ms) for the young cohort of Chapter 5 and older cohort of Chapter 6 at baseline of visit 1 for the RVIP task (*p<.05).

Stroop: The t test revealed the young cohort (M = 702.65) responded significantly quicker for correct responses on the Stroop task [t (46) = 4.872, p <.001] in comparison to the older cohort (M = 962.93).

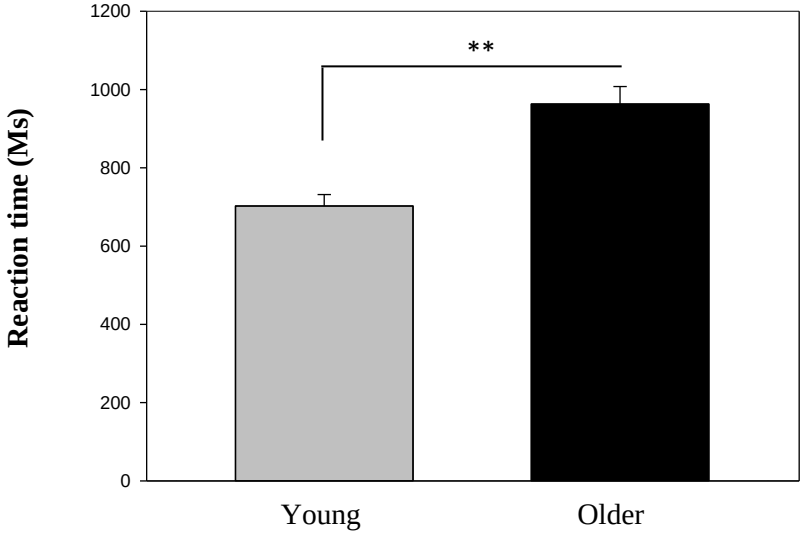

Figure 6.7 The difference between average reaction time to correct responses entered on the Stroop task for both young and older cohorts. Figure displays the baseline means (with SEM error bars) for reaction time (in ms) for the young cohort of Chapter 5 and older cohort of Chapter 6 at baseline of visit 1 for the Stroop task (**p<.01).

6.5 Discussion

This final experimental chapter assessed whether an acute dose of resveratrol could induce significant increases in CBF and cognitive performance in healthy, older adults. It was argued that the CBF effects of resveratrol would provide increased utility in ageing populations, above and beyond those in hitherto utilized younger samples; leading to improved cognitive functioning. The findings here do not support this. Indeed, contrasting with all previous literature (Kennedy et al., 2010; Wightman et al., 2014; Wightman et al., 2015), and Chapter 5 of this thesis, a single dose of 500 mg resveratrol was unable to modulate CBF in the prefrontal cortex during either the absorption period or task performance. In addition, cognitive performance was found to decrease following a single dose of resveratrol, relative to placebo.

Two key methodological factors of the current trial differ from those in previous chapters and may explain these unanticipated findings, particularly the lack of CBF effects; 1, the use of a different NIRS device and 2, the older sample of participants. As little evidence exists to support the use of FD NIRS in the measurement of CBF from nutritional interventions, it is possible that the use of the current FD NIRS device may have contributed to the abovementioned unanticipated findings. This device has been noted to produce a greater level of noise within the data in comparison to its CW counterpart (Davies et al., 2016), whilst also being unable to provide depth specific measurements (Bakkers et al., 2004). It is therefore unlikely that the current study has reliably measured the same area of the prefrontal cortex as recorded in previous investigations; making it difficult to provide an accurate comparison between studies. The benefits of this device (see section 2.2.2) make it particularly practical for the measurement of CBF with the prefrontal cortex in older cohorts but further research is clearly required to confirm the efficacy of the measure of this NIRS in the field.

The second explanation, is that it is also possible that the CBF effects reported here reflect differences emanating from the older sample themselves. Although rCBF has been found to decline with age in areas such as the prefrontal cortex (Lu et al., 2010), it has been proposed that older adults who score highly on cognitive tasks, may be counteracting such age-related neural declines through a plastic reorganisation of neurocognitive networks (Cabeza et al., 2002). Indeed, changes in CBF across the brain are not uniform, with subcortical regions remaining relatively unaffected by reduced blood flow (Chen et al., 2011), which may contribute to neural reorganisation. To add further ambiguity, the

diversity of rCBF reductions are still subject to debate, with several researchers reporting large variance in the extent of rCBF decline, which may result from several individual differences (Sonntag et al., 2007). Despite the uncertainty surrounding the cerebrovascular efficacy of the current sample, it is noteworthy that the older cohort employed within this study were found to perform significantly poorer on several the cognitive tasks in comparison to the young (18-35 years) sample of Chapter 5. Indeed, the current sample were found to score significantly fewer correct entries on both Serial subtraction tasks, while displaying significantly poorer reaction time on CRT, RVIP and Stroop tasks in comparison to the younger participants of Chapter 5. These results support the notion that the participants did indeed possess the anticipated reduction to cognitive performance.

With regards to treatment related effects on cognition, resveratrol was found to significantly worsen cognitive performance in this cohort. This was indexed by significantly poorer accuracy and reaction time on the RVIP task in comparison to placebo. In the face of no effect on cerebral haemodynamics, this may actually point towards a high, acute dose of resveratrol being detrimental in older adults. Interestingly, decreases in executive functioning following long term polyphenol consumption (catechins, proanthocyanidins & flavonols) have been reported previously (Kesse-Guyot et al., 2011). Despite this evidence stemming from a longitudinal study, the authors do highlight that, certain polyphenols, under certain conditions, may exert pro-oxidant effects that may explain the inverse relation in performance (Kesse-Guyot et al., 2011). Similarly, recent evidence would imply a potential hormesis dose effect of resveratrol where by high doses may induce pro-oxidant effects (Plauth et al., 2016); this may (albite tentatively) suggest a high dose of resveratrol may result in a negative impact on cognitive performance.

Building on the above, the CBF and subsequent cognitive benefits of resveratrol may be observed more consistently following smaller doses. Indeed, Wong et al. (2016) examined a range of acute doses of resveratrol (75, 150, and 300 mg) in individuals with type 2 diabetes mellitus (aged 49-78 years); a disorder acknowledged to reduce the ability of cerebral vessels to supply blood to brain regions during local metabolic demand (Janson et al., 2016). All 3 doses showed significantly increased blood flow velocity in the middle cerebral arteries. However, only the lower 75 mg dose was found to additionally increase blood flow velocity in the posterior cerebral arteries. Moreover, when this dose was supplemented for an extended period (14 weeks), Evans et al. (2017)

found significant overall improvements in cognition which correlated with increased cerebral vasodilatory responsiveness in menopausal women. Two further studies have also demonstrated cognitive benefits from resveratrol following 23 weeks of supplementation in dosages of ≤200 mg (Witte et al., 2014; Kobe et al., 2017) in older adults. Taken together, the above evidence may point towards prolonged supplementation of resveratrol in smaller doses to obtain maximal benefit; especially in the age group utilized here.

Additionally and alternatively, it is finally postulated that the standardised diet may have also contributed to the unanticipated findings in this chapter. As all participants were asked to refrain from phenolic containing foods for 24 hours prior to testing, this would have naturally lowered their circulating polyphenol levels and, consequently, may have reduced cognitive performance and attention. This is supported by the notion that polyphenols may not accumulate over time and only reflect the last meal (Rendeiro et al., 2015). Higher intake of polyphenols has been found to lead to significantly better cognitive functioning in comparison to lower intake (Letenneur et al., 2007). Indeed, although average polyphenol intake was not officially monitored, several participants reported the diet to be 'unpleasant' or 'difficult', due to having to avoid regularly consumed foods such as fruit and vegetables. Lamport et al. (2012) report that restriction in habitual phenolic intake may impact a polyphenol intervention and its underlying mechanisms of action. As withdrawal may lead to a relative deficit, it is postulated that additional supplementation within trials may only serve to attenuate the effects of polyphenols removed from the diet; much in the same way that acute caffeine supplementation, following short-term abstinence, simply removes withdrawal effects rather than exerting net effects on the consumer. It is therefore suggested that future acute investigations may be better served recruiting individuals who regularly consume low quantities of polyphenols, in order to obtain the optimum benefits of resveratrol and avoid interference from withdrawal effects.

In conclusion, the current study aimed to investigate whether an acute dose of resveratrol was capable of augmenting CBF and, in turn, cognitive performance in healthy, ageing adults. The results of this study revealed that a single dose of resveratrol was unable to improve CBF and lead to worsened cognitive performance in this cohort. Both findings are surprising; given the consistent CBF findings observed in previous investigations, and the small, but growing literature pointing towards cognitive enhancement following resveratrol supplementation.

Chapter 7

General discussion

The current PhD thesis aimed to assess the potential cognitive enhancing effects of the polyphenol resveratrol, in young and ageing adults. This was in response to a growing body of research which had demonstrated that oral supplementation of resveratrol could induce NO-dependent vasodilation and, consequently, increases in CBF. Despite consistent previous observations of increased CBF and O_2 extraction, no study at the time of undertaking this thesis had found these in conjunction with any consistent modulation of cognitive performance. It was proposed that the young, healthy samples employed in previous studies may have been unable to benefit from increased CBF for two reasons. Firstly, such cohorts are hypothesised to be the in the peak of their cognitive abilities. Secondly, they are also likely able to maintain an effective CBF response during cognitive demand; leaving any improvements from resveratrol to be so small as to be unobservable.

As naturally ageing populations are noted to suffer a reduction in rCBF, $CMRO_2$ and / or a subsequent decline in cognitive performance, it was proposed that the CBF effects of resveratrol could attenuate this decline and therefore improve cognitive performance. However, given the multifactorial nature of cognitive ageing, it was suggested that a model of cognitive ageing would be more appropriate to first test this hypothesis. Indeed, it was proposed that an acute bout of hypoxia to reduce cognitive functioning in young, healthy populations would sufficiently model the age-related decline in neural O_2 delivery and extraction. By first compromising cognitive functioning in young, healthy populations, this would create a clearer picture of the capacity of resveratrol to mitigate a reduced $CMRO_2$, via an enhanced CBF response. This could then be established in healthy, older cohorts directly.

The current thesis therefore, primarily aimed to assess the metabolic, cerebral-haemodynamic and cognitive effects of resveratrol administration in healthy, young and (naturally) ageing cohorts. The ancillary aim of this thesis was to examine the efficacy of a hypoxic model as a representative, experimental model of cognitive ageing.

7.1 Summary of empirical findings

The first empirical chapter (Chapter 3) was a double-blind, placebo-controlled, crossover investigation, which employed ICa to provide a proxy for cerebral metabolism during high cognitive demand. Here, the aim was to establish whether administration of

resveratrol could induce a dose (250 mg, 500 mg) dependent increase in metabolic and substrate expenditure during rest and cognitive performance, within a sample of 27 healthy, young (M age = 22 years) participants. The results here showed that 500 mg resveratrol was capable of evincing significant increases in CHO oxidation during cognitive performance (as indexed by higher RER), 45 minutes and 3 hours post administration, relative to placebo. However, cognitive performance and metabolic / substrate expenditure at rest were unaffected by either dose. The overall conclusion of this chapter was that resveratrol is capable of influencing substrate oxidation during cognitive performance and this provides evidence for its effect on cerebral metabolism. Although, as resveratrol failed to improve cognitive functioning in a young, healthy cohort, it was proposed that administration may be more beneficial in populations who suffer a reduction in the supply of neural fuel substrates; such as naturally ageing populations.

The second empirical study (Chapter 4), a double-blind, placebo-controlled, crossover investigation, aimed to address the conclusions of the previous chapter by employing mild hypoxia (16% F_iO_2) to model the role of decreased rCBF and subsequent $CMRO_2$, in naturally ageing populations from a sample of 24 young (M age = 22 years), healthy participants. It was proposed that a reduction in O_2 would lead to impaired cognitive performance and that administration of a single 500 mg dose of resveratrol would attenuate this decline with an increase in CBF (monitored throughout the session via CW NIRS). Cerebral oxygenation was found to decrease in the hypoxia condition, as indexed by significantly lower concentrations of oxy-Hb relative to the normoxia condition. However, this did not lead to any decline in cognitive performance. As increased O_2 extraction (indexed by significantly higher concentrations of deoxy-Hb) was present throughout the absorption and post dose task period, it was postulated that this may have naturally mitigated the hypoxic environment and the subsequent decreases in cognitive functioning. This suggests that the hypoxic level employed in this chapter was not disruptive enough to model the age-related decline in cognitive functioning.

Administration of resveratrol exaggerated the increase in deoxy-Hb concentrations across the absorption period, but was unable to modulate CBF during the post dose task period in either hypoxia or normoxia. Strikingly however, administration of resveratrol was found to significantly reduce the number of errors entered on both the Serial 3 and 7 subtraction tasks, irrespective of the environmental condition (O_2 status); suggesting an effect of resveratrol itself. This is perhaps the first study to identify cognitive

improvements resulting from a single dose of resveratrol in healthy, young humans. However, in the absence of any modulation of CBF, it was proposed that the ability of resveratrol to offer anxiolytic effects (via modulation of MOA), may have offset the potential anxiogenic environment of the environmental chamber and subsequently provided cognitive enhancement.

The following investigation (Chapter 5); a double-blind, placebo-controlled, crossover investigation, took note of the preceding findings and employed a more disruptive (12.7% O_2) hypoxic environment, in order to model the age-related regional reductions to $CMRO_2$ and consequential deficits in cognitive functioning more effectively. It was hypothesised again that the lower O_2 availability would induce cognitive deficits which resveratrol would then mitigate with an increased CBF response. In keeping with the previous chapter, a further sample of 24 young (M age = 23) healthy participants were employed. Contrasting with the previous chapter, the results showed a significant increase in cerebral oxygenation within the prefrontal cortex (evidenced by significantly higher oxy-Hb concentrations) across both the absorption and post dose task period within hypoxia, in comparison to normoxia. This may have been as a result of the CNS protecting itself from the insult of hypoxia with an exaggerated CBF response.

Despite this, exposure to the hypoxia condition did reveal clear reductions in cognitive functioning and mood. Performance was found to decrease on individual tasks (Serial subtractions & Delayed word recall), while composite cognitive Z score analysis revealed a significant decrease in overall accuracy performance across the tasks. Moreover, participants reported significantly higher levels of mental fatigue when in hypoxia in comparison to normoxia. This provided confirmation of the success of the hypoxic model of cognitive ageing.

In keeping with previous investigations, a single 500 mg dose of resveratrol was found to significantly increase total-Hb and oxy-Hb concentrations in the prefrontal cortex during post dose task performance in the normoxia condition. However, in contrast to the initial hypothesis, resveratrol administration was unable to exaggerate the CBF response to hypoxia across both the absorption and task periods. As the same dose of resveratrol has been shown to clearly increase CBF in normoxia, it is more likely that the hypoxic condition itself must have contributed to the current findings, rather than a lack of efficacy of resveratrol to enhance CBF. It was surmised that, as the sole purpose of the autoregulatory response to hypoxia is to protect to the CNS from hypoxic damage, this

restricts any exogenous factors from increasing this reflex further, blunting any effect of resveratrol.

A single treatment related difference to cognitive performance was observed in Chapter 5. However, this showed that administration with resveratrol significantly worsened reaction time on the correct responses of the choice reaction time task, in comparison to placebo. As this represented only 1 of 17 outcome measures, it was deemed likely a type I error. Moreover, on further inspection of the difference between the means, the worsening in reaction time during the task was so minimal, it would be difficult to attribute this as a cognitive enhancing effect. Despite the lack of modulation of CBF and cognitive performance by resveratrol, investigation into its effect on ageing populations was still deemed to be warranted. The hypoxic model may have inadvertently interfered with the vascular mechanism of resveratrol. Further, exaggerating an already increased CBF response does not fully compare to attenuating reductions in blood flow capacity observed consistently in ageing populations. The success and the constraints of the use of hypoxia as a representative, experimental model of cognitive ageing will be discussed further in section 7.5.

The final empirical study (Chapter 6), also a double-blind, placebo-controlled, crossover investigation, utilized a sample of 24 (M age = 59 years) naturally ageing adults. It was hypothesized that a single dose of resveratrol would be capable of inducing significant increases in CBF and, in turn, improve cognitive functioning. However, the results of Chapter 6 showed no significant resveratrol-mediated modulation of CBF nor of cognitive performance. In fact, resveratrol lead to a decrease in cognitive performance.

This loss of the previously observed resveratrol-mediated CBF effects was proposed to have been the result of the constraints of the FD NIRS apparatus, which may not have measured the same area of the prefrontal cortex as monitored previously. The advantages and constraints of both NIRS devices will be discussed in section 7.6.2. Contrary to previous predictions, resveratrol was found to significantly worsen both accuracy and correct response reaction time on the RVIP task, relative to placebo. However, the requirement to abstain from phenolic containing foods for 24 hours prior to testing, may have only served to contribute to the unpredictable impact on cognitive performance. A summary of the findings across the four experimental chapters can be found below in Table 7.0.

Table 7.0 The summary of effects of 500 mg resveratrol against placebo on cognitive performance, cerebral haemodynamics and metabolism across the 4 experimental chapters. Abbreviations: ↑ increase in performance or physiological measure; ↓ decreases in performance or physiological measure. A diagonal line denotes that this measure was not an outcome in this chapter.

	Chapter 3	Chapter 4		Chapter 5		Chapter 6
		Normoxia	Hypoxia	Normoxia	Hypoxia	
Cognition	No significant effects.	**↑ Improved error rate (S3 & S7 subtractions)**		**↓ RT (CRT)**		**↓ RT (RVIP)** **↓ Accuracy (RVIP)**
Total-Hb		No significant effects.	No significant effects.	**↑ During post dose task performance**	No significant effects.	No significant effects.
Oxy-Hb		No significant effects.	No significant effects.	**↑ During post dose task performance**	No significant effects.	No significant effects.
Deoxy-Hb		No significant effects.	**↑ During absorption period**	No significant effects.	No significant effects.	No significant effects.
RER	**↑ RER (45 mins & 3hrs)**					

7.2 The metabolic profile of resveratrol

The rationale behind investigating the metabolic effects of resveratrol in Chapter 3, was to provide insight into the capacity of resveratrol to enhance cerebral metabolic and substrate utilisation during cognitive demand. It was noted that previously observed increases in CBF and O_2 extraction during both task performance and at rest (Kennedy et al., 2010; Wightman et al., 2014) only reflect the pattern of cerebral metabolic activity and therefore do not quantify the extent of cerebral metabolic change or cerebral EE (Al Naher et al., 2016). Given that the brain is the most metabolic organ in the body, the monitoring of whole body metabolism, via ICa, provided a proxy of resveratrol-mediated increases in blood flow capacity in response to cerebral metabolic change from increased substrate delivery and utilisation. The use of ICa and the methodological restraints of its use will be discussed further in section 7.6.1.

The results of Chapter 3 revealed that both 250 mg and 500 mg doses of resveratrol could significantly increase the oxidation of CHO during task performance in a dose dependent manner. This was evidenced by significant increases in RER and subsequent percentage of CHO of EE during Serial 3 and 7 subtraction performance. This was seen 45 minutes and 3 hours post administration. Although there was no resveratrol-mediated increase in EE, the results still provided interesting confirmation that a single dose of resveratrol can evince a subtle, yet significant, shift in fuel utilization during cognitive demand. Although this was attributed to the CBF effects of resveratrol, it is notable that the shift in oxidation of CHO from resveratrol may also have been as a result of its ability to modulate AMPK; a key regulator in energy sensing procedures (Kulkarni & Cantó, 2015), glycaemic response and energy homeostasis (Hardie et al., 2012). In support, resveratrol has previously been shown to activate AMPK and subsequently stimulate insulin signalling and glucose uptake in both skeletal muscle and neuronal cells *in vitro* (Patel et al., 2011).

Two previous investigations have also reported that acute doses of resveratrol can increase RER at rest (Williams et al. 2013; Scribbans et al., 2014). However, as neither investigation measured cognitive performance, it remains unclear whether this has been beneficial. Indeed, due to the novelty of the paradigm, no clear consensus exists within the literature as to whether an increase in RER during cognitive demand correlates with improved performance. Only two investigations at the time of writing have reported modulation of RER during cognitive performance, with one reporting a significant increase (Delistraty et al., 1991) and the other a significant decrease (Troubat et al., 2009)

in RER across cognitive demand. In a recent review, Grassman et al. (2016) note that the excretion of CO_2 is the only capnographic measure which has shown sensitivity to increasing task difficulty. This is particularly interesting as this implies that cognitive effort not only involves additional EE, but also that increased CO_2 excretion may provide a proxy for increased exertion. As RER is characterised by an increased ratio of $CO_2 : O_2$, it may be that resveratrol can increase the rate of glucose metabolism during cognitive demand and, consequently, may offer a means to boost cognitive performance.

The increase in glucose utilisation observed here may also imply an additional method of enhancement by resveratrol. Other polyphenols have been credited with the regulation of blood glucose and it is proposed that the capacity for polyphenols to do so may allow for a plausible mechanism of cognitive enhancement; given the importance of glucose for cellular function and cognitive performance (Bell et al., 2015). It is hypothesized that the availability of glucose can be enhanced when consumed in a polyphenol-rich food / beverage, as polyphenols may slow the rate of absorption of sugar which consequently results in a greater availability of glucose over an extended period (Bell et al., 2015). In support, polyphenol rich blackcurrant extract has been found to increase blood glucose levels and cognitive performance (Watson et al., 2015), while ~40% and ~17% of the cognitive benefits of cocoa-flavanols observed in elderly adults were attributed to insulin resistance respectively (Desideri et al., 2012; Mastroiacovo et al., 2015).

The ability of resveratrol to shift to a higher CHO fuel utilisation during cognitive performance may imply a possible interaction with glucose utilisation and insulin regulation, and thus, provide an alternative means of physiological effects. Although, as the effects of resveratrol were observed under fasted conditions in Chapter 3, it is interesting to consider that resveratrol could also influence glucose utilisation in conjunction with the consumption of glucose; which may have potentially beneficial ramifications for cognitive performance. Moreover, such resveratrol-meditated modulation of glucose could exist outside of CBF effects and, therefore, warrants further study, particularly in older cohorts who are acknowledged to suffer a reduction in glucose tolerance; an established cause of age-related cognitive decline (Lamport et al., 2009).

Regarding the metabolic effects of resveratrol during rest, previous investigations had observed increased concentrations of deoxy-Hb, 35 minutes following administration (Kennedy et al., 2010) while pharmacokinetic evidence suggests the metabolites of resveratrol peak within ~30 minutes of consumption (Walle, 2011), suggesting that the

onset of resveratrol effects may emerge around this time point; even at rest. This led to the tentative suggestion that the metabolic effects of resveratrol may also be apparent prior to engagement in task performance. However, no treatment related differences were observed 35 minutes following consumption of either the 250 mg or 500 mg dose relative to placebo. It is possible that relative changes in metabolism are subtle and, as this data was not a change from baseline measure, it is likely that this has greatly reduced the sensitivity of the assessment, blunting any potentially measurable treatment related effects.

7.3 The cerebral haemodynamic effects of resveratrol

The premise that acute administration of resveratrol would be capable of exerting an amplified CBF response and increased O_2 extraction / utilization was supported by Chapter 5 and (albeit to a weaker extent) Chapter 4 respectively. However, the additional hypotheses that resveratrol would be able to (1) exaggerate the CBF response and O_2 extraction induced by a hypoxic condition and (2) enhance CBF in samples which experience a reduction in CBF during cognitive performance, were not supported by any study in this thesis. All studies which measured CBF in the current thesis monitored changes in cerebral haemodynamics across both the absorption and post dose cognitive task periods. Chapter 4 revealed that 500 mg resveratrol could enhance O_2 extraction at rest in hypoxia (as indexed by significantly higher concentrations of deoxy-Hb) after only 10 minutes of administration. This was observed to continue until the end of the 45 minute absorption period. However, this effect was seen to diminish immediately upon the start of the post dose cognitive task performance period (see Figure 4.1). Chapter 5 observed that the same dose significantly increased both oxy-Hb and total-Hb concentrations during task performance when in normoxia, indexing an increase in CBF and cerebral oxygenation. However, this finding was not reproduced during task performance within hypoxia (see Figure 5.2 & Figure 5.3). Finally, Chapter 6 saw no treatment related change in cerebral haemodynamics across the absorption or post dose task period in a cohort of older, naturally ageing volunteers.

The resveratrol-mediated increase in both total-Hb and oxy-Hb concentrations in the prefrontal cortex reported in Chapter 5 demonstrated the CBF mechanism of resveratrol; supporting its capacity to increase the availability of neural fuel substrates during cognitive performance. Moreover, as the CBF effects of resveratrol were observed during cognitive performance only, and not during resting / absorption periods, this supports the

notion that resveratrol can increase the NO-mediated endothelial vasodilatory response to neural demand specifically. Although significant increases in O_2 extraction were seen throughout the absorption period in Chapter 4, this was within hypoxia only, and an explanation for this is offered below. The findings of Chapter 5 are broadly in line with that of previous investigations into resveratrol (Wightman et al., 2015) and research into other vaso-active polyphenols (Francis et al., 2006; Sorond et al., 2008). Distinctions can be made however where, despite also finding significant increases in concentrations of total-Hb, both Kennedy et al. (2010) and Wightman et al. (2014) reported complementary increases in deoxy-Hb alongside oxy-Hb concentrations from resveratrol administration. Why the additional increase in deoxy-Hb concentrations has not been replicated here is unclear.

The lack of CBF modulation during cognitive performance in both hypoxia and normoxia in Chapter 4 was unanticipated. The explanation offered was that the anxiogenic nature of the environmental chamber may have added a level of noise to the neuroimaging data when recorded by the NIRS device. Why this has occurred in Chapter 4 and not Chapter 5, is not clear and will be discussed further below. The results of Chapter 4 did demonstrate that 500 mg of resveratrol could enhance the natural increase in O_2 extraction and utilisation (as indexed by increases in deoxy-Hb concentrations) and this started after only 10 minutes following administration. As the hypoxic level was set at the start of the absorption period in Chapter 4, this would demonstrate that resveratrol can respond to the slow but steady decrease to atmospheric O_2 with increased O_2 extraction; thus, offsetting the immediate hypoxic environment, and may suggest a beneficial adaptive effect of resveratrol. However, as this effect was seen to diminish at the end of the absorption period, which consequently was also the time that the hypoxic level would have reached the prescribed mild hypoxic level, this is unlikely to have any bearing on neurocognitive performance in the young sample employed.

Regarding the CBF response during task performance in hypoxia, resveratrol was unable to enhance CBF in either mild (Chapter 4) or moderate (Chapter 5) hypoxia. It is first important to note that the cerebral haemodynamic response during both investigations was measured within the prefrontal cortex only and, therefore, it is possible that resveratrol could have modulated CBF within areas not measured in either study. However, as the autoregulation of the CBF response operates to protect the CNS from hypoxic damage, it appears more likely that the natural vasodilatory reflex, induced upon exposure to hypoxia, cannot be exaggerated by exogenous means and this would likely

be tightly regulated across all areas of the brain. It has been proposed that this autoregulatory response only becomes disturbed once capillary O_2 saturation reaches <60% (Querido & Sheel, 2007) and therefore attempting to augment CBF further, via resveratrol administration, may be redundant (Masschelein et al., 2012). To put this into perspective, the mild hypoxia (16% F_iO_2) employed in Chapter 4 would be the equivalent of a capillary O_2 saturation of ~94%, while the moderate hypoxia of Chapter 5 (12.7% F_iO_2) would equate to ~86% capillary O_2 saturation. Therefore, neither hypoxic level was sufficient to disrupt the autoregulatory response.

This is supported by the observation that other potent vasodilators have been unable to enhance neurovascular coupling in hypoxia. Lefferts et al. (2015) found that acute supplementation of dietary nitrates was unable to promote increased neurovascular coupling or cognitive performance in moderate-high hypoxia (11.6% F_iO_2). Moreover, 6 days of nitrate supplementation prior to exposure to high hypoxia (11% F_iO_2) has been found to improve arterial and muscle oxygenation status, but not cerebral oxygenation, during exercise (Masschelein et al., 2012). This would suggest that even nitrate or resveratrol 'loading' prior to exposure cannot modulate NO-dependent vasodilation. Despite the lack of CBF effects of nitrite supplementation in humans, an increased CBF response within hypoxia has been found in rodent models. However, this is only seen when normal NO bioavailability / production was compromised within the brain (Piknova et al. 2011). An alternative explanation could be that the CBF response of hypoxia is not solely mediated by NO. Indeed, the hypoxia-induced vasodilatory response has also been found to be uninterrupted even when in the presence of a NO inhibitor, questioning whether NO is the sole contributor to the vasodilatory response to hypoxia (Umbrello et al., 2013). A recent review has proposed that several other factors may play a role in the autoregulated CBF response to hypoxia; including adenosine and anaerobic neuronal metabolism (Willie et al., 2014). This would certainly offer a reasonable explanation as to why resveratrol was able to increase deoxy-Hb concentrations in Chapter 4 and not in Chapter 5. However, this would question the use of hypoxia as a model for the ageing brain utilised in the current thesis (this will be discussed further in section 7.5) especially when investigating the use of NO-dependent vasodilators to overcome a compromised O_2 supply.

Finally, the efficacy of resveratrol to augment CBF in naturally ageing populations was not supported in this thesis. This finding was certainly unanticipated and would contradict much previous literature which has seen resveratrol-mediated increases in cerebral

perfusion (Wong et al., 2016) and / or endothelial vasodilation (Wong et al., 2011, 2012) in older or vascular impaired cohorts. Given the consistent ability of resveratrol administration to induce vascular effects following both acute and chronic doses, the most plausible explanation for this observation here is methodological issues. In contrast to the other two studies which monitored CBF (Chapter 4 & 5), a FD NIRS was preferred to a CW device for Chapter 6 (the rationale for this device has been outlined in section 2.1.2) and it is therefore proposed that this may have contributed to the current findings (see section 7.6.2).

7.4 Cognitive and mood effects of resveratrol

The hypothesis of the current PhD thesis was that the engendered CBF effects of resveratrol would enhance cognitive performance via the increased access and utilisation of neural fuel substrates (namely O_2 and glucose) as, when consumed alone, these have been previously found to improve several aspects of cognitive functioning (Scholey et al., 1998; Moss et al., 1998; Sünram-Lea et al., 2001; Scholey et al., 2001). The resulting cognitive benefits of resveratrol however, were proposed to be more observable in populations with a notable reduction in CBF and subsequent $CMRO_2$ e.g. ageing populations. This was also extended to a preceding model of ageing via acute hypoxia (where cerebral oxygenation would also be lower), as previous investigations have revealed an attenuation of behavioural performance following supplementation with additional O_2 (Legg et al., 2016). However, in contrast to predictions, no cognitive benefits of resveratrol were seen in young, healthy participants within hypoxia (Chapter 4 & 5), nor were any cognitive effects of resveratrol established in a sample of healthy, older (50-70 years) participants (Chapter 6). To add further ambiguity, the cognitive enhancing effects of resveratrol were observed, in the absence of any modulation of CBF, in Chapter 4.

Chapter 3 saw no cognitive effects from either 250 mg or 500 mg doses of resveratrol in young, healthy populations on three Serial subtraction tasks in an increasing gradation of difficulty. Chapter 5 also observed no resveratrol-mediated modulation of cognitive functioning when assessed across 8 different tasks, with a combined total of 17 outcome measures, in both hypoxia or normoxia, in a similar cohort.[7] This is perhaps unsurprising

[7] A single treatment related increase in performance was observed, where administration of resveratrol was found to significantly worsen reaction time on the choice reaction time task relative to placebo. However, as this represented 1 of 17 outcome measures, this was deemed more likely to be a type I error.

given that no investigation previously had observed consistent improvements in cognitive performance resulting from an acute or chronic dose of resveratrol in young, healthy cohorts. However, Chapter 6 utilized the same battery of cognitive tasks as Chapter 5 in a healthy, older sample and found that 500 mg resveratrol had a negative impact on performance, in the form of slower reaction time and reduced accuracy on the RVIP task in comparison to placebo (see Figure 6.3). Interestingly, despite no modulation of CBF during task performance, administration of 500 mg resveratrol was found to significantly reduce the number of incorrect responses entered during both the Serial 3 and 7 subtraction tasks (see Figure 4.5 & Figure 4.6). As this was a main effect of treatment, and not an interaction with O_2 status, it was proposed this was an effect of resveratrol in and of itself.

The absence of modulation of cognitive functioning in young, healthy samples observed in 2 out of 3 studies is consistent with the previous studies investigating the cognitive effects of resveratrol prior to undertaking this thesis (Kennedy et al., 2010; Wightman et al., 2014; Wightman et al., 2015). Further still, this would also generally correspond with the majority of investigations into the cognitive effects of other vaso-active polyphenols such as cocoa-flavanols (Francis et al., 2006; Decroix et al., 2016). The current thesis therefore, has provided further confirmation that the CBF mechanism underpinning the proposed mechanism of action of resveratrol, and indeed many other polyphenols (Bell et al., 2015), is unable to provide consistent cognitive improvements in young, healthy cohorts. As proposed initially, and supported by the increase in fuel utilisation and cerebral oxygenation from resveratrol in Chapters 3 and 5 respectively, this is due to the already superior supply of CBF beyond that of the demand for $CMRO_2$ and ATP consumption (Raichle & Gusnard, 2002; Leithner & Royl, 2014) in the young, healthy samples employed; which are more than likely at the peak capacity of their cognitive functioning (Rönnlund et al., 2005). Although some authors have proposed that the lack of cognitive improvements seen in this population are due to inadequate cognitively demanding paradigms (Massee et al., 2015), the weight of so many investigations, would suggest otherwise.

The enhancement of cognitive performance in the young, healthy sample observed in Chapter 4 was unanticipated. In the absence of the CBF mechanism and / or any interaction with the O_2 status, the results here were attributed to the anxiolytic effects of resveratrol, which may have functioned to offset the potential anxiogenic environment of the environmental chamber. Although most evidence stems from rodent models,

resveratrol has been found to inhibit MOA activity in a dose dependent manner; increasing serotonin and noradrenaline levels (Xu et al., 2010). Moreover, although the body of evidence to support this claim is small, single doses of other polyphenols such as EGCG have been found to significantly improve self-reported stress and calmness (Scholey et al., 2012). Interestingly, a 30 day supplementation of 500 mg cocoa polyphenols was found to significantly improve self-rated calmness and contentedness (Pace et al., 2013). Although this does not imply anxiolytic effects specifically, the authors of this study proposed that polyphenols may prove beneficial in situations of high stress or anxiety (Pace et al., 2013). Such claims could be supported by the findings of Chapter 4 yet, as the majority of evidence for polyphenol-mediated mood enhancement comes predominately from chronic (>28 days) supplementation, and no significant treatment related finding was found on self-reported anxiety in Chapter 5, this finding remains ambiguous and could be the result of an unseen / unknown mechanism of resveratrol.

The cognitive enhancement observed in Chapter 4 is the first to be seen in a young, healthy population from 3 published studies (Kennedy et al., 2010; Wightman et al. 2014; Wightman et al., 2015[8]) and a further unpublished study (Wightman, 2013). Moreover, the findings represent 1 less error across both Serial subtraction tasks; representing only a single improvement across a variety of cognitive tasks / domains. The above is not intended to take away from the significant finding here, but instead contextualises the cognitive enhancement. Additionally, as this finding was observed within a hypothesized anxiogenic environment, it is also emphasised that the effects of resveratrol observed here in a young, healthy cohort were a result of anxiolytic properties rather than direct cognitive enhancement per se.

Due to the relative novelty of the hypoxic model employed in the current thesis, there is little previous polyphenol research to shed light on the lack of cognitive improvements seen within hypoxia in both Chapter 4 and 5. However, a number of trials have been performed to examine the efficacy of vaso-active nitrate supplementation. Lefferts et al. (2015), for example, found that an acute dose of dietary nitrate was unable to promote increased neurovascular coupling or cognitive performance during exposure to hypoxia. More recently, Shannon et al. (2017) found that nitrate rich beetroot juice was able to increase exercise performance but not cognition in either moderate (~14% O_2) or high

[8] Wightman et al. (2014) did observe a single resveratrol-mediated increase on 3back performance but the authors attributed this as a type I error.

(~11% O_2) hypoxia. It is also noteworthy that the lack of cognitive enhancement seen in hypoxia in both Chapter 4 and 5 from resveratrol, were not found in conjunction with any increase in CBF or O_2 extraction during task performance. As the mechanism underpinning the cognitive enhancing effect of resveratrol appeared to be thwarted within hypoxia (see section 7.3), the findings here perhaps suggest a recurring trend for all vaso-active polyphenols that interact with NO signalling.

The lack of cognitive performance modulation by resveratrol within the naturally ageing populations (Chapter 6) would conflict with the small, but growing, body of literature which has found cognitive improvements following consumption of other vaso-active polyphenols; including fruit flavanones (Lamport et al., 2015; Kean et al., 2015) and cocoa-flavanols (Desideri et al., 2012; Mastroiacovo et al., 2015; Brickman et al., 2014). However, the cognitive benefits reported from these investigations appear to be evident following chronic doses (≥8 weeks)[9]. As the current study only investigated the efficacy of a single, bolus acute dose, this may suggest that the cognitive effects of resveratrol will be more apparent following prolonged supplementation, especially in older adults. Indeed, 3 studies to date have observed significant improvements in cognitive performance following prolonged resveratrol supplementation (14-23 weeks) (Witte et al., 2014; Kobe et al., 2017) with one investigation demonstrating cognitive enhancement in direct conjunction with enhanced CBF responsiveness (Evan et al., 2017). However, this does not totally undermine the benefit of single doses. Scholey et al. (2014) found significant modulation of cognitive performance in a cohort of older adults (mean age = 70.44 years) following acute supplementation of resveratrol enhanced wine. These results should be viewed with caution however, as there was no dealcoholized control, nor measurement of CBF. Finally, it is possible that there is simply a lack of research into acute doses rather than a lack of efficacy from acute resveratrol administration.

The results of Chapter 6 revealed that resveratrol worsened aspects of cognitive performance in healthy, older participants. Although no immediate explanation presents itself for this, it was proposed that the dietary requirements of the study may have contributed to these ambiguous cognitive effects. All participants were asked to refrain from consuming phenolic containing foods for 24 hours prior to testing. Lamport et al. (2012) argue against such dietary restrictions of habitual phenolic intake, as this would naturally lower circulating levels of polyphenols and, in turn, may impact the polyphenol

[9] With the exception of Mastroiacovo et al. (2015), who found significant improvements in cognitive functioning following an acute dose of (both 993 mg & 520 mg) cocoa-flavanols.

intervention and its underlying mechanisms of action. This could be the result of withdrawal from regular circulating levels of polyphenols which would mean that subsequent polyphenol administration would serve only to attenuate any withdrawal-induced deficits. In the case of Chapter 6, such deficits could pertain to cognition and may have produced the abovementioned unanticipated results.

In addition, it could be argued seeking out samples that are particularly healthy do not represent 'normal' ageing as the natural ageing process is co-morbid with several other health complaints; including a diminishing efficacy of the cardiovascular system. In fact, it is more likely that, given the strict inclusion / exclusion criteria, the current cohort possessed a higher than normal cerebrovascular functioning. Despite this, the older adults employed in Chapter 6 did possess the anticipated deficits to cognition, as indexed significantly less correct entries on the Serial subtraction tasks and poorer reaction time on the CRT, RVIP and Stroop tasks in contrast to the younger adults of Chapter 5. However, it is noteworthy that the average task scores of the older cohort do meet norms associated with high cognitive functioning for within this age group, and given the cohort's superior (particularly cardiovascular) health, it is unlikely this would allow scope for improvements to cognitive performance via resveratrol-mediated increases to CBF. Both the above points raise an interesting questions for the methodological rigour of future investigations (see section 7.8).

7.5 Discussion of the success of the hypoxic model of ageing

The rationale behind the use of hypoxia to model the cognitive ageing process was based on the premise that ageing is commonly characterized by a decrease in O_2 supply due to diminishing functionality of the human vasculature (Valli et al., 2015). Therefore, the use of hypoxia has been suggested as an experimental model sufficient for studying ageing processes (Cataldi & Di Giulio, 2009). In support of this, altitude induced hypoxia has been found to induce cognitive deficits (Petrassi et al., 2012; McMorris et al., 2017) which interestingly coordinate with those observed during cognitive ageing (Glisky, 2007; Harada et al., 2013).

Due to the multifactorial nature of cognitive ageing, and the various extraneous variables that can exacerbate cognitive decline, it was proposed that the above model would be an appropriate first step. By first modelling these reductions, this would provide a clearer representation of the role of $CMRO_2$ and cognitive decline. The anticipated deficits from

this model would then be more controlled and allow investigation of the capacity of resveratrol to attenuate such deficits; hypothesized to be via increased CBF.

The success of the model was analysed by comparing the placebo conditions to assess the differences in cerebral haemodynamics between hypoxia and normoxia. The impact of hypoxia on cognitive performance was assessed via composite Z scores, representing the cognitive domains assessed in the cognitive batteries: accuracy, speed of processing, errors and secondary memory. All of these domains had previously shown reductions during bouts of altitude induced hypoxia (Petrassi et al., 2012). Chapter 4 employed a mild (16% F_iO_2 = 2134 m above sea level) hypoxia and observed a significant reduction in cerebral oxygenation within the prefrontal cortex. However, this was not found in conjunction with any significant reductions in cognitive performance across individual tasks or overall task domains. Chapter 5 induced a moderate hypoxia (12.7% F_iO_2 = 4000 m above sea level) and successfully demonstrated significant reductions in behavioural performance. This was evidenced by decreases in the performance of individual tasks, including Serial 3 subtractions and delayed word recall, and overall accuracy across tasks. Additionally, participants reported significantly higher levels of mental fatigue when in hypoxia in comparison to normoxia. Contrasting to the previous chapter however, oxygenation and CBF were found to increase when in hypoxia relative to normoxia.

Before undertaking the investigation in Chapter 4, it remained ambiguous as to what level of hypoxia should be used to model age-related cognitive decline. Previous research had only found consistent reductions in cognitive performance at altitudes of >3000 m (Bahrke & Shukitt-Hale, 1993); potentially due to the efficacy of the body to successfully adapt to milder bouts of hypoxia. However, it was also proposed that the inconsistency of results found at mild hypoxia was the result of the durations (1-24 hours) employed, and a lack of control over extraneous variables (e.g. physical exertion). It was suggested therefore, that a short period of exposure to a hypoxic condition would still evince cognitive deficits due to immediate exposure allowing for minimal adaptations (Petrassi et al., 2012). The lack of cognitive reductions observed in Chapter 4 would certainly support the notion that mild hypoxia is unable to induce cognitive deficits at rest. This was proposed to be the result of the high efficacy of young, healthy populations in addition to inability of mild hypoxia to induce cognitive deficits.

The moderate hypoxic level of Chapter 5 induced several convincing reductions to performance both in individual tasks and overall accuracy of performance. However,

these cognitive deficits were not observed in conjunction with a significant decrease in cerebral oxygenation, rather an increase. The increases observed may reflect the cerebral perfusion reflex of the brain which aims to avoid potential damage to the CNS (Reis et al., 1994; Harris et al., 2013). This also potentially lends further support to the argument that the mild hypoxia was not sufficient to induce neurocognitive deficits in Chapter 4, as no comparative adaptive CBF response was induced during the lower hypoxia condition. The significant reductions observed in cognitive performance in chapter interestingly correspond to that of age-related deficits. Therefore, the results of Chapter 5 do provide confirmation that hypoxia could be used to model cognitive ageing.

Despite such convincing reductions in cognitive performance during hypoxia, administration of resveratrol was unable to modulate either cognitive functioning or CBF. The explanation behind the latter has already been discussed in more detail (see section 7.3) but it is logical to hypothesize that the lack of effects in the former is due to the latter. The results of both studies, therefore, beg the question whether the use of hypoxia is an appropriate model of age-related impairment of the cerebral vasculature and subsequent cognitive performance. It would appear from the above discussed results that the use of hypoxia has been a double-edged sword. Firstly, the adaptive NO-dependant vasodilatory response to hypoxia appears unmodifiable by resveratrol (or other NO-dependant vasodilators), which could have nullified the CBF mechanism of cognitive enhancement under investigation in this thesis. Moreover, it could be argued that resveratrol-induced exaggeration of an already heightened CBF response does not fully reflect the reduction in blood flow capacity experienced during ageing.

Indeed, the whole basis of the rationale behind utilizing resveratrol in ageing cohorts was based on its efficacy to induce an enhanced vasodilatory response within individuals who directly suffer a reduction in eNOS expression or an impaired vasodilatory response. For example, individuals who suffer from endothelial dysfunction (and therefore an impaired NO response), such as obese or hypertensive cohorts have been shown to have an increased vasodilatory response from acute doses of resveratrol (Wong et al., 2011; Wong et al., 2013). However, by assessing the capacity to further increase CBF during an event of already heightened blood flow, rather than impaired CBF, does not allow for a fair comparison.

In summary, the results here suggest that the use of hypoxia for an experimental model of cognitive ageing can induce age-related cognitive deficits in a young, health cohort.

However, the interference from the natural protective increase in CBF, induced to protect the CNS during bouts of hypoxia, has inadvertently muddied the interpretation of the CBF effects of resveratrol in hypoxia. Therefore, it becomes difficult to argue for the suitability of hypoxia as a model for the ageing brain when investigating the effects of vaso-active compounds to overcome such reductions; especially those which rely on the interaction with NO signalling to exert their effects. Moreover, the comparison to sea level was also arguably compromised by potential anxiogenic effects induced by the use of an environmental chamber. Given that anxiety has been found to interfere with neuroimaging data, this also makes it difficult to compare the findings within the chamber to previous studies. However, this should not deter further investigation of resveratrol in ageing cohorts directly, nor should it discourage the use of hypoxia as a model for the ageing brain external to the field of nutritional neuroscience.

7.6 Discussion of methodologies

7.6.1 Indirect Calorimetry (ICa)

ICa is a non-invasive method which monitors pulmonary gases to provide an accurate estimate of whole body EE and fuel substrate utilisation. Before now, this method has predominantly been used in sporting and exercise contexts (Westerterp & Plasqui, 2004). However, a small number of studies have employed ICa to evaluate changes to whole body metabolism during cognitive demand. The rationale behind the use of ICa was to build upon the growing CBF evidence of resveratrol measured previously by NIRS which, despite reflecting the overall pattern of cerebral metabolic activity, does not quantify the extent of cerebral metabolic change, nor the relative change of EE as a whole (Al Naher et al., 2016). Therefore, the use of ICa was to gain an insight to any resveratrol mediated changes in fuel utilization and EE during cognitive demand, providing further insight into the CBF effects under investigation in the current thesis.

Previous investigations have demonstrated that ICa can examine subtle changes in EE during cognitive task performance and even during varying task demand (Al Naher et al., 2016). Although the measurement of metabolic changes from nutritional interventions during cognitive performance remains novel, support did stem from a previous investigation which has demonstrated that ICa was sensitive enough to detect subtle changes in fat oxidation from acute and chronic multivitamin supplementation during task performance (Kennedy et al., 2016). ICa was only employed in Chapter 3 of this thesis, but it was capable of detecting resveratrol-mediated increases in RER during task

performance, 45 minutes and 3 hours post administration. Although, again, it remains unclear whether this increase in RER was beneficial (see section 7.2).

As first outlined in section 7.2, no consensus exists within the literature as to what a 'typical' response to cognitive demand entails beyond that of an increase in EE. The direction of results from Chapter 3 demonstrated that RER gradually decreased across the 3 post-treatment task intervals (45 min, 2hr & 3hrs). This would support the findings of Troubat et al. (2009), who reported an initial higher RER upon starting a cognitively demanding game of chess, which then gradually decreased throughout the match. As the cerebral haemodynamic response to cognitive demand typically elevates levels of total-Hb and oxy-Hb (Obrig & Villringer, 1997) and, in turn, lowers deoxy-Hb concentrations (Obrig & Villringer, 2003), ICa data seen in this study and by Troubat et al. (2009) would therefore complement the natural CBF response measured previously by NIRS.

7.6.2 Near-Infrared Spectroscopy (NIRS)

NIRS is a non-invasive neuroimaging technique that is capable of monitoring cerebral haemodynamics in response to varying neural demand on different cognitive tasks (Cui et al., 2011). Typically, the CBF response to neural activation is greater than that of $CMRO_2$ (Leithner & Royl, 2014). Thus, neural demand evinces an increase in both total-Hb and oxy-Hb concentrations and, consequently, lowers deoxy-Hb concentrations (Obrig & Villringer, 1997; Obrig & Villringer, 2003). NIRS is also sensitive to the CBF effects of nutritional interventions during cognitive performance (Jackson & Kennedy, 2013). This is supported by the observation that NIRS can detect both increases in CBF (as indexed from higher levels of total-Hb) from DHA-rich fish oil (Jackson et al., 2012a) and multivitamins (Kennedy et al., 2016) and reductions in CBF from caffeine (Kennedy & Haskell, 2011) and low doses of the polyphenol EGCG (Wightman et al., 2012). The current thesis employed the use of the two different NIRS devices: CW NIRS (the device used in all the above-referenced research) and the novel FD NIRS. The former is the most commercially available of the two, and allows for the observation of concentration change in regional CBF (Hoshi, 2003). The latter however, measures the quantification of the CBF response; thus, permitting the measurement of absolute concentrations of cerebral haemodynamics.

The CW NIRS device has demonstrated previously to be an appropriate instrument to detect the CBF effects of resveratrol including the novel observation of resveratrol-

mediated increases in oxy-Hb, deoxy-Hb and total-Hb concentrations at rest and during cognitive performance (Kennedy et al., 2010; Wightman et al., 2014; Wightman et al., 2015). The same NIRS was also detected CBF effects of resveratrol in response to neural demand (Chapter 5), and at rest, in hypoxia (Chapter 4) within this thesis, adding further support for its use in intervention studies. Moreover, consistent with other investigations (Davies et al., 2016), the CW device also effectively measured the CBF response to hypoxia in the prefrontal cortex; as indexed by decreased oxygenation and increased O_2 extraction (Chapter 4). The consistency of this device across previous trials supports the assertion that CBF measurements were scuppered in the final study which investigated older participants (aged 50-70 years). However, it is likely that the issue here was not with the NIRS per se, but rather the change in NIRS device which was implemented in this age group for 2 key reasons: (1) the standard formula utilised by the CW NIRS is based upon those recommended Duncan et al. (1995), which are only valid for the measurement of brain tissue in individuals aged 17-50 years. (2) The CW device must remain attached throughout the course of a full testing session, making the restrictive nature of the equipment difficult to commit to an extended testing session (~2 ¼ hours).

The measurement of absolute haemoglobin concentrations obtained by the FD device overcame both of these constraints and offered an alternative method of measuring CBF in the prefrontal cortex. Despite the sensitivity of FD NIRS to measure the cerebral haemodynamic effects of nutritional interventions being unestablished prior to its employment in Chapter 6, such FD devices have been reported capable of monitoring neural demand on certain of cognitive tasks (Power et al., 2010). However, as outlined in section 7.3, the efficacy of the FD device to measure the CBF effects of resveratrol is questionable, as no significant treatment-related changes to cerebral haemodynamic were observed in the naturally ageing cohort. In the absence of any data into the CBF effects of resveratrol with the CW NIRS in this age group, it is not possible to determine whether this lack of effect seen here is due to this age-group, the FD NIRS device or, indeed, a combination of the two.

The multi-distance principle of FD NIRS (see section 2.1.2) has been reported to produce a greater level of noise within the data set in comparison to its CW counterpart (Davies et al., 2016). This may result in the measure of superficial tissue, as the device is unable to provide depth specific measurements (Bakkers et al., 2004). The 4 cm separation distance between transmitter and receiver of the CW NIRS has been credited with sufficient spatial resolution reach a depth of 0.5-2 cm (Fukui et al., 2003). This depth is

sufficient to reach the blood vessels and capillaries of the prefrontal cortex (Haque et al., 1998) and reflects neural activity of the prefrontal cortex; as evidenced by the wealth of supporting evidence outlined above and the results of Chapter 5 of this thesis. However, it is unclear whether this precise depth has been replicated with the FD device; therefore, it is unclear whether the same cortical area of the prefrontal cortex has been recorded in comparison to that of previous investigations and those in Chapter 4 and 5 of this thesis.

On the face of such results it appears likely the use of CW NIRS is more appropriate for acute, single dose investigations due to its superior sensitivity to nutritional interventions. This does not discount the use of FD NIRS for future research, rather that its use appears to be better suited to chronic investigations where changes over time can be better measured. Further research is of course needed to understand the efficacy of the FD device and its use in measuring the cerebral haemodynamic response of nutritional interventions. To this researcher's knowledge, several ongoing investigations are currently utilizing the same FD device to examine the CBF response of DHA-rich fish oils, iron and phenolic-rich tea. The results of these forthcoming studies certainly will provide further insight into the use of FD NIRS in the field of nutritional neuroscience.

7.6.3 Environmental chamber

In order to deliver a hypoxic model of aging, the current thesis employed the use of the state of the art environmental chamber at Northumbria University. The use of an environmental chamber was preferred over other methods of inducing hypoxia as this allowed for control over the extraneous environmental factors that can impact cognitive performance in addition to hypoxic conditions (Taylor et al., 2015). Moreover, other artificial methods of inducing hypoxia (i.e. hypoxic gases via face masks) may have provided discomfort to the participant due to the constrains of already wearing a neuroimaging (NIRS) device.

The results of Chapter 4 and 5 demonstrated that the chamber could provide an effective method of inducing hypoxia and the desired effects to mental functioning. This was evidenced by reductions in cerebral oxygenation in the prefrontal cortex (Chapter 4) and cognitive performance (Chapter 5) from the hypoxic condition. Interestingly, some unanticipated results also took place within the chamber, in both environmental conditions, suggesting a psychological influence of the chamber in and of itself. For the first time, clear resveratrol-mediated increases in cognitive performance were observed

in a young, healthy cohort, irrespective of the O_2 status. Moreover, the previously observed resveratrol-mediated increases in CBF at rest, and during task performance, were not observed in the normoxic condition of Chapter 4[10]. These unanticipated cognitive and CBF effects observed within the chamber have raised some interesting questions surrounding its potential anxiety-inducing nature, and whether this may have influenced the neuroimaging and cognitive data measured during the testing sessions. It is noteworthy that exposure to altitude-induced hypoxia (artificial or otherwise) has been found to induce a wide range of adverse psychological changes; including increased feelings of anxiety (Shukitt, & Banderet, 1987). Although, as declining mood states have only been consistently observed at higher levels of hypoxia (>2800 m altitude; ~14.7% F_iO_2) it is unclear why this could have occurred in Chapter 4, which employed a mild hypoxia, but not in Chapter 5 where a moderate hypoxia was used.

In both investigations, participants were introduced to the chamber briefly at the initial training session. This was to familiarise subjects with the testing environment and consequently reduce any physiological and psychological noise that may occur from the anticipation and/or anxiety of the first testing visit. Despite this, it was proposed that an anxiogenic effect induced by the chamber may have played a role in the results of Chapter 4. Although the environmental chamber possesses hypoxic silencers, a continuous level of noise was still present as a result of the regulation of air in both hypoxia and normoxia conditions. Additionally, as the O_2 status was blind to the participant, even the perceived reduction in O_2 may have induced feelings of tension and anxiety. Finally, the laboratory itself is a 20 m^2 space which may have added a further claustrophobic or confining nature to the testing procedure. These factors may all have contributed to the facilitation of an anxiogenic effect, perhaps even more so in individuals who are of a nervous disposition (Roth et al., 2002).

Anxiety induced in simulated altitude can lead to hyperventilation and consequently hypocapnia (vasoconstriction). This can then be exacerbated further by individuals who misinterpreted symptoms of the hypoxia for 'dangerous' side effects (Roth et al. 2002). Interestingly, however, Asmaro et al. (2015) reported that participants were unaware of their behavioural decline during exposure to hypoxia in a hypobaric chamber. In fact, participants reportedly believe that they had continued to perform well on a cognitive battery even at 5334 m and 7620 m stimulated altitude. This may suggest that the hypoxic

[10] Why resveratrol was unable to increase CBF during hypoxia in the same study has been discussed previously in section 7.3.

conditions employed in the current thesis were sufficiently blind to the participants and that the chamber itself may have induced anxiety. This is further supported by the observation that no differences in self-reported anxiety were noted between the hypoxia and normoxia conditions in Chapter 5. As higher hypoxic levels like those induced in Chapter 5 (12.7% F_iO_2) have been seen to induce anxiety and tension, the fact that self-reported anxiety did not increase in the hypoxic condition, in comparison to the normoxic condition, would certainly suggest that the chamber itself was anxiety inducing. It may just be that a greater number of participants were sensitive to this in Chapter 4 than in Chapter 5.

The solution going forward appears to be that more extensive prior exposure to the environmental chamber itself is needed before testing (i.e. all training/ familiarisation sessions to be undertaken within the chamber). Monitoring breathing rate in hypoxia and normoxia also appears essential to further understand and interpret the cerebral haemodynamic response within hypoxic conditions. However, at this time it is not clear how this could be achieved without the potential claustrophobic addition of a mask over the mouth.

7.6.4 Cognitive tasks

The rationale behind the cognitive tasks employed in this thesis was four-fold. Firstly, all tasks employed across the chapters have previously shown sensitivity to nutritional interventions. Secondly, all tasks (with the exception of memory tasks) employed across Chapters 4-6 were capable of activating the prefrontal cortex and were sensitive enough to be detected by NIRS (CW NIRS only) (Wightman et al., 2012; Jackson et al., 2012). Thirdly, the tasks used in Chapters 4 and 5 reflected cognitive domains that were sensitive to reductions in O_2, whilst also being directly relevant to the cognitive domains that see a decline in naturally ageing adults. Finally, the use of these tasks was also proposed to induce sufficient cognitive demand to require additional neural fuel resources which resveratrol was hypothesized to provide.

With reference to the first and third rationale, no previous cognitive improvements had been observed directly from resveratrol consumption, thus an expansive battery of tasks and cognitive domains was used in Chapters 4-6. This included the use of the three tasks in the cognitive demand battery (CBD; which comprises Serial 3 and 7 subtractions and RVIP); all of which were completed for 2 minutes each. The use of these tasks has shown

sensitivity to cognitive improvements following consumption of polyphenol-rich supplements such as cocoa-flavanols (Scholey et al., 2010) and ginkgo biloba (Scholey & Kennedy, 2002) in young, healthy samples. Improvements (on Serial subtractions only) have also been seen following administration with resveratrol-enriched red wine (Scholey et al., 2014) in ageing cohorts. As these tasks are considered to measure working memory and executive functioning, the employment of these tasks across the final 3 chapters was appropriate given that both cognitive domains have shown to decline in hypoxia (Aquino et al., 2013) and during ageing (Peters, 2006). This was also supported by the significant reductions in performance observed on the Serial subtraction tasks in both hypoxia (Chapter 5) and the older adults (Chapter 6). The resveratrol-mediated improvement in the number of incorrect responses entered during both Serial subtraction tasks in Chapter 4 demonstrates that resveratrol may enhance working memory performance.

Memory is particularly subject to decline during ageing (John & Cole, 1986); as evidenced by poorer recall performance in naturally ageing samples (Howieson, 2015). Recall tasks are sensitive to improvements from additional supplementation of O_2 in young adults (Moss et al., 1998) whilst being particularly susceptible to declining performance in response to a reduction in O_2 supply from a hypoxic condition (Aquino et al., 2013). Thus, Chapters 4-6 included immediate and delayed word recall and word recognition; involving the encoding, storage and retrieval of 12 words per repetition of the cognitive battery. No resveratrol-mediated improvements were found on memory performance within the older cohort of Chapter 6, nor were any observable reductions in memory performance found in the older cohort of Chapter 6 in comparison to the younger adults of Chapter 5. Despite this, the moderate hypoxic condition of Chapter 5 did show significantly reduced performance on delayed word recall, in comparison to the normoxic condition, supporting previous findings.

The final task employed in the cognitive battery of Chapter 4 was the 3back task. The 3back task was replaced with CRT and the Stroop task in Chapter 5 and 6 and the aim here was to utilize tasks / cognitive domains that were sensitive to a reduction in F_iO_2. Indeed, Stroop and reaction time tasks are all sufficiently sensitive to observe the reductions in executive functioning and speed of processing within hypoxic conditions (Atkinson, 2016; de Aquino Lemos et al., 2012). The results of Chapter 5 showed a significant reduction in accuracy across the Serial subtraction, Stroop, RVIP and CRT tasks, although, no significant main or interaction effect was found on reaction time / speed of processing. However, significantly poorer reaction times on Stroop, RVIP and

CRT tasks were observed in the older adults in Chapter 6, in contrast to the young adults of Chapter 5; supporting claims that older adults will exhibit slower reaction times / speed of processing (Filley & Cullum, 1994; Salthouse, 1996; Berardi et al., 2001).

Regarding the final aforementioned rationale, all tasks were employed in a continuous demanding battery that was repeated 3 times to ensure that an adequate level of demand was implemented across all 4 studies. Although the use of these tasks, within this format, have not provided previous support for inducing sufficient to evince the benefits of resveratrol in young, healthy populations, this demanding paradigm was hypothesized to be sufficiently taxing to ageing populations in order to draw upon an increased demand for neural fuel resources.

Finally, Chapter 3 utilized the Serial subtraction tasks (3, 7 & 17), each running for 5 minutes each. The use of Serial subtraction tasks has been reported previously to be cognitively demanding by participants and demonstrated to increase both glucose utilisation and heart rate beyond that of peripheral mechanisms (Scholey et al., 2001; Kennedy & Scholey, 2004; Kennedy et al., 2016). As there was a main effect of time (pre vs post completion) on 'mental fatigue' across post-dose cognitive performance in Chapter 3, it can be argued that the young sample employed also found the subtraction battery sufficiently taxing and it may be deemed 'cognitively demanding'. Moreover, Wightman (2013) proposed the objective increase in workload can be observable from the increase in the cerebral haemodynamic response across task performance (or in the case of Chapter 3, the increase in fuel oxidation) which details an increase in demand for neural resources; supporting this point further.

7.7 Discussion of the statistical approach

The statistical method adopted across this current thesis for physiological data (NIRS & ICa) was planned comparisons, but only upon the emergence of a significant F test from the initial ANOVA. Arguably, planned comparisons may be carried out irrespective of the significance of the initial ANOVA and to rely on this may even be deemed 'overly conservative' (Keppel, 1991). However, it could be argued that the CBF effects of resveratrol have now been established and, as such, a more conservative statistical approach should be utilized going forward.

A case could be made to argue for a less conservative approach for Chapter 3 due to the novelty of the paradigm. Yet, as the small number of planned comparisons required was

so small (9 in total), the analysis of ICa data, regardless of the initial ANOVA, would have inflated the risk of a misinterpreting a pattern of significant results as a 'true' effect (Coolican, 2014). Despite this more rigorous approach, resveratrol was found to significantly increase cerebral haemodynamics and RER in the direction of previous investigations. Similarly, regarding behavioural data, given that there is more scope to observe a pattern of effects with large comparisons (i.e. the NIRS data), the small number of comparisons required across the cognitive and mood sub-measures (3 post dose repetitions per chapter) would again increase the risk of misinterpreting patterns of significant follow up t-tests, for true effects. Therefore, given the large number of sub measures and subsequent analysis undertaken, and to protect against this increase risk of a type I error, all cognitive and mood sub measures were analysed via Bonferroni corrected pairwise comparisons rather than planned comparisons.

7.8 Future directions

Upon starting this thesis, research investigating the use of polyphenols for cognitive enhancement was growing rapidly. What was surprising, however, was that only a small number of trials had supported the proposed CBF mechanism of vaso-active phenolics with appropriate physiological data (Bell et al., 2015). Hence, the current thesis aimed to employ a supporting measurement of cerebral activity during each investigation of this thesis. The number of trials investigating resveratrol within humans still remains relatively small; with research predominantly focusing on young, healthy populations. In the face of consistent increases in cerebral perfusion, alongside no subsequent modulation of cognitive performance, in younger cohorts, it appeared axiomatic that the benefits of resveratrol would be more pronounced in those who suffer a reduction in CBF; namely ageing populations. The current thesis aimed to investigate this gap, but with a specific focus on providing clear support for the proposed CBF mechanism for cognitive enhancement of resveratrol.

The findings of the four investigations reported here do not support the argument that resveratrol can provide cognitive enhancement in healthy, naturally ageing populations. However, when taking this forward, if research is to continue in naturally ageing samples, studies should look to relax the extensive exclusion / inclusion criteria set out in the current thesis. Seeking out samples that are particularly healthy does not represent 'normal' ageing as the natural ageing process itself, is co-morbid with several other health complaints. Although the older adults employed in Chapter 6 did possess the anticipated

deficits to cognition, it is noteworthy that the average task scores of the older cohort do meet norms associated with high cognitive functioning for this age group. Moreover, the reductions observed cannot be credited to a decrease in rCBF. In fact, it is more likely that given the strict inclusion / exclusion criteria and the lack of a resveratrol-mediated increase in CBF that the current cohort possess a higher than normal cerebrovascular functioning. Therefore, despite employing a cohort with adequate reductions to cognitive performance adopting an overly conservative approach to sample recruitment may not be representative of the wider population, nor allow any scope for improvement from resveratrol administration, due to the cohort's superior (particularly cardiovascular) health. Nevertheless, this sample group was a key first step prior to investigating more varied cohorts.

Further investigation may be better suited in populations that either self-report mild cognitive decline or possess evidence of an impairment in the cerebral vasculature; such as ageing individuals that are overweight or hypertensive. Indeed, the vasodilatory effects of resveratrol are found to be more pronounced in obese and hypertensive cohorts; as evidence by an increased vasodilatory response from both acute and chronic doses of resveratrol (Wong et al., 2011; Wong et al., 2013). Further, individuals with mild cognitive impairment are more likely than their younger counterparts to exhibit cognitive benefits from vaso-active cocoa-flavanols (Desideri et al., 2012). Although, it should be noted that this does not necessarily restrict the benefits of resveratrol to 'unhealthy' populations, but may also encompass cohorts who generally consume less phenolics in their diet. Indeed, as this thesis has shown, acute investigations may be better served recruiting individuals who regularly consume low quantities of polyphenols and, from a real-world perspective, these cohorts are probably more likely to reap the benefits of polyphenol supplementation yet are, by definition, not likely to receive them from the diet. Chapter 6 has evidenced that restricting phenolic consumption in regular consumers serves only to disrupt or interfere with the CBF / cognitive effects of resveratrol. Therefore, future investigations should be cautious of implementing 'beige' diets and may gain further understanding from measuring phenolic intake prior to enrolment and use this information as a covariate (Lamport et al., 2012).

In addition, the cognitive enhancing effects of resveratrol do not seem confined to the ability to bolster NO-dependant vasodilation. Indeed, the results from Chapter 3 suggest that there may be merit in investigating the potential glucose-regulatory effects of resveratrol further (fasted or postprandial) particularly in older adults who are noted to

suffer a reduction in glucose tolerance; which has shown to be a contributor to cognitive decline. Furthermore, the results of Chapter 4 propose that acute resveratrol administration may interact with mood, which may indirectly provide cognitive enhancement; particularly in events of high stress and or anxiety. Research into the mood enhancing effects of resveratrol (and indeed other polyphenols) is small but, on the face of promising evidence, further investigation is warranted to assess the extent to which resveratrol can improve mood parameters; especially under established stress paradigms. With regards to the future use of the hypoxic model, it is hoped that findings here spark further research into the use of hypoxia to investigate the cognitive ageing process. Although its use to measure reductions in cerebral vascular activity and the capacity of resveratrol to attenuate such deficits does appear to be flawed, this should not discourage the use of hypoxia to model cognitive decline of ageing populations outside the field or indeed other 'hypoxic' populations.

7.9 Concluding remarks

Previous research has observed consistent CBF effects of resveratrol, yet no subsequent cognitive improvements in young, healthy cohorts. The current thesis aimed to examine whether the CBF effects of resveratrol would provide cognitive enhancement in older adults, who are considered to suffer a reduction in rCBF, $CMRO_2$ and consequently cognitive performance. Additionally, this series of investigations also aimed to examine the efficacy of employing hypoxia as a representative, experimental model of ageing in young, healthy populations. This hypoxic model was employed in order to provide a clearer picture into the potential CBF mechanism of cognitive enhancement by resveratrol; to directly attenuate reductions is $CMRO_2$ in an otherwise healthy cohort. The 4 intervention studies reported here demonstrated that an acute dose of 500 mg can increase fuel utilisation (Chapter 3) and CBF (Chapter 5) during cognitive demand in young, healthy cohorts in normoxia. However, no resveratrol-mediated CBF or cognitive benefits were found in hypoxia (Chapter 4 & 5) or in comparatively healthy older adults (Chapter 6). Nevertheless, it was argued that the heightened CBF response in hypoxia may have inadvertently interfered with the CBF effects of resveratrol here, questioning the suitability of hypoxia as a representative model of ageing. Questions about the novel NIRS device utilized, offer potential explanations for the lack of resveratrol-mediated cognitive and CBF effects in this sample. Alternatively, it is possible high, acute doses of resveratrol may be detrimental to aspects of cognition in this cohort; although, further

research is needed to elucidate this claim. Interestingly, cognitive benefits (in the form of reduced error responses) were found irrespective of the previously observed CBF effects (Chapter 4). As this was found within a proposed anxiogenic environment, and irrespective of the O_2 status, it is likely that this cognitive benefit is an indirect result of anxiolytic effects, rather than direct cognitive enhancement.

In summary, the results here would suggest that resveratrol may be unable to evince cognitive enhancement within hypoxic conditions or in healthy, older cohorts. Future research may benefit from further exploration into unanticipated cognitive deficits seen here in older adults. Although this may be a result of the methods employed in this thesis, smaller, more frequent doses may unlock the cognitive benefits of resveratrol.

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Appendix

Appendix 1.0 The summary of the behavioural results of Chapter 3: the effects of 250 mg, 500 mg resveratrol and placebo on mood, with baseline scores and the three post-dose repetitions. Table displays baseline scores, and the change from baseline scores (*with SEM values in brackets*) for the 3 post-dose repetitions after administration of treatment for 26 young, healthy adults. Scores were calculated change from baseline, using the post-baseline scores as a baseline. Table displays ANOVA's F and P values for main effects of treatment (T), repetition (R), Time (PP) and their subsequent interactions (T*R) (*<.05, **<.01 and t= trend).

				Repetition			ANOVA		
Measure	**Treatment**	**Baseline**	**Time**	**1**	**2**	**3**	**Effect**	**F**	**P**
Mental Fatigue	**250 mg Resveratrol**	45.46 (*8.92*)	**Pre**	-8.12 (*2.25*)	-11.92 (*2.66*)	-9.08 (*3.95*)	R	3.206	.062t
			Post	-1.42 (*3.28*)	-5.73 (*3.30*)	1.27 (*3.08*)	PP	10.711	.003**
	500 mg Resveratrol	46.15 (*9.05*)	**Pre**	-6.00 (*3.66*)	-11.46 (*3.96*)	-5.38 (*3.41*)	T	.850	.434
			Post	-4.12 (*4.06*)	-5.23 (*4.03*)	1.08 (*4.00*)	T*R	.580	.678
	Placebo	43.31 (*8.49*)	**Pre**	-3.54 (*2.89*)	-11.15 (*2.51*)	-3.92 (*2.76*)	T*PP	.618	.543
			Post	-1.65 (*2.94*)	1.27 (*3.08*)	5.42 (*2.82*	T*PP*R	1.484	.213
Concentration	**250 mg Resveratrol**	53.85 (*10.56*)	**Pre**	.08 (*2.50*)	4.38 (*3.42*)	6.04 (*3.47*)			
			Post	5.65 (*2.63*)	6.73 (*4.42*)	6.81 (*3.98*)	R	1.157	.323
	500 mg Resveratrol	57.38 (*11.25*)	**Pre**	-1.96 (*3.76*)	-1.69 (*4.68*)	-1.46 (*4.58*)	PP	4.073	.054t
			Post	4.08 (*4.49*)	8.69 (*3.48*)	-1.04 (*5.53*	T	1.502	.233
	Placebo	56.35 (*11.05*)	**Pre**	-7.15 (*2.85*)	-1.62 (*3.27*)	-4.35 (*3.43*)	T*R	1.391	.243
			Post	1.58 (*2.93*)	.58 (*4.22*)	-1.88 (*3.82*)	T*PP	.519	.598
							T*PP*R	1.162	.329

Appendix 1.1 The summary of the behavioural results of Chapter 3: the effects of 250 mg, 500 mg resveratrol and placebo on cognitive performance across baseline and the three post dose repetitions. Table displays baseline and change from baseline scores (*with SEM values in brackets*) for the 3 post-dose repetitions after administration of treatment for 27 young, healthy adults. Table also displays ANOVA's F and P values for main effects of treatment (T), repetition (R) and their subsequent interactions (*<.05, **<.01 and t= trend (nearing significance)).

			Repetition			ANOVA		
Measure	**Treatment**	**Baseline**	**1**	**2**	**3**	**Effect**	**F**	**P**
Serial 3's Total	**250 mg Resveratrol**	110.88 (*8.18*)	7.58 (*2.06*)	8.65 (*2.33*)	14.04 (*2.69*)	R	2.458	.096
	500 mg Resveratrol	112.15 (*8.18*)	10.19 (*1.86*)	9.46 (*2.79*)	9.85 (*2.27*)	T	.145	.865
	Placebo	112.65 (*9.00*)	9.42 (*3.83*)	10.58 (*2.26*)	13.23 (*2.41*)	T*R	.974	.405
Serial 3's Correct	**250 mg Resveratrol**	107.35 (*8.18*)	6.19 (*2.13*)	7.38 (*2.80*)	12.15 (*2.96*)	R	2.251	.116
	500 mg Resveratrol	108.58 (*8.21*)	7.77 (*2.07*)	7.27 (*2.94*)	7.92 (*2.33*)	T	.612	.546
	Placebo	107.00 (*8.99*)	8.69 (*3.93*)	9.54 (*2.24*)	12.88 (*2.41*)	T*R	.734	.521
Serial 3's Errors	**250 mg Resveratrol**	3.54 (*.61*)	1.15 (*.85*)	1.27 (*.92*)	1.88 (*.75*)	R	.029	.972
	500 mg Resveratrol	3.58 (*.56*)	2.42 (*.90*)	2.19 (*.95*)	1.92 (*.76*)	T	1.557	.221
	Placebo	5.65 (*.62*)	.73 (*.70*)	1.04 (*.81*)	.35 (*.89*)	T*R	.499	.736
Serial 7's Total	**250 mg Resveratrol**	77.46 (*7.06*)	4.65 (*1.64*)	6.15 (*1.63*)	8.42 (*2.18*)	R	2.634	.082t
	500 mg Resveratrol	78.50 (*7.71*)	8.00 (*2.02*)	7.88 (*2.10*)	7.77 (*2.13*)	T	1.059	.354
	Placebo	76.04 (*7.65*)	7.85 (*1.78*)	9.19 (*2.17*)	11.54 (*2.46*)	T*R	.839	.504
	250 mg Resveratrol	72.35 (*7.29*)	3.50 (*1.66*)	5.73 (*1.75*)	8.12 (*2.05*)	R	2.108	.132

Serial 7's Correct	**500 mg Resveratrol**	73.50 (*7.54*)	8.65 (*1.87*)	6.96 (*2.10*)	8.12 (*2.12*)	T	.999	.376
	Placebo	70.19 (*7.83*)	7.96 (*1.73*)	8.38 (*2.29*)	10.65 (*2.62*)	T*R	1.044	.388
Serial 7's Errors	**250 mg Resveratrol**	5.12 (*.94*)	1.15 (*.52*)	.42 (*.65*)	.31 (*.98*)	R	.729	.487
	500 mg Resveratrol	5.00 (*.64*)	-.65 (*.75*)	.92 (*.55*)	-.35 (*.75*)	T	.468	.629
	Placebo	5.85 (*.75*)	-.12 (*.70*)	.81 (*.82*)	.88 (*.79*)	T*R	1.531	.199
Serial 17's Total	**250 mg Resveratrol**	48.73 (*5.73*)	4.54 (*1.16*)	6.92 (*1.34*)	7.35 (*1.57*)	R	1.899	.160
	500 mg Resveratrol	51.23 (*5.48*)	4.00 (*1.71*)	5.19 (*1.34*)	4.35 (*1.99*)	T	2.368	.104
	Placebo	47.92 (*5.19*)	8.12 (*1.25*)	6.81 (*1.44*)	10.12 (*1.77*)	T*R	1.989	.102
Serial 17's Correct	**250 mg Resveratrol**	43.96 (*5.63*)	4.38 (*1.24*)	6.69 (*1.49*)	6.38 (*1.82*)	R	1.598	.212
	500 mg Resveratrol	46.00 (*5.41*)	3.50 (*2.07*)	4.73 (*1.65*)	4.69 (*2.11*)	T	2.599	.084t
	Placebo	42.31 (*5.19*)	8.65 (*1.45*)	7.96 (*1.67*)	10.27 (*1.89*)	T*R	.772	.546
Serial 17's Errors	**250 mg Resveratrol**	4.77 (*.92*)	.38 (*.75*)	.23 (*.76*)	.96 (*.84*)	R	.347	.708
	500 mg Resveratrol	5.23 (*.52*)	.50 (*.68*)	.46 (*.64*)	-.35 (*.65*)	T	.871	.425
	Placebo	5.62 (*.73*)	-.54 (*.77*)	-1.15 (*.54*)	-.15 (*.61*)	T*R	1.244	.297

Appendix 2.0 The summary of the behavioural results of Chapter 4: the effects of 500 mg resveratrol and placebo on cognitive performance in both hypoxic and normoxic conditions. Table displays baseline and change from baseline scores (*with SEM values in brackets*) for the 3 post-dose battery repetitions after placebo and 500 mg resveratrol for 23 healthy, young adults. Table also displays ANOVA F and P values for main effects of treatment (T), oxygen status (OS) the interaction between the two (T*OS), repetition (T*R) all three (T*OS*R). *<.05, **<.01 and t= trend (nearing significance).

Measure	Treatment	N	Repetition				ANOVA		
			Baseline	1	2	3	Effect	F	P
3's Correct (number)	Placebo Hypoxia	23	48.04 (*2.95*)	.09 (*1.15*)	.48 (*1.59*)	-2.13 (*1.84*)	T	.858	.364
	Placebo Normoxia	23	44.87 (*3.04*)	1.22 (*1.83*)	.35 (*1.50*)	1.43 (*1.96*)	OS	2.585	.122
	Resveratrol Hypoxia	23	45.78 (*2.52*)	-1.00 (*1.45*)	2.17 (*1.35*)	.22 (*1.54*)	T*R	1.431	.250
	Resveratrol Normoxia	23	44.70 (*3.05*)	2.78 (*1.32*)	3.39 (*1.91*)	1.09 (*1.87*)	T*OS	.029	.866
							T*OS*R	2.145	.129
3's Incorrect (number)	Placebo Hypoxia	23	1.09 (*.29*)	.91 (*.50*)	.96 (*.42*)	2.61 (*.73*)	T	4.813	.039*
	Placebo Normoxia	23	1.65 (*.50*)	.22 (*.59*)	1.52 (*.59*)	1.35 (*.59*)	OS	2.261	.147
	Resveratrol Hypoxia	23	2.87 (*.42*)	-.26 (*.38*)	.48 (*.67*)	1.00 (*.62*)	T*R	.489	.617
	Resveratrol Normoxia	23	1.57 (*.47*)	-.91 (*.62*)	-1.00 (*.50*)	1.04 (*.65*)	T*OS	.067	.798
							T*OS*R	3.321	.062t
7's Correct (number)	Placebo Hypoxia	23	26.43 (*1.97*)	1.61 (*1.10*)	1.35 (*1.24*)	3.00 (*1.14*)	T	1.355	.257
	Placebo Normoxia	23	25.61 (*1.97*)	1.26 (*1.41*)	.22 (*1.42*)	1.17 (*1.44*)	OS	.732	.401
	Resveratrol Hypoxia	23	26.04 (*1.97*)	.00 (*1.23*)	3.17 (*1.32*)	.87 (*1.84*)	T*R	2.284	.114
	Resveratrol Normoxia	23	25.48 (*2.30*)	3.57 (*.93*)	3.65 (*1.04*)	4.57 (*1.01*)	T*OS	3.266	.084t
							T*OS*R	1.526	.229
7's Incorrect	Placebo Hypoxia	23	2.17 (*.54*)	.43 (*.73*)	1.83 (*.47*)	.65 (*.63*)	T	5.447	.029*

(number)	Placebo Normoxia	23	2.39 *(.43)*	.04 *(.46)*	.52 *(.53)*	.78 *(.55)*	OS	.773	.389
	Resveratrol Hypoxia	23	2.65 *(.66)*	-.17 *(.66)*	.00 *(.62)*	.04 *(.73)*	T*R	.933	.401
	Resveratrol Normoxia	23	2.91 *(.44)*	-.65 *(.45)*	-.17 *(.47)*	-.26 *(.42)*	T*OS	.066	.799
							T*OS*R	.940	.398
RVIP % Correct	Placebo Hypoxia	19	55.92 (*5.71*)	5.92 *(2.89)*	-.99 *(4.44)*	4.93 *(3.96)*	T	.231	.637
	Placebo Normoxia	19	61.51 (*5.22*)	.33 *(3.22)*	1.97 *(3.38)*	-4.28 *(4.54)*	OS	.001	.975
	Resveratrol Hypoxia	19	61.51 (*5.04*)	-.99 *(3.74)*	-1.32 *(4.16)*	-4.61 *(3.99)*	T*R	.593	.558
	Resveratrol Normoxia	19	60.53 (*6.48*)	3.29 *(4.26)*	4.28 *(4.51)*	-1.97 *(4.71)*	T*OS	1.194	.289
							T*OS*R	.629	.539
RVIP % Correct RT (msecs)	Placebo Hypoxia	19	494.77 (*10.66*)	3.36 *(10.42)*	-21.56 *(28.90)*	7.63 *(13.77)*	T	1.717	.207
	Placebo Normoxia	19	485.10 (*8.63*)	18.69 *(14.04)*	13.78 *(13.50)*	24.32 *(11.15)*	OS	.190	.668
	Resveratrol Hypoxia	19	484.92 (*11.30*)	-12.56 *(9.28)*	5.63 *(12.14)*	3.01 *(14.77)*	T*R	2.867	.070t
	Resveratrol Normoxia	19	511.03 (*11.87*)	-16.57 *(9.17)*	-4.50 *(12.79)*	-23.64 *(10.76)*	T*OS	3.060	.097
							T*OS*R	.397	.675
RVIP Incorrect (nmber)	Placebo Hypoxia	19	.53 *(.18)*	-.26 *(.20)*	-.26 *(.17)*	-.05 *(.20)*	T	1.163	.295
	Placebo Normoxia	19	.47 *(.12)*	.00 *(.20)*	-.26 *(.19)*	.05 *(.21)*	OS	1.138	.300
	Resveratrol Hypoxia	19	.42 *(.16)*	-.11 *(.13)*	-.05 *(.16)*	-.16 *(.16)*	T*R	3.034	.061t
	Resveratrol Normoxia	19	.21 *(.10)*	.16 *(.12)*	.16 *(.12)*	.05 *(.12)*	T*OS	.208	.654
							T*OS*R	.164	.775

3 Back % Correct	Placebo Hypoxia	21	87.30 (*3.56*)	1.06 *(.85)*	-.32 *(1.48)*	.95 *(1.29)*	T	.022	.883
	Placebo Normoxia	21	87.20 (*3.23*)	2.12 *(1.23)*	-.53 *(1.41)*	4.23 *(.93)*	OS	.700	.794
	Resveratrol Hypoxia	21	87.09 (*3.30*)	1.48 *(1.25)*	.74 *(1.38)*	3.07 *(1.21)*	T*R	1.227	.304
	Resveratrol Normoxia	21	88.57 (*3.33*)	.00 *(1.70)*	.95 *(1.71)*	1.90 *(1.20)*	T*OS	.616	.442
							T*OS*R	1.496	.236
3 Back Correct RT (msecs)	Placebo Hypoxia	21	766.24 (*58.72*)	-93.11 *(34.63)*	-62.84 *(35.33)*	-94.27 *(39.14)*	T	1.166	.293
	Placebo Normoxia	21	764.52 (*63.22*)	-95.37 *(42.22)*	-128.20 *(43.69)*	-125.25 *(40.67)*	OS	.403	.533
	Resveratrol Hypoxia	21	736.52 (*64.89*)	-69.54 *(30.02)*	-82.53 *(28.95)*	-75.59 *(39.06)*	T*R	.326	.724
	Resveratrol Normoxia	21	726.38 (*57.34*)	-55.59 *(25.36)*	-73.19 *(28.65)*	-88.32 *(30.18)*	T*OS	.548	.468
							T*OS*R	1.297	.285
Word Recognition % Correct	Placebo Hypoxia	22	84.09 (*2.10*)	-5.91 *(1.40)*	-2.27 *(2.18)*	-3.64 *(1.94)*	T	.002	.967
	Placebo Normoxia	22	82.12 (*2.02*)	-1.36 *(2.25)*	-1.36 *(2.32)*	.30 *(2.71)*	OS	3.078	.094
	Resveratrol Hypoxia	22	81.97 (*1.69*)	-3.49 *(1.27)*	-3.64 *(2.38)*	-3.94 *(1.99)*	T*R	.303	.740
	Resveratrol Normoxia	22	82.57 (*1.83*)	-2.42 *(2.08)*	.30 *(2.14)*	-1.36 *(2.26)*	T*OS	.037	.849
							T*OS*R	1.550	.224
Word Recognition % Correct RT (msecs)	Placebo Hypoxia	22	798.45 (*52.87*)	-36.19 *(29.25)*	-22.97 *(34.66)*	-60.86 *(41.85)*	T	.535	.472
	Placebo Normoxia	22	744.54 (*27.79*)	26.31 *(25.35)*	45.33 *(48.19)*	4.64 *(20.96)*	OS	1.291	.269
	Resveratrol Hypoxia	22	750.54 (*31.53*)	4.00 *(27.07)*	-22.45 *(24.60)*	-36.31 *(29.86)*	T*R	.406	.669
	Resveratrol Normoxia	22	772.73 (*47.55*)	-23.31 *(32.21)*	-12.34 *(38.39)*	-37.89 *(31.03)*	T*OS	1.373	.254
							T*OS*R	.144	.866

Immediate Word Recall	Placebo Hypoxia	23	8.33 *(.39)*	-1.65 *(.48)*	-1.07 *(.43)*	-.174 *(.46)*	T	.157	.696
	Placebo Normoxia	23	7.67 *(.43)*	-.57 *(.43)*	-.50 *(.45)*	-.67 *(.41)*	OS	.086	.772
	Resveratrol Hypoxia	23	8.33 *(.39)*	-.96 *(.37)*	-.94 *(.49)*	-.65 *(.41)*	T*R	.335	.717
	Resveratrol Normoxia	23	8.15 *(.46)*	-1.13 *(.54)*	-1.04 *(.50)*	-.67 *(.56)*	T*OS	.359	.555
							T*OS*R	1.758	.184
Delayed Word Recall	Placebo Hypoxia	23	6.43 *(.51)*	-2.35 *(.51)*	-2.59 *(.70)*	-1.76 *(.70)*	T	1.735	.201
	Placebo Normoxia	23	6.02 *(.50)*	-1.78 *(.43)*	-1.89 *(.53)*	-1.67 *(.57)*	OS	.015	.905
	Resveratrol Hypoxia	23	5.63 *(.49)*	-1.07 *(.42)*	-1.20 *(.52)*	-1.41 *(.52)*	T*R	1.864	.167
	Resveratrol Normoxia	23	5.96 *(.52)*	-1.54 *(.58)*	-1.65 *(.54)*	-2.13 *(.51)*	T*OS	1.110	.304
							T*OS*R	.078	.925

Appendix 2.1. The summary of the behavioural Z score analysis of Chapter 4: the effects of 500 mg resveratrol and placebo on overall cognitive domains in both hypoxic and normoxic conditions. Table displays change from baseline scores (*with SEM values in brackets*) for the 3 post-dose battery repetitions after placebo and 500 mg resveratrol for 19 healthy, young adults. Table also displays ANOVA F and P values for main effects of treatment (T), oxygen status (OS) the interaction between the two (T*OS), repetition (T*R) all three (T*OS*R). *<.05, **<.01 and t= trend (nearing significance).

			Repetition		ANOVA		
Measure	**Treatment**	**1**	**2**	**3**	**Effect**	**F**	**P**
Accuracy	Placebo Hypoxia	.092 *(.11)*	-.149 *(.14)*	-.010 *(.14)*	T	.229	.638
	Placebo Normoxia	.028 *(.13)*	-.124 *(.12)*	.018 *(.11)*	OS	2.163	.159
	Resveratrol Hypoxia	-.206 *(.10)*	.012 *(.11)*	-.078 *(.13)*	T*R	4.441	.019*
	Resveratrol Normoxia	.155 *(.13)*	.174 *(.17)*	.087 *(.15)*	T*OS	.966	.339
					T*OS*R	1.148	.328
Speed	Placebo Hypoxia	-.055 *(.10)*	-.071 *(.22)*	-.103 *(.18)*	T	.196	.663
	Placebo Normoxia	.188 *(.16)*	.061 *(.16)*	.119 *(.11)*	OS	.153	.701
	Resveratrol Hypoxia	.028 *(.09)*	.014 *(.10)*	.031 *(.15)*	T*R	.668	.226
	Resveratrol Normoxia	-.048 *(.13)*	.009 *(.14)*	-.173 *(.15)*	T*OS	1.474	.240
					T*OS*R	.699	.511
Error	Placebo Hypoxia	-.156 *(.14)*	.156 *(.11)*	.259 *(.19)*	T	1.461	.242
	Placebo Normoxia	-.56 *(.15)*	.011 *(.15)*	.207 *(.18)*	OS	.043	.839
	Resveratrol Hypoxia	-.151 *(.10)*	.023 *(.14)*	-.068 *(.10)*	T*R	1.114	.339
	Resveratrol Normoxia	-.206 *(.11)*	-.112 *(.11)*	.092 *(.13)*	T*OS	.011	.919
					T*OS*R	.685	.511

Secondary Memory	Placebo Hypoxia	-.385 *(.14)*	-.259 *(.19)*	.044 *(.19)*	T	.047	.831
					OS	3.637	.073t
	Placebo Normoxia	.191 *(.16)*	.164 *(.18)*	.168 *(.19)*	T*R	.530	.593
	Resveratrol Hypoxia	-.046 *(.10)*	-.090 *(.17)*	-.40 *(.15)*	T*OS	.489	.493
	Resveratrol Normoxia	.069 *(.19)*	.079 *(.18)*	.102 *(.17)*	T*OS*R	1.378	.265

Appendix 3.0 The summary of the behavioural results of Chapter 5: the effects of 500 mg resveratrol and placebo on cognitive performance in both hypoxic and normoxic conditions. Table displays baseline and change from baseline scores (*with SEM values in brackets*) for the 3 post-dose battery repetitions after placebo and 500 mg resveratrol for 24 healthy, young adults. Table also displays ANOVA F and P values for main effects of treatment (T), oxygen status (OS) the interaction between the two (T*OS), repetition (T*R) all three (T*OS*R). *<.05, **<.01 and t= trend (nearing significance).

Measure	Treatment	N	Repetition				ANOVA		
			Baseline	1	2	3	Effect	F	P
3's Correct (number)	Placebo Hypoxia	24	48.29 (*3.37*)	.92 *(1.34)*	-.58 *(1.11)*	1.08 *(1.40)*	T	.603	.445
	Placebo Normoxia	24	46.88 (*3.87*)	-.04 *(1.39)*	1.79 *(1.64)*	4.92 *(1.72)*	OS	5.872	.024*
	Resveratrol Hypoxia	24	49.12 (*4.10*)	-1.58 *(1.26)*	-1.21 *(1.12)*	-.96 *(1.11)*	T*R	2.344	.107
	Resveratrol Normoxia	24	48.25 (*3.76*)	2.38 *(1.23)*	2.79 *(1.16)*	2.04 *(1.30)*	T*OS	.628	.436
							T*OS*R	3.467	.040*
3's Incorrect (number)	Placebo Hypoxia	24	2.00 (*.48*)	.71 *(.54)*	.83 *(.45)*	1.04 *(.70)*	T	.015	.904
	Placebo Normoxia	24	1.63 (*.31*)	.13 *(.48)*	-.21 *(.40)*	.00 *(.47)*	OS	11.797	.002*
	Resveratrol Hypoxia	24	1.63 (*.39*)	.71 *(.41)*	1.00 *(.47)*	1.17 *(.42)*	T*R	1.108	.328
	Resveratrol Normoxia	24	2.46 (*.45*)	-.83 *(.53)*	-.04 *(.44)*	.25 *(.52)*	T*OS	.102	.753
							T*OS*R	.624	.540
7's Correct (number)	Placebo Hypoxia	24	28.04 (*2.49*)	2.42 *(.70)*	3.21 *(.87)*	2.08 *(1.34)*	T	.016	.900
	Placebo Normoxia	24	27.88 (*2.54*)	2.29 *(1.13)*	1.71 *(1.13)*	2.29 *(1.39)*	OS	.473	.499
	Resveratrol Hypoxia	24	27.17 (*2.53*)	2.42 *(1.49)*	2.96 *(1.12)*	1.79 *(2.15)*	T*R	.306	.738
	Resveratrol Normoxia	24	29.08 (*3.00*)	1.58 *(.84)*	1.08 *(1.24)*	3.42 *(1.08)*	T*OS	.002	.966
							T*OS*R	.388	.681
7's Incorrect	Placebo Hypoxia	24	2.29 (*.45*)	-.08 *(.36)*	.29 *(.49)*	.17 *(.44)*	T	.579	.455

(number)	Placebo Normoxia	24	2.04 (*.38*)	.21 *(.58)*	-.04 *(.34)*	.54 *(.55)*	OS	.109	.745
	Resveratrol Hypoxia	24	2.54 (*.48*)	-.33 *(.55)*	-.17 *(.58)*	2.04 *(1.85)*	T*R	1.283	.279
	Resveratrol Normoxia	24	1.58 (*.36*)	.13 *(.51)*	1.38 *(.72)*	.50 *(.40)*	T*OS	.001	.973
							T*OS*R	2.240	.137
Choice Reaction Time % Correct	Placebo Hypoxia	24	96.46 (*1.01*)	-.10 *(.68)*	.83 *(.78)*	.42 *(.84)*	T	.326	.573
	Placebo Normoxia	24	96.46 (*.94*)	1.67 *(.76)*	1.67 *(.78)*	.42 *(.78)*	OS	1.424	.245
	Resveratrol Hypoxia	24	97.08 (*.65*)	-.31 *(.63)*	-.10 *(.57)*	.94 *(.69)*	T*R	.861	.429
	Resveratrol Normoxia	24	97.08 (*.76*)	.83 *(.36)*	.94 *(.49)*	.10 *(.65)*	T*OS	.309	.583
							T*OS*R	.342	.712
Choice Reaction Time % Correct RT (msecs)	Placebo Hypoxia	24	428.05 (*17.22*)	-15.02 *(10.65)*	-13.44 *(8.68)*	-1.72 *(14.64)*	T	4.827	.038*
	Placebo Normoxia	24	430.02 (*19.63*)	-14.80 *(15.29)*	.17 *(12.21)*	-1.56 *(9.51)*	OS	.189	.668
	Resveratrol Hypoxia	24	409.23 (*12.44*)	9.51 *(6.57)*	11.06 *(6.91)*	9.77 *(7.10)*	T*R	.530	.602
	Resveratrol Normoxia	24	422.21 (*10.70*)	8.69 *(7.98)*	9.25 *(7.19)*	17.60 *(9.70)*	T*OS	.024	.861
							T*OS*R	1.581	.243
RVIP % Correct	Placebo Hypoxia	23	72.56 (*12.44*)	.82 *(3.30)*	-.82 *(3.13)*	-6.79 *(3.13)*	T	.108	.746
	Placebo Normoxia	23	72.28 (*3.85*)	1.09 *(2.74)*	-.54 *(3.31)*	1.63 *(3.95)*	OS	4.221	.052t
	Resveratrol Hypoxia	23	78.26 (*2.99*)	-1.90 *(2.79)*	-5.98 *(3.32)*	-8.42 *(3.44)*	T*R	1.325	.276
	Resveratrol Normoxia	23	70.38 (*3.67*)	6.52 *(2.68)*	-.27 *(2.41)*	1.63 *(2.92)*	T*OS	.550	.466
							T*OS*R	.831	.442
	Placebo Hypoxia	23	463.38	14.44	33.34	3.68	T	1.079	.310

RVIP Correct RT (msecs)			(*12.98*)	*(7.07)*	*(9.43)*	*(9.15)*	OS	.000	.983
	Placebo Normoxia	23	471.36 (*12.72*)	17.58 *(10.21)*	13.89 *(12.06)*	13.03 *(13.45)*	T*R	2.781	.073t
	Resveratrol Hypoxia	23	468.77 (*10.87*)	5.00 *(9.23)*	3.47 *(10.50)*	14.74 *(11.22)*	T*OS	.109	.745
	Resveratrol Normoxia	23	469.85 (*11.49*)	1.03 *(8.61)*	13.69 *(11.35)*	14.17 *(12.17)*	T*OS*R	5.406	.016*
RVIP Errors (number)	Placebo Hypoxia	23	1.48 (*.63*)	-.83 *(.55)*	-.52 *(.53)*	-.48 *(.59)*	T	.150	.871
	Placebo Normoxia	23	.87 (*.30*)	.39 *(.23)*	.17 *(.20)*	.35 *(.36)*	OS	2.225	.437
	Resveratrol Hypoxia	23	1.17 (*.31*)	-.30 *(.24)*	-.13 *(.22)*	.04 *(.24)*	T*R	.058	.398
	Resveratrol Normoxia	23	1.13 (*.28*)	-.30 *(.30)*	-.04 *(.29)*	-.04 *(.33)*	T*OS	1.720	.203
							T*OS*R	1.333	.274
Stroop % Correct	Placebo Hypoxia	24	95.63 (*1.18*)	.73 *(.87)*	-.83 *(.98)*	.21 *(.91)*	T	.242	.627
	Placebo Normoxia	24	95.10 (*1.11*)	1.23 *(.60)*	1.04 *(1.06)*	1.67 *(.70)*	OS	.424	.521
	Resveratrol Hypoxia	24	95.52 (*.96*)	.21 *(.74)*	1.04 *(.58)*	.42 *(.72)*	T*R	1.426	.251
	Resveratrol Normoxia	24	95.83 (*.87*)	-.63 *(1.58)*	.42 *(.73)*	.63 *(.63)*	T*OS	1.314	.263
							T*OS*R	.210	.728
Stroop % Correct RT (msecs)	Placebo Hypoxia	24	669.81 (*30.52*)	-25.41 *(12.06)*	-6.94 *(19.37)*	-44.97 *(14.19)*	T	.732	.401
	Placebo Normoxia	24	655.96 (*24.99*)	-23.98 *(14.40)*	-53.95 *(14.54)*	-45.69 *(19.37)*	OS	2.746	.111
	Resveratrol Hypoxia	24	640.32 (*20.58*)	5.15 *(9.94)*	-15.00 *(12.37)*	-28.04 *(9.54)*	T*R	.359	.654
	Resveratrol Normoxia	24	658.93 (*23.16*)	-20.74 *(19.71)*	-30.79 *(18.90)*	-48.60 *(18.55)*	T*OS	.039	.845
							T*OS*R	3.090	.055t

Word Recognition % Correct	Placebo Hypoxia	24	81.53 (*2.21*)	-4.72 *(2.30)*	-4.45 *(1.93)*	-2.22 *(1.60)*	T	.758	.393
	Placebo Normoxia	24	81.94 (*2.07*)	-3.33 *(1.82)*	.69 *(2.07)*	-1.11 *(2.37)*	OS	1.507	.323
	Resveratrol Hypoxia	24	82.92 (*2.14*)	-1.53 *(1.76)*	-1.67 *(1.95)*	-2.36 *(1.78)*	T*R	1.127	.333
	Resveratrol Normoxia	24	81.25 (*2.23*)	-.28 *(1.87)*	-.42 *(1.39)*	-.42 *(1.98)*	T*OS	.113	.740
							T*OS*R	.806	.453
Word Recognition % Correct RT (msecs)	Placebo Hypoxia	24	832.57 (*43.30*)	68.03 *(26.16)*	-27.87 *(29.83)*	-18.88 *(38.47)*	T	.223	.641
	Placebo Normoxia	24	878.45 (*44.32*)	-40.41 *(37.20)*	-58.89 *(32.61)*	-71.43 *(32.14)*	OS	2.452	.131
	Resveratrol Hypoxia	24	831.21 (*42.02*)	38.46 *(44.03)*	-15.60 *(25.47)*	-4.61 *(28.89)*	T*R	.811	.451
	Resveratrol Normoxia	24	843.63 (*2.21*)	-19.93 *(20.63)*	-60.19 *(28.84)*	-23.41 *(27.63)*	T*OS	.267	.611
							T*OS*R	.824	.445
Immediate Word Recall Correct (number)	Placebo Hypoxia	24	8.15 (*.52*)	-1.67 (*.44*)	-1.23 (*.57*)	-.42 (*.43*)	T	.125	.727
	Placebo Normoxia	24	7.81 (*.39*)	-.48 (*.44*)	-.04 (*.36*)	.21 (*.45*)	OS	2.776	.109
	Resveratrol Hypoxia	24	8.19 (*.49*)	-.52 (*.57*)	-1.23 (*.44*)	-.78 (*.41*)	T*R	3.137	.053t
	Resveratrol Normoxia	24	8.21 (*.36*)	-.46 (*.33*)	-.46 (*.37*)	-.81 (*.28*)	T*OS	1.757	.198
							T*OS*R	.389	.680
Immediate Word Recall Incorrect (number)	Placebo Hypoxia	24	.29 (*.14*)	.00 (*.17*)	.17 (*.19*)	.13 (*.16*)	T	.073	.789
	Placebo Normoxia	24	.29 (*.11*)	-.21 (*.12*)	.08 (*.18*)	-.04 (*.13*)	OS	.195	.663
	Resveratrol Hypoxia	24	.38 (*.17*)	.13 (*.14*)	-.08 (*.08*)	.08 (*.18*)	T*R	.889	.418
	Resveratrol Normoxia	24	.21 (*.11*)	-.21 (*.06*)	.13 (*.14*)	.17 (*.13*)	T*OS	1.061	.314
							T*OS*R	.177	.838

Delayed Word Recall Correct (number)	Placebo Hypoxia	24	5.94 (*.09*)	-2.54 (*.48*)	-2.54 (*.61*)	-1.44 (*.60*)	T	.106	.748
	Placebo Normoxia	24	5.78 (*.45*)	-1.17 (*.42*)	-.65 (*.47*)	-.98 (*.54*)	OS	4.787	.039*
	Resveratrol Hypoxia	24	6.06 (*.46*)	-1.21 (*.55*)	-2.75 (*.45*)	-2.02 (*.62*)	T*R	1.946	.410
	Resveratrol Normoxia	24	6.17 (*.55*)	-1.56 (*.38*)	-1.23 (*.47*)	-1.40 (*.40*)	T*OS	1.192	.286
							T*OS*R	1.946	.154
Delayed Word Recall Incorrect (number)	Placebo Hypoxia	24	.33 (*.12*)	.42 *(.22)*	.62 *(.24)*	.46 *(.21)*	T	.874	.359
	Placebo Normoxia	24	.50 (*.14*)	-.08 *(.24)*	.42 *(.31)*	.25 *(.30)*	OS	.008	.929
	Resveratrol Hypoxia	24	.75 *(.23*)	-.29 *(.20)*	.13 *(.27)*	.21 *(.23)*	T*R	.850	.410
	Resveratrol Normoxia	24	.21 (*.09*)	.13 *(.09)*	.33 *(.17)*	.42 *(.16)*	T*OS	2.552	.124
							T*OS*R	.952	.394

Appendix 3.1. The summary of the behavioural results of Chapter 5: the effects of 500 mg resveratrol and placebo on mood in both hypoxic and normoxic conditions. Table displays baseline and change from baseline scores (*with SEM values in brackets*) for the 3 post-dose battery repetitions after placebo and 500 mg resveratrol for 23 healthy, young adults. Table also displays ANOVA F and P values for main effects of treatment (T), oxygen status (OS) the interaction between the two (T*OS), repetition (T*R) all three (T*OS*R). *<.05, **<.01 and t= trend (nearing significance).

Measure	Treatment	N	Repetition				ANOVA		
			Baseline	1	2	3	Effect	F	P
Difficulty	Placebo Hypoxia	24	39.83 (*4.41*)	3.96 *(2.37)*	9.63 *(2.68)*	8.54 *(3.17)*	T	.031	.862
	Placebo Normoxia	24	36.88 (*3.52*)	4.71 *(2.56)*	2.67 *(3.40)*	9.79 *(3.57)*	OS	2.863	.104
	Resveratrol Hypoxia	24	35.46 (*3.24*)	9.54 *(3.46)*	12.50 *(3.98)*	13.62 *(3.64)*	T*R	.727	.539
	Resveratrol Normoxia	24	39.46 (*4.40*)	2.50 *(2.31)*	1.67 *(2.32)*	5.50 *(2.75)*	T*OS	1.796	.193
							T*OS*R	.762	.483
Mental Fatigue	Placebo Hypoxia	24	34.71 (*3.58*)	12.79 *(2.59)*	17.02 *(3.28)*	15.54 *(3.96)*	T	.353	.904
	Placebo Normoxia	24	32.50 (*3.86*)	1.21 *(3.52)*	7.13 *(3.47)*	15.54 *(3.95)*	OS	25.326	<.001*
	Resveratrol Hypoxia	24	33.75 (*3.82*)	12.33 *(2.87)*	19.42 *(2.92)*	18.08 *(4.29)*	T*R	.422	.663
	Resveratrol Normoxia	24	40.17 (*4.10*)	2.04 *(2.16)*	6.37 *(3.32)*	9.00 *(3.91)*	T*OS	.008	.930
							T*OS*R	.288	.834
Anxiety	Placebo Hypoxia	24	25.54 (*4.16*)	-2.12 *(1.62)*	-3.17 *(2.28)*	-5.00 *(2.81)*	T	1.240	.277
	Placebo Normoxia	24	16.79 (*2.05*)	-1.79 *(2.13)*	-2.50 *(1.88)*	-2.13 *(1.91)*	OS	.084	.775
	Resveratrol Hypoxia	24	24.21 (*3.38*)	5.33 *(3.05)*	1.75 *(3.22)*	.63 *(3.68)*	T*R	2.582	.060t
	Resveratrol Normoxia	24	22.08 (*3.23*)	-.54 *(1.23)*	1.63 *(3.71)*	-4.17 *(2.04)*	T*OS	.636	.433
							T*OS*R	1.564	.206
	Placebo Hypoxia	24	70.38 (*2.61*)	-4.67 *(3.17)*	-4.92 *(2.55)*	-5.58 *(3.32)*	T	3.306	.082t

Friendliness	Placebo Normoxia	24	69.38 (*3.08*)	-.42 *(2.04)*	-1.33 *(2.17)*	-1.54 *(3.35)*	OS	2.543	.124
	Resveratrol Hypoxia	24	67.25 (*3.06*)	.92 *(2.30)*	-.42 *(2.10)*	-4.13 *(3.65)*	T*R	.227	.820
	Resveratrol Normoxia	24	67.50 (*3.06*)	1.46 *(1.00)*	.75 *(1.67)*	1.87 *(1.74)*	T*OS	.115	.737
							T*OS*R	.489	.636
Aggression	Placebo Hypoxia	24	16.63 (*3.14*)	1.33 *(1.55)*	2.46 *(3.08)*	4.00 *(3.82)*	T	.273	.606
	Placebo Normoxia	24	12.54 (*1.88*)	.83 *(1.37)*	2.63 *(2.96)*	.04 *(1.87)*	OS	2.836	.106
	Resveratrol Hypoxia	24	17.29 (*2.48*)	2.50 *(1.55)*	4.92 *(1.77)*	.42 *(2.06)*	T*R	1.237	.300
	Resveratrol Normoxia	24	17.00 (*2.71*)	-.12 *(1.43)*	-.58 *(1.70)*	-1.67 *(2.29)*	T*OS	.544	.468
							T*OS*R	.854	.429

Appendix 3.2. The summary of the behavioural Z score analysis of Chapter 5: the effects of 500 mg resveratrol and placebo on overall cognitive domains in both hypoxic and normoxic conditions. Table displays change from baseline scores (*with SEM values in brackets*) for the 3 post-dose battery repetitions after placebo and 500 mg resveratrol for 23 healthy, young adults. Table also displays ANOVA F and P values for main effects of treatment (T), oxygen status (OS) the interaction between the two (T*OS), repetition (T*R) all three (T*OS*R). *<.05, **<.01 and t= trend (nearing significance).

					ANOVA		
Measure	**Treatment**	**1**	**2**	**3**	**Effect**	**F**	**P**
Accuracy	Placebo Hypoxia	-.004 *(.07)*	-.061 *(.08)*	-.107 *(.10)*	T	1.451	.241
	Placebo Normoxia	.100 *(.10)*	.114 *(.10)*	.203 *(.10)*	OS	7.324	.013*
	Resveratrol Hypoxia	-.146 *(.07)*	-.122 *(.08)*	-.162 *(.10)*	T*R	.40	.961
	Resveratrol Normoxia	.072 *(.19)*	.04 *(.08)*	.073 *(.09)*	T*OS	.063	.958
					T*OS*R	.624	.540
Speed	Placebo Hypoxia	-.101 *(.12)*	.145 *(.14)*	-.169 *(.15)*	T	1.511	.232
	Placebo Normoxia	-.063 *(.17)*	-.103 *(1.16)*	-.107 *(.13)*	OS	.278	.604
	Resveratrol Hypoxia	.150 *(.14)*	.061 *(.11)*	.087 *(.10)*	T*R	1.035	.364
	Resveratrol Normoxia	.020 *(.12)*	.050 *(.14)*	.029 *(.16)*	T*OS	.008	.931
					T*OS*R	2.498	.094
Error	Placebo Hypoxia	-.147 *(.14)*	-.063 *(.14)*	.005 *(.15)*	T	.462	.504
	Placebo Normoxia	.053 *(.08)*	-.073 *(.08)*	.049 *(.11)*	OS	.103	.752
	Resveratrol Hypoxia	-.056 *(.01)*	.021 *(.11)*	.318 *(.20)*	T*R	2.752	.075t
	Resveratrol Normoxia	-.197 *(.11)*	.072 *(.10)*	.018 *(.12)*	T*OS	.823	.374
					T*OS*R	1.327	.276

Secondary Memory	Placebo Hypoxia	-.329 *(.18)*	-.346 *(.21)*	.011 *(.18)*	T	.147	.705
					OS	3.829	.063t
	Placebo Normoxia	.031 *(.15)*	.295 *(.16)*	.198 *(.19)*	T*R	1.954	.154
	Resveratrol Hypoxia	.102 *(.18)*	-.190 *(.14)*	-.073 *(.17)*	T*OS	1.320	.263
	Resveratrol Normoxia	.122 *(.13)*	.115 *(.14)*	.065 *(.13)*	T*OS*R	.756	.476

Appendix 4.0 The summary of the behavioural results of Chapter 6: the effects of 500 mg resveratrol and placebo on cognitive performance. Table displays baseline and change from baseline scores (*with SEM values in brackets*) for the three post-dose battery repetitions after placebo and 500 mg resveratrol for 24 healthy, older adults. Table also displays ANOVA F and P values for main effects of treatment (T) and repetition (R) and an interaction between the two (T*R) with *<.05, **<.01 and t= trend (nearing significance).

Measure	Treatment	N	Task Battery				ANOVA		
			Baseline	1	2	3	Effect	F	P
3's Correct (number)	Placebo	24	27.25 (*2.41*)	.92 (*1.04*)	.58 (*1.06*)	1.83 (*1.24*)	R	3.256	.048*
	Resveratrol	24	27.25 (*2.08*)	.17 (*1.30*)	.00 (*1.21*)	2.58 (*1.13*)	T	.023	.880
							T*R	.475	.625
3's Incorrect (number)	Placebo	24	1.50 (*.40*)	.25 (*.71*)	1.00 (*.38*)	.17 (*.39*)	R	2.322	.110
	Resveratrol	24	1.25 (*.23*)	.25 (*.79*)	1.13 (*.48*)	.83 (*.47*)	T	.490	.491
							T*R	.691	.506
7's Correct (number)	Placebo	23	19.17 (*1.53*)	-.46 (*.71*)	.33 (*1.01*)	1.08 (*.93*)	R	5.446	.008**
	Resveratrol	23	19.38 (*1.57*)	-.79 (*.93*)	.92 (*.88*)	1.13 (*.94*)	T	.006	.939
							T*R	.379	.687
7's Incorrect (number)	Placebo	23	1.67 (*.38*)	.04 (*.48*)	.71 (*.46*)	.04 (*.48*)	R	.333	.718
	Resveratrol	23	1.38 (*.32*)	1.08 (*.56*)	.75 (*.42*)	.92 (*.52*)	T	.972	.334
							T*R	1.593	.214
CRT % Correct	Placebo	24	97.71 (*.60*)	.52 (*.54*)	1.15 (*.48*)	.94 (*.58*)	R	1.144	.318
	Resveratrol	24	96.97 (*.89*)	1.46 (*.90*)	1.88 (*.76*)	1.98 (*.92*)	T	1.261	.273
							T*R	.153	.858
CRT % Correct RT (msecs)	Placebo	24	507.56 (*20.97*)	-12.40 (*16.64*)	-2.16 (*16.32*)	-17.80 (*14.95*)	R	2.464	.114
	Resveratrol	24	484.95 (*18.24*)	34.70 (*18.47*)	13.22 (*16.18*)	-4.33 (*11.91*)	T	2.474	.129
							T*R	1.527	.232
RVIP % Correct	Placebo	24	56.77 (*5.16*)	.52 (*2.68*)	6.51 (*2.42*)	8.33 (*2.37*)	R	1.579	.217

	Resveratrol	24	63.28 (*5.00*)	-2.86 *(3.19)*	-6.77 *(3.49)*	-2.60 *(2.98)*	T T*R	9.514 3.026	.005** .058t
RVIP Correct RT (msecs)	Placebo	24	529.73 (*14.81*)	-28.32 *(23.46)*	-5.73 *(10.40)*	-8.55 *(10.67)*	R T	.026 6.991	.935 .015*
	Resveratrol	24	494.05 (*14.72*)	38.72 *(13.69)*	20.59 *(9.59)*	19.23 *(12.42)*	T*R	1.352	.265
RVIP Errors (numbers)	Placebo	24	2.08 (*.35*)	.46 *(.56)*	.04 *(.42)*	.08 *(.41)*	R T	.893 .001	.416 .978
	Resveratrol	24	1.85 (*.47*)	.33 *(.52)*	.33 *(.45)*	-.04 *(.32)*	T*R	.615	.545
Stroop % Correct	Placebo	23	97.72 (*.77*)	.22 *(.86)*	-.22 *(.72)*	-.76 *(1.18)*	R T	.866 .660	.374 .425
	Resveratrol	23	97.39 (*.68*)	.44 *(.58)*	.87 *(.88)*	-.33 *(1.56)*	T*R	.389	.613
Stroop % Correct RT (msecs)	Placebo	23	921.34 (*40.53*)	-31.60 *(21.30)*	-60.56 *(24.58)*	-81.33 *(26.26)*	R T	4.180 .161	.035* .692
	Resveratrol	23	906.61 (*47.66*)	-28.33 *(26.85)*	-52.61 *(23.64)*	-57.11 *(28.07)*	T*R	.201	.773
Word Recognition % Correct	Placebo	23	82.03 (1.*78*)	-5.07 *(2.24)*	-4.93 *(2.00)*	-6.09 *(2.10)*	R T	.556 .077	.577 .784
	Resveratrol	23	82.03 (1.*63*)	-7.68 *(1.89)*	-4.64 *(1.55)*	-5.65 *(1.81)*	T*R	.710	.497
Word Recognition RT % Correct	Placebo	23	993.61 (*68.21*)	37.74 *(37.60)*	33.08 *(50.14)*	-33.26 *(44.91)*	R T	4.541 .390	.016* .539
	Resveratrol	23	1008.01 (*56.75*)	22.90 *(40.98)*	-13.74 *(45.78)*	-38.29 *(40.32)*	T*R	.482	.621
Immediate Word Recall Correct (number)	Placebo	24	6.58 (*.53*)	-1.17 *(.50)*	-.67 *(.43)*	-.44 *(.46)*	R T	1.370 3.397	.264 .078t
	Resveratrol	24	7.25 (*.45*)	-1.63 *(.44)*	-1.77 *(.35)*	-1.38 *(.50)*	T*R	.528	.593

Immediate Word Recall Incorrect (number)	Placebo	24	.58 (*.18*)	.17 *(.28)*	.29 *(.18)*	.21 *(.22)*	R	.853	.408
							T	3.235	.085t
	Resveratrol	24	.33 (*.13*)	.46 *(.29)*	.58 *(.19)*	.96 *(.24)*	T*R	1.304	.278
Delayed Word Recall Correct (number)	Placebo	24	4.92 (*.55*)	-2.13 *(.44)*	-2.79 *(.50)*	-2.21 *(.52)*	R	.968	.388
							T	1.492	.234
	Resveratrol	24	5.60 (*.53*)	-2.81 *(.42)*	-3.04 *(.46)*	-2.85 *(.53)*	T*R	.376	.688
Delayed Word Recall Incorrect (number)	Placebo	24	.71 (*.19*)	.87 *(.26)*	2.12 *(.41)*	1.17 *(.37)*	R	8.021	.001**
							T	1.965	.174
	Resveratrol	24	.88 (*.20*)	.46 *(.32)*	1.08 *(.36)*	1.29 *(.48)*	T*R	2.034	.142

Appendix 4.1 Summary of the behavioural analysis comparing the younger and the ageing cohort on cognitive task performance. Table displays the average baseline scores for each task outcome from visit 1 (with SEM values in brackets) for the 24 healthy, young (18-35 years old) adults of Chapter 5 and the 24 healthy, ageing (50-70 years old) adults of Chapter 6. Table also displays t-test T and P values. *<.05, **<.01 and t= trend (nearing significance).

Measure	Cohort	Analysis			
		N	Mean (SE)	T	P
Serial 3 Correct	18-35 years	24	41.17 *(3.38)*	5.093	>.001**
	50-70 years	24	23.83 *(2.15)*		
Serial 3 Error	18-35 years	24	2.21 *(.36)*	.385	.702
	50-70 years	24	2.00 *(.41)*		
Serial 7 Correct	18-35 years	24	24.17 *(2.45)*	2.086	.044*
	50-70 years	24	18.04 *(1.56)*		
Serial 7 Error	18-35 years	24	2.17 *(.20)*	.579	.565
	50-70 years	24	1.83 *(1.95)*		
CRT Correct %	18-35 years	24	97.60 *(2.90)*	.515	.609
	50-70 years	24	97.08 *(4.02)*		
CRT Correct RT	18-35 years	24	441.47 *(104.03)*	2.102	.041*
	50-70 years	24	501.57 *(93.82)*		
RVIP Correct %	18-35 years	24	68.75 *(25.93)*	1.364	.179
	50-70 years	24	58.85 *(24.30)*		
RVIP Correct RT	18-35 years	24	448.58 *(114.08)*	2.047	.046*
	50-70 years	24	504.67 *(70.78)*		
RVIP errors	18-35 years	24	2.08 *(3.32)*	.771	.445
	50-70 years	24	1.50 *(1.64)*		
Stroop Correct %	18-35 years	24	95.83 *(4.87)*	.234	.816
	50-70 years	24	95.31 *(9.76)*		

Stroop Correct RT	18-35 years	24	702.65 *(143.02)*	4.872	>.001**
	50-70 years	24	962.93 *(219.21)*		
Word Recognition Correct %	18-35 years	24	81.11 *(10.80)*	.554	.582
	50-70 years	24	82.64 *(8.10)*		
Word Recognition Correct RT	18-35 years	24	865.06 *(212.59)*	1.808	.077T
	50-70 years	24	993.08 *(274.14)*		
Imm word recall correct	18-35 years	24	7.73 *(.44)*	.849	.400
	50-70 years	24	7.17 *(.49)*		
Imm word recall errors	18-35 years	24	.25 *(.11)*	.731	.468
	50-70 years	24	.38 *(.13)*		
Delayed word recall correct	18-35 years	24	5.63 *(.50)*	.417	.678
	50-70 years	24	5.92 *(.49)*		
Delayed word recall errors	18-35 years	24	.54 *(.13)*	.8459	.395
	50-70 years	24	.75 *(.20)*		