The cognitive and cerebral blood flow effects of the polyphenol resveratrol in healthy, young humans.

Emma Louise Wightman

PhD

2013

The cognitive and cerebral blood flow effects of the polyphenol resveratrol in healthy, young humans.

Emma Louise Wightman

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

Research undertaken in the Faculty of Health and Life Sciences: Department of Psychology.

September 2013

Abstract

The polyphenol *trans*-resveratrol interacts with a number of mechanisms relevant to brain function and has demonstrated preserved and enhanced cognitive function in animal models as a result. Two of these mechanisms also suggest that resveratrol may be capable of acute cognitive enhancement: firstly via nitric oxide (NO)-mediated vasodilation leading to increased cerebral blood flow (CBF) and, in turn, increased neural access to the metabolic substrates oxygen and glucose; and secondly via enhanced mitochondrial oxidative phosphorylation which would be expected to increase the utilization of this enhanced provision of neural fuel.

To date, research has yet to investigate the potentially CBF and cognitive enhancing effects of resveratrol in humans. This thesis aimed to redress this paucity and reports the findings from five placebo-controlled, double-blind, multiple-dose, acute and chronic resveratrol supplementation studies; all conducted in young, healthy human volunteers. Throughout this programme of studies the novel neuroimaging technique near-infrared spectroscopy (NIRS) has been utilized to monitor the effects of resveratrol on CBF in the prefrontal cortex. The cognitively demanding tasks utilized to assess cognitive function are all predominantly sub-served by this region of the brain.

The consistent finding emerging from this thesis is that, acutely, resveratrol is a potent enhancer of the natural demand-driven increase in CBF and, in support of the hypothesis, also evinces significant enhancement of oxygen utilization. The lack of strong, replicable cognitive effects of resveratrol in this thesis however, suggests that resveratrol is not able to translate this increased access and utilization of metabolic substrates into improved cognitive performance in healthy, young adults.

List of contents

Appendix contents listing

Tables and figures contents listing

Tables

Figures

after 1, 2, 3 and 4 weeks supplementation.

142 **5.10.** Bioavailability of resveratrol metabolites after 500mg *trans*-resveratrol acutely, and after a 28 day supplementation period.

156 **6.1.** Chapter 6 testing session timeline.

157 **6.2.** The effects of 500mg resveratrol and placebo on delayed word recall.

Acknowledgements

This programme of studies was undertaken to address the paucity of literature on the effects of resveratrol, specifically with regards cerebral blood flow and cognitive function- of which there was none at the time of commencing this PhD- in humans. It is hoped that these studies represent only the beginning of a fruitful research area into the effects of resveratrol, and indeed other polyphenols in humans.

I have many thanks to give to those who have supported me during this PhD. I am very grateful to my supervisors; David Kennedy, Jonathon Reay and Crystal Haskell for tolerating my strange ways and stubbornness. I consider myself lucky to have had a supervisory team that has given me just the right amount of freedom to work independently and shape my own PhD, and sufficient support for when that went awry. I would also like to thank my line manager Mark Moss for, first of all, employing me as a graduate tutor and, secondly, for managing my career such that I was able to complete this PhD to a level that I am very proud of. Thanks should also go to Dr's Edward Okello and Georg Lietz at the school of Agriculture, Food and Rural Development at Newcastle University and Professor Gary Williamson and Dr Tristan Dew at the School of Food Sciences and Nutrition at the University of Leeds for their expertise in analysing the plasma samples for many of the studies in this PhD. James Betz at Biotivia^{TM} also deserves thanks for providing financial support for the chronic supplementation study. I would lastly like to thank my partner David for providing much needed lols and for being the invaluable calming influence in my life.

Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. This is with the exception of sections *3.3.4*, *4.3.4* and *5.2.8* where technical assistance was provided by Drs Georg Lietz, Edward Okello and Tristan Dew and Professor Gary Williamson. Here the plasma analysis of the related chapters was conducted at the universities of Newcastle and Leeds respectively and the aforementioned sections prepared by the above persons. I also confirm that this work fully acknowledges opinions, ideas and contributions from 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 70,233 words

Name _____

Signature _____

Date _____

Chapter 1.
General Introduction

1.1 Classification, synthesis and botanical function of polyphenols

1.1.1 Classification

Polyphenols are a group of plant phytochemicals which are characterised chemically as having molecular weights ranging from 500-4000 and possessing between 12-16 phenolic groups with two or more aromatic rings attached (Haslam, 1998). Currently ~10,000 compounds meet this definition. The term polyphenol is therefore very much an umbrella for this abundance of compounds which can be subcategorised into smaller and more appropriate groups. Figure 1.1 (page 13) provides a simplified diagram of these groups and some examples of dietary sources. Phenolic acids are included in the diagram for completeness although by definition, i.e. comprising only 1 phenol ring, they are not '*poly*'phenols.

As the diagram demonstrates; most polyphenols are flavonoids and comprise: isoflavones, flavones, flavanones, flavanols (which can be further sub-categorised into flavan-3-ols and proanthocyanidins), flavonols and anthocyanins. In terms of dietary sources, isoflavones like daidzein and genistein are found most abundantly in soy (Setchell, 1998); flavones, such as luteolin, in capsicum pepper; flavanones such as naringenin, in lemon (Kanaze, Bounartzi, Georgarakis, & Niopas, 2006); flavanols, like the catechins epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG), in green tea; flavonols, such as quercetin and kaempferol, are abundant in vegetables like onions (Hollman et al., 1997); and anthocyanins, such as cyanidin, delphinidin, malvidin, pelargonidin and petunidin, are found most abundantly in berries such as grapes, raspberries, cherries and strawberries (Mazza & Miniati, 1993).

Considering this ubiquity of polyphenols throughout the fruit and vegetable kingdom it is not surprising that they can form a significant proportion of our daily food intake. The process of calculating these levels however is problematic. As Manach et al. (2004) outline, in part this is due to individual food preferences and diets but the greatest difficulty emerges when attempting to assess polyphenol levels based on food questionnaires alone. The latter data collection tool relies on the accuracy of participant reports (e.g. with regards portion size) and is based on the premise that all products consumed will contain the same polyphenol levels. The conditions during the growth and transport of polyphenol-containing fruits and vegetables will of course differentially

affect the levels of polyphenols and the cooking method will influence this further. These factors, and more, render the calculation of average daily polyphenol consumption levels difficult but Manach et al. (2004) estimate that, in the west, those who consume several portions of fruit and vegetables per day likely achieve ~1g/d. With regards levels of resveratrol consumption specifically, the Phenol-Explorer website estimates that the average red wine contains ~0.27mg/100ml and white ~0.04mg/ml. Grapes reportedly contain between ~0.15mg/100g (black) and 0.02mg/kg and peanuts 0.08mg/100g. Thus, it is not inconceivable that ~1-2mg of the above 1g/d polyphenol daily consumption estimation is derived from resveratrol alone. (See Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004 for comprehensive review of food sources and bioavailability of individual polyphenols.)

Despite the dominance of flavonoid polyphenols, the diagram does delineate a non-flavonoid group; the stilbene polyphenols. The stilbenes comprise the polyphenol under investigation in this thesis, resveratrol, as well as including other stilbenes derived from the grape (*vitis vinifera*), Japanese knotweed (*polygonum cuspidatum*), pine (*pinaceae*) and peanut (*fabaceae*) such as piceid, pterostilbene and pinosylvin (Chong, Poutaraud, & Hugueney, 2009).

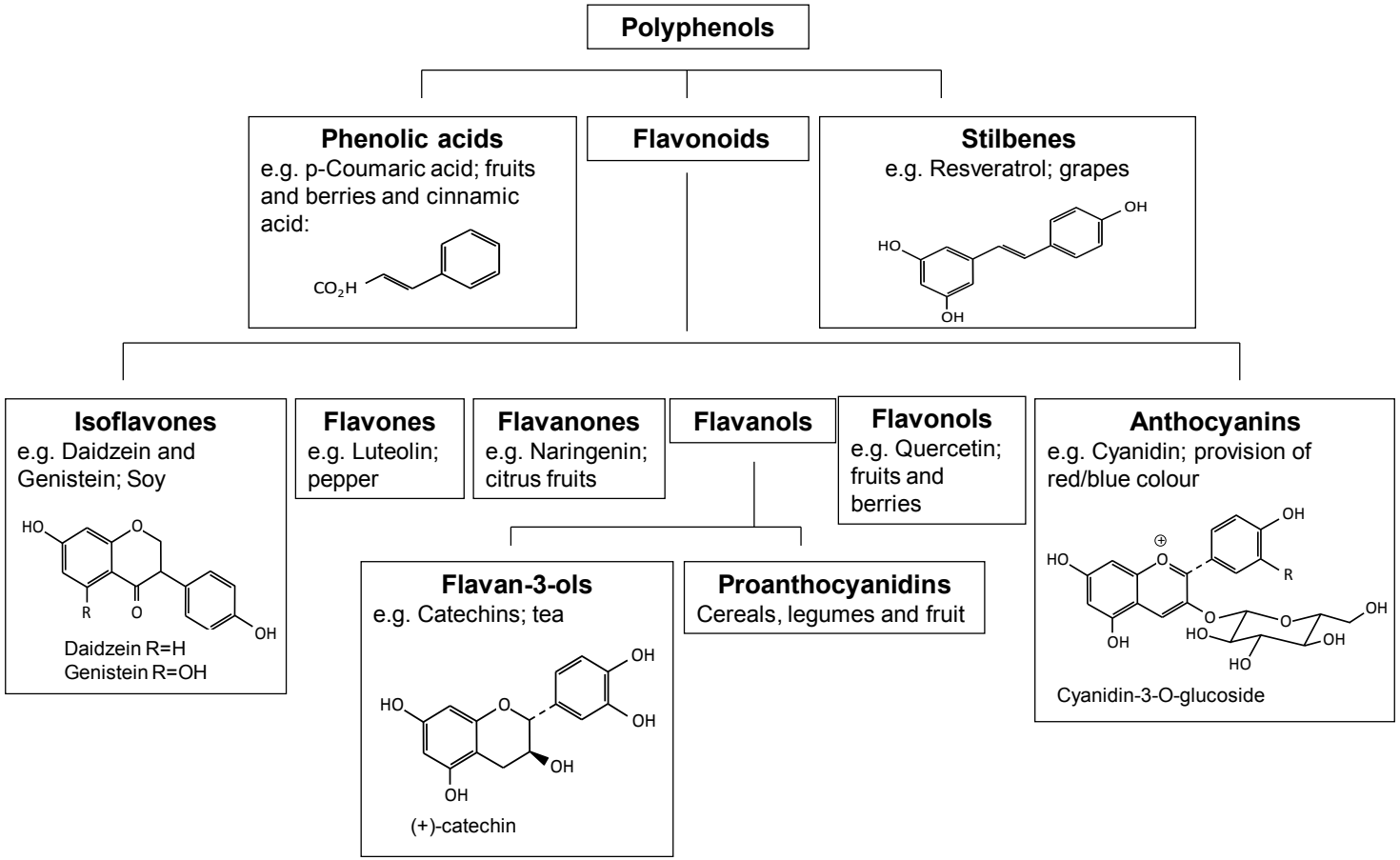


Figure 1.1. Main sub-groups of polyphenols and examples of dietary sources. Phenolic acids are included for completeness but are not technically 'poly'phenols due to the presence of only 1 phenol group. Chemical structures in diagram are adapted from Kennedy, D.O. (In press). *Plants and the Human Brain*. Oxford University Press. New York.

1.1.2 Synthesis and botanical function

Plant polyphenols are derived from the phenylpropanoid pathway and begin with cinnamic acid and two or three malonyl-CoA units. This synthetic pathway culminates in the production of either chalcone; which forms the basis of all flavonoid polyphenols, or stilbene synthase; which forms the basis of stilbenes such as resveratrol (Dewick, 1994). This pathway is not related to the plants primary metabolism of compounds which are vital for its immediate survival; e.g. in the same way as photosynthesis, but represent a secondary pathway where the production of phenolic chemicals increases the survivability of the plant nonetheless. As such, the term 'secondary metabolite' has been applied to polyphenolic compounds and the survivability-enhancing actions they confer are associated with three main areas. The first is that of deterring potentially damaging herbivores and competitors; the second is, conversely, in attracting potentially beneficial symbiotes; and the third is phytoalexin-mediated protection against potentially damaging stressors.

1.1.2a Deterrent function

The use of secondary metabolites in chemical communication is also termed 'allelopathy' and is defined as a complex of subtle communications between plants and between plants and other organisms (Lovett, 1990; Lovett, Ryuntyu, & Liu, 1989). This communication has a number of potential roles although it has been argued that defence against potentially damaging agents is chief amongst them (Harborne, 1993). Evidence indicates that one of the main roles of allelopathic chemicals is in the deterrence of feeding by insects, conferred by their astringent and unpalatable taste (Bate-Smith, 1973). Some phenolics also have the capacity to deter other plants from growing in the immediate vicinity, thus preventing competition for resources. For instance, *Centaurea maculosa* (spotted knapweed) is able to displace native plant species by exuding catechins from its roots. The result of this in the offending plant is a wave of reactive oxygen species (ROS) which disturbs calcium signalling and gene expression, ultimately resulting in death (Bais, Vepachedu, Gilroy, Callaway, & Vivanco, 2003).

1.1.2b Attractant function

Phenolic secondary metabolites also have the reverse effect of attracting beneficial symbiotic organisms, in order to improve the fitness of the plant via pollination. The main attractant properties of the plant are provided by anthocyanins; one of three classes of plant pigment (along with chlorophyll which confers green colouring and carotenoids (terpene compounds) which are responsible for the yellow to red spectrum) which provide blue, red, purple and black pigmentation (Schaefer & Rolshausen,

2006). With regards flowers, colour preference by insects and birds has been reported (Gori, 1989; Meléndez-Ackerman, Campbell, & Waser, 1997 respectively) with colour representing an indication of the level of reward in terms of pollen levels etc. Birds also appear to use fruit colour as a cue to underlying nutritional value. In a study by Schaefer, McGraw and Catoni (2008), a food choice test demonstrated that birds consumed significantly more food which was rich in anthocyanins than a food which was not. The authors assert this demonstrates that birds may actively select for anthocyanins in their food using colour as a cue.

1.1.2c Protective function

The final role of phenolic secondary metabolites is to provide biotic and abiotic phytoalexin protection to the host plant. As such, many of these compounds are synthesized and expressed *de novo* during times of stress. Biotic stressors include fungal, bacterial and viral agents with polyphenols employing a number of techniques to protect against these invaders. With regards anti-viral actions, tea catechins can inhibit the level of infection and multiplication of tobacco and cucumber mosaic viruses by bonding to the nucleic acids of the virus (Okada, 1978) and can also block the activation of cellular signalling pathways incited by viral agents (see Friedman, 2007 for review). Tea catechins have also demonstrated antibacterial activity which may be as a result of perturbing cell wall membranes (Friedman, 2007) and antifungal actions by inhibiting initial germination of spores (Brownlee, McEuen, Hedger, & Scott, 1990). Interestingly, these anti-fungal/bacterial/viral actions seem to be effective against human pathogens (Friedman, 2007). Research indicates that tea catechins have inhibitory activity against HIV, hepatitis B and the herpes simplex virus *in vitro* (Tao, 1992) and that green tea catechins specifically are potent inhibitors of influenza virus replication (Song, Lee, & Seong, 2005) which suggests a possible future role in human viral/fungal/bacterial treatment.

Abiotic plant stressors include ultraviolet (UV) light (Adrian, Jeandet, Douillet-Breuil, Tesson, & Bessis, 2000), the presence of heavy metals (Adrian, Jeandet, Bessis, & Joubert, 1996), chemicals such as ozone (Schubert et al., 1997), water deprivation and dramatic changes in temperature (see Steyn, Wand, Holcroft, & Jacobs, 2002 for review). Plants are observed to accumulate anthocyanins in all of the above instances which may be instigated by two main mechanisms: carbohydrate accumulation and oxidative stress. Both factors often follow abiotic stress and may therefore signal anthocyanin synthesis in order to protect against the deleterious effects of these two processes (Steyn et al., 2002). These protective mechanisms may also benefit mammals under abiotic stress following consumption, particularly with regards UV light damage. In rodents, oral and topical administration of green tea polyphenols was

shown to protect against sunburn and to significantly lower the incidence of UVB-induced skin tumours (Sevin et al., 2007; Wang, Agarwal, Bickers, & Mukhtar, 1991 respectively). Katiyar et al. (2001) report that topical application of EGCG to human skin, before UV exposure, significantly reduced markers of stress in the dermis and epidermis of tissue. For instance, application decreased production of hydrogen peroxide, and nitric oxide and prevented lipid peroxidation. In the plant, anthocyanins reduce photo-inhibition and photo-bleaching of vital chlorophyll cells by acting as a photo-protecting light shield and absorbing the potentially damaging rays. In mammalian skin cells this protection is posited to function in one or more of the following ways: by increasing the barrier for UV light, i.e. absorbing UV rays; by protecting target molecules via antioxidant actions; by repairing UV damaged cells and/or by suppressing the cellular response to UV damage e.g. anti-inflammatory mediators (Black & Rhodes, 2001).

1.1.2d Determinants of secondary metabolite expression

The variety and levels of polyphenols within each plant is dependent on a number of factors. It could be that genetic control is the ultimate underlying influence in phenolic expression but that external factors may act to modify them (e.g. Macheix, Fleuriet, & Billot, 1990). Such factors, according to the resource allocation theory, include the nutrient resources available to plants which will largely determine the quantity and type of allelochemicals they can produce (Bryant, Chapin III, & Klein, 1983). The plant apparency theory, however, purports that plants and plant chemistry varies in the degree to which the plant is available to, or likely to be discovered by, other organisms (Coley, Bryant, & Chapin, 1985). The truth of the matter probably encompasses all of the aforementioned factors in that the type of phenolic compound a plant is able to express will of course depend upon its genetic makeup and the constituent parts available to it in the form of nutrients drawn from the ground, but the type of phenolic that a plant expresses will also depend upon millions of years of co-evolution with the plants/insects/herbivores that it encounters in the environment.

1.2 Historical use of polyphenols in humans

Polyphenols have been consumed by humans throughout their existence and have especially been utilized over the last several millennia for the prevention and treatment of disease. For instance, traditional Chinese medicine has utilized an abundance of phenolic compounds as medicinal treatments. Liu et al. (2008) investigated the polyphenol content of 68 Chinese herbals and reported that the total phenolic levels ranged from ~0.57-280mg/g; with the highest levels of total flavonoids observed in the Chinese white olive. This particular herbal has been used predominantly to cure pain and swelling in the throat, an action which may be related to its antioxidant capacity- indeed the Chinese white olive also had the highest Ferric reducing ability of plasma (FRAP) value of all the herbals investigated.

The tradition of tea drinking has a long history which dates back to the Han period (206 BDC-220 CE) in China where it was consumed as a medicinal concoction. The consumption of tea was intertwined with the Taoist principals of healing, and indeed it seems that those who consumed tea were aware of its stimulatory effects and correlated this with the bitter taste of the beverage (which is related to the content of condensed tannin polyphenols in the leaf). It was not until the middle of the T'ang period (618-907) that tea was widely consumed as a refreshing beverage in China and not until the Nara period (710-794) that the custom of tea drinking arrived in Japan, reportedly with immigrating Buddhist priests. It was some centuries later (between 1185-1573) that it became an integral part of the Japanese culture. In part, this tradition seems to have passed between the two cultures because of the health promoting properties of the tea leaf. The Japanese Buddhist priest Myōan Eisai (1141-1215) is reported to have written that the good health and longevity of the Chinese people could be attributed to their high consumption of tea. This was relative to his own Japanese people who he described as having much heart trouble, a short life-span and being generally thin and 'sickly looking'. His remedy was to drink tea and indeed henceforth it became common practice in Japan to prescribe tea as a health-promoting concoction. (see Ludwig, 1981 for review of history of the tea ceremony.)

Ayurveda; the science (*veda*) of life (*ayu*), is the traditional practice of medicine in India. As with the aforementioned traditional Chinese medicinal treatments, when the compounds utilised in Ayurvedic medicine were analysed, they too revealed an abundance of polyphenols amongst their bioactive components. As an example, the historical process of Panchakarma (a method of detoxification) utilizes a range of plants which contain multiple phenolic compounds. Chief amongst these are a number

of flavonoids and, specifically, high levels of quercetin (Gupta & Shaw, 2009). Western research has recently reported that this flavonoid is beneficial in a number of disorders for which it has traditionally been used to in Ayurvedic medicine (see Boots, Haenen, & Bast, 2008; and Sies, 2010 for beneficial health effects of quercetin and general polyphenols respectively).

Darakchasava is another well-known Indian herbal preparation, the main ingredient of which is *vitis vinifera* (grape vine). This preparation has historically been used to treat a number of diverse disorders. For instance, anaemia, worm infestation, tuberculosis and heart disease. This is particularly interesting when one considers the more recent association between red wine (Lippi, Franchini, Favaloro, & Targher, 2010; Wollin & Jones, 2001), and resveratrol (Wu et al., 2001), and beneficial effects with regards coronary heart disease (CHD); a phenomenon known as the 'French paradox' (Kopp, 1998). A number of mechanisms by which resveratrol and other red wine polyphenols may exert this protection are likely, including: antioxidant (Holthoff et al., 2010; Jia et al., 2008), anti-inflammatory (Das & Das, 2007; Udenigwe, Ramprasath, Aluko, & Jones, 2008), reduction of platelet aggregation (Pace-Asciak, Hahn, Diamandis, Soleas, & Goldberg, 1995), anti-atherosclerotic (Fan, Zhang, Jiang, & Bai, 2008) and vasorelaxatory (Chen & PaceAsciak, 1996; Novakovic, Bukarica, Kanjuh, & Heinle, 2006; Novakovic, Gojkovic-Bukarica, et al., 2006) effects. These mechanisms will be discussed in more depth in the relevant sub-sections of the following section on polyphenols and health.

1.3 Polyphenols and health

The above demonstrates that the beneficial effects of protective plant polyphenols have long been known to extrapolate to humans. It seems axiomatic that the ubiquity of polyphenols in the foods and beverages that we consume on a daily basis also make them well placed to potentially influence health parameters. Indeed, evidence from epidemiological studies suggests that the consumption of food-stuffs rich in polyphenols is correlated with positive health outcomes. To support this, an emerging body of controlled intervention trials has demonstrated significant direct effects of polyphenols on human health parameters.

1.3.1 Epidemiological correlations between polyphenol consumption and general health

In terms of single food products, the consumption of flavanol-containing chocolate (Djousse, 2012) and catechin-containing tea (Deka & Vita, 2011) is inversely related to the incidence of cardiovascular disease. Further, a positive correlation between general polyphenol consumption and reduced mortality due to cardiovascular disease has also been reported (Bauer, Ding, & Smit, 2011). This includes data from a 7 year longitudinal study in a cohort aged between ~50-70yrs (at the outset of the study) which observed that the consumption of anthocyanidins, flavan-3-ols, flavones, flavonols, and proanthocyanidins were associated with a protection against death from cardiovascular disease (McCullough et al., 2012).

Epidemiological data suggests that cultures which naturally consume a diet high in polyphenol levels might also be afforded cardiovascular protection. The Mediterranean diet, for example, is typified by the relatively high consumption of fruits and vegetables, (alongside legumes, nuts and olive oil and moderate consumption of fish and dairy and low consumption of meat and poultry (Willett et al., 1995)) which are naturally high in polyphenols. Sofi et al. (2010), reporting a meta-analysis of 18 prospective studies, found that higher adherence to the 'typical' Mediterranean diet was associated with significantly reduced mortality when assessed between 4-20yrs at follow-up. Specifically, this reduced mortality manifested itself as a protection against cardiovascular disease, tumours and cerebro-vascular and neurodegenerative diseases such as dementia. This cardioprotection also seems to extend to wine consumption, as part of the diet, in these particular cultures. Here the 'French paradox' is a term coined to explain the relatively low incidence of coronary heart disease (CHD) in those cultures which consume high levels of fat; potentially as a result of concomitant wine consumption. However, the argument that this protection might be the result of ethanol rather than, or including some synergistic, protection afforded by

red wine polyphenols such as resveratrol and quercetin, has been made. The results of correlations between CHD and the consumption of wine, beer spirits, dietary fats and fruit, made in the second half of the 20th century, concluded that wine ethanol was most strongly inversely associated with CHD (Criqui & Ringel, 1994). The issue with epidemiological data of course is that correlations are merely drawn, in this case, between dietary habits and certain health outcomes which may be the product of other unknown factors and/or some complicated interaction between lifestyle and dietary factors. Further, whilst this data provides a fascinating account of associations between the levels of polyphenols consumed in particular cultures, this data cannot inform on the efficacy of individual polyphenols and health outcomes. Here instead we look to the testing of individual polyphenols in intervention trials.

1.3.2 Evidence of health effects of polyphenols from controlled intervention studies

An increasing amount of evidence from controlled clinical trials is emerging to support the above observations of the protective health effects of polyphenols in humans. The bulk of this research is concerned with cocoa-derived flavanols and demonstrates significantly improved health outcomes after both acute and chronic consumption. Shrime et al. (2011), reporting a meta-analysis of 24 short-term cocoa intervention studies, found significantly improved blood pressure, insulin resistance, lipid profiles, and peripheral blood flow (i.e. endothelial function) outcomes in humans. Longer-term supplementation of cocoa-flavanols is also observed to decrease insulin levels, and improve insulin resistance, diastolic blood pressure and mean arterial pressure; these latter effects are likely to be the result of the observed improvements in blood flow (Hooper et al., 2012).

Mechanisms here may include anti-inflammatory, antioxidant and anti-platelet effects (Sudano, Flammer, Noll, & Corti, 2012); which would serve to reduce the build-up of fatty deposits in the blood, associated damage to the endothelial lining (and the vasodilatory response) alongside reducing the viscosity of blood. A further potential mechanism is in influencing the vascular tone of the endothelial lining directly by interacting with the cellular signalling molecule nitric oxide (NO) and inducing a vasorelaxatory response. In support of this, Fisher et al. (2003) report that 4 days supplementation with 821mg cocoa flavanols daily induces peripheral vasodilation in healthy humans; a response which was reversed following the NO inhibitor N-Nitro-L-Arginine Methyl Ester (LNAME).

1.3.3 Polyphenols and brain function

It seems axiomatic that these beneficial vascular and blood flow effects of flavonoids in the periphery must also extend to the cerebro-vasculature. It follows then that polyphenols may also be capable of conferring positive neurological effects as a result of improving blood flow to the brain. Importantly, reduced cerebral blood-flow and disorders of the cerebro-vasculature contribute to the cognitive decline seen in neurodegenerative dementias (O'Brien et al., 2003). Cerebral blood volume and metabolism of oxygen is also observed to decline in healthy, human ageing (Marchal et al., 1992) alongside cognitive performance (Hedden & Gabrieli, 2004). Thus, an argument could be made for the potentially beneficial effects of polyphenols in attenuating this decline in cognitive functioning as a result of cerebro-vascular protection.

In support of this, if we again consider epidemiological data, the consumption of tea (Arab, Liu, & Elashoff, 2009; Kuriyama et al., 2006), fruit and vegetables and total levels of flavonoids (Hollman, Geelen, & Kromhout, 2010) are reported to be associated with protection against, or slowed progression of cerebro-vascular diseases such as strokes, and neurological disorders such as Alzheimer's disease and other dementias (Barberger-Gateau et al., 2007; Commenges et al., 2000; Letenneur, Proust-Lima, Le Gouge, Dartigues, & Barberger-Gateau, 2007; Ng, Feng, Niti, Kua, & Yap, 2008a). In terms of the outcomes of this cerebro-vascular protection, cognitive impairment is observed to be inversely associated with tea consumption in elderly cohorts (Kuriyama et al., 2006; Ng, Feng, Niti, Kua, & Yap, 2008b) and better cognitive function has been shown to be associated with the consumption of polyphenol-rich foods such as chocolate, red-wine, and tea (Nurk et al., 2009). General flavonoid consumption has also been associated with reduced rates of cognitive decline in a cohort of 16,000 >70's (Devore, Kang, Breteler, & Grodstein, 2012) and a positive relationship has been found between overall consumption of polyphenols in 2574 middle-aged adults and cognitive function, specifically relating to language and verbal memory, assessed 13yrs later (Kesse-Guyot et al., 2012).

If we now look at the increasing evidence from intervention studies with cocoa flavanols we see that, acutely, their consumption is also associated with increased CBF and cognitive performance. In terms of CBF, 2 weeks supplementation with 900mg flavanols daily can increase cerebral blood flow volume (CBFV), as assessed by trans-cranial Doppler (TCD) sonography (Sorond, Lipsitz, Hollenberg, & Fisher, 2008). An increase in CBF during cognitive task performance has also been reported by Francis

et al. (2006) in 16 healthy, young females, following 5 days supplementation with 172mg flavanols daily, as assessed by functional magnetic resonance imaging (FMRI).

In terms of the effects of polyphenols on cognitive performance in humans, a number of randomised, placebo-controlled, intervention studies have assessed both the acute and chronic effects of cocoa-flavanols. Acute improvements on spatial memory performance, detection of stimuli movement and sensitivity to visual contrast have been found following 720mg cocoa flavanols (Field, Williams, & Butler, 2011). Improved performance on cognitively demanding mental arithmetic tasks (rapid visual information processing (RVIP)) and reduced task-induced mental fatigue has been seen following doses of 994- and 520mg (Scholey et al., 2010) in healthy, young humans 90-minutes following consumption. An increase in errors in performance was seen following the higher dose on Serial 7s subtractions in the latter study, however, and a later study failed to find any cognitive enhancing effects following 30-days consumption with 500- or 250mg cocoa polyphenols in healthy, young adults (Camfield et al., 2012) although mood was reported to be improved with the higher dose in a separate study by the same group (Pase et al., 2013). Non-significant cognitive effects of cocoa flavanols have also been reported in healthy young adults elsewhere, e.g. after a five day regimen of 150mg (Francis et al., 2006) and in healthy older (≥60yrs) adults with no reported cognitive impairment following 6 weeks consumption of a 397.30mg- and 357.41mg total proanthocyanidins chocolate bar and beverage respectively (Crews, Harrison, & Wright, 2008). However, improved verbal fluency and performance on the trail maker task is reported to be improved in a cohort of older adults suffering from mild cognitive impairment following 990- and 520mg cocoa flavanol consumption for 8-weeks (Desideri et al., 2012).

In terms of cognitive effects of other polyphenols, evidence is scarce and, to the best of current knowledge, comes only from other catechins and isoflavones. Epigallocatechin-3-gallate (EGCG), for example, has been reported to improve cognitive function in a sub-sample of participants suffering from mild cognitive impairment after supplementation with 1,680mg daily, for 16 weeks, of an EGCG and L-theanine combination (Park et al., 2011). Modest cerebro-electrical activity as assessed by EEG was also observed in this study and elsewhere (Scholey et al., 2012) although the latter study did not report any concomitant cognitive performance effects after a 300mg dose in healthy, young humans. A 500ml, *Rivella green*®, green tea extract drink (dose of green tea not specified) is also reported to modulate cerebral activity, as assessed by FMRI, but again reports no significant effects on cognitive performance (Borgwardt et al., 2012). This finding is similar to another recent study which demonstrated that a 135mg dose of pure EGCG evinced significant modulation of the hemodynamic

response in the prefrontal cortex during cognitively demanding task completion, alongside no cognitive performance effects (Wightman, Haskell, Forster, Veasey, & Kennedy, 2012).

With regards isoflavones, supplementation seems to afford similar cognitive protection/enhancement to that of oestrogen supplementation in post-menopausal women (Steffens et al., 1999). Kritz-Silverstein et al. (2003), for example, report that 6-month supplementation of 110mg total isoflavones daily to post-menopausal women (55-74yrs) resulted in higher within-treatment improvements to cognitive task performance as compared to placebo. In support of this, File et al. (2005) report that a shorter, 6 week, supplementation of 60mg total isoflavones daily to post-menopausal women (51-66yrs) significantly increased aspects of memory and 'frontal' cognitive function as well as reducing somatic symptoms of the menopause.

1.3.4 Summary remarks

This section has demonstrated an epidemiological link between polyphenol consumption and positive health outcomes. Alongside this, results from controlled intervention studies support the beneficial health effects of some classes of flavonoids in humans; specifically with regards cardio- and cerebro-vascular protection. One of the mechanisms underlying this is dilation of the vasculature, and the resulting augmentation in CBF has been putatively linked to improved cognitive performance, after both acute and chronic supplementation, in humans.

This introduction will now focus specifically on the polyphenol resveratrol; a close structural relative of the flavonoids, which is the focus of investigation in this thesis. Whilst resveratrol has not garnered as much evidence of beneficial effects on human health as, for instance, the cocoa-flavanols, evidence pertaining to the latter group was presented in this thesis to demonstrate that structurally related polyphenols are capable of vasodilatory effects, leading to increased CBF and potentially to improved cognitive function. The following will outline the health effects of resveratrol (via a brief foray into the pharmacokinetics of resveratrol and other important information), demonstrating the overlap with cocoa flavanols. The ensuing sections will culminate with the hypothesis that resveratrol too should be capable of improving cognitive performance via vasodilation and augmented CBF in healthy humans.

1.4 Pharmacokinetics of resveratrol

1.4.1 Metabolism and absorption

Once consumed orally, resveratrol is conjugated (paired with a hydrophilic molecular species) from its aglycone (or 'parent') form to sulfated-, glucuronidated and potentially methylated conjugates (Wu, Kulkarni, Basu, Zhang, & Hu, 2011). This first pass/phase II metabolism takes place in both the jejunum and ileum of the small intestine and conversion to these hydrophilic conjugates may represent a mechanism to facilitate entry into the blood stream, diffusion through the body, and excretion (Wu et al., 2011).

Conjugation and de-conjugation of resveratrol is observed to take place numerous times *in vitro* and it has been argued that whilst the former is necessary for absorption, the latter may be needed to convert resveratrol back to its active aglycone form in order to exert biological effects (van de Wetering et al., 2008). This theory finds evidence in the fact that aglycone resveratrol is often absent, or present only in very low concentrations, in plasma. Abd El- Mohsen et al. (2006) believe this phenomenon, which they observed in their own results, supports the possibility that ubiquitous enzymes, such as β-glucuronidase, could convert conjugates back to resveratrol aglycone locally or systematically once they have travelled through the blood in metabolite form. As an aside, if it is the case that conjugates are 're-activated' in target tissue after being transported through the body in the form of benign conjugates, then taking plasma levels of aglycone resveratrol (which has consistently been observed to have extremely low bioavailability) as a proxy for bioactivity, would not provide a completely accurate picture. This might also explain the apparent paradox within the resveratrol literature; that resveratrol exerts a wealth of significant physiological effects despite poor bioavailability.

Whilst the majority of aglycone resveratrol undergoes extensive first pass metabolism in the small intestine, a certain amount is able to bypass this and reaches the liver un-conjugated (Wu et al., 2011). Here, aglycone resveratrol is glucuronidated/ sulfated/methylated and either excreted in bile or absorbed into systemic circulation as conjugates. After intestinal and hepatic conjugation resveratrol may then reach the colon. Here bacterial microflora de-conjugate to aglycone resveratrol which then has the potential to be absorbed into systemic circulation or, alternatively, to be returned to the intestines (Wu et al., 2011).

This enterohepatic recirculation (enteric recirculation by re-absorption after intestinal hydrolysis) has been demonstrated in many animal and human investigations. Marier et al. (2002) argue that enterohepatic recirculation contributes significantly to the exposure of rats to aglycone resveratrol; as demonstrated by a second plasma peak between 4- and 8hrs post-dose, following an initial peak at 0.29hrs. The pharmacokinetic observations described by Boocock et al. (2007) in humans support this finding with a second plasma peak of resveratrol at approximately 5hrs post-dose, following an initial peak at 0.8hrs. Walle et al. (2004) also observed an initial peak at 1hr following the oral consumption of 25mg of resveratrol in adults followed by a second plasma peak at approximately 6hrs post-dose. Interestingly this was not the case if resveratrol was administered intravenously which may suggest the importance of the aforementioned digestive and absorption processes before resveratrol reaches the blood stream.

With regards absorption from the intestine, several transporters have been highlighted as demonstrating the capacity to displace resveratrol. Van de Wetering et al. (2008) theorised that the intestinal Breast Cancer Resistance Protein (BCRP) and Multidrug Resistance Protein 3 (MRP3) pumps are optimally placed to handle sulfo- and glucuronic acid conjugates of resveratrol. These transporters are known as ATP binding cassettes (ABC) and their role is to efflux xenobiotics back to the intestinal lumen (via BCRP on the Apical side of intestinal membrane) which reduces bioavailability, or into the blood (via MRP3 on the basolateral side) facilitating absorption. *In vitro* 'knock-out' studies of these pumps concluded that BCRP and MRP3 are two major determinants of the pharmacokinetics of resveratrol and its metabolites. BCRP was found to transport resveratrol in its parent form, as well as sulfate metabolites, with high affinity whereas resveratrol-3-glucuronide had high affinity for MRP3. This phenomenon would explain the relatively higher plasma levels of glucuronidated resveratrol explained in more detail below.

1.4.2 Bioavailability

The efficient metabolism and excretion of resveratrol described above could be considered a product of the bodies' identification of this polyphenol as a xenobiotic with potentially toxic effects (van de Wetering et al., 2008). The result of this rapid excretion is extremely low bioavailability of resveratrol, especially of the aglycone, in serum and plasma after various means of ingestion. With regards the aglycone, after oral ingestion of 50mg/kg, levels were reported to reach a mean of 6.57μmol/L with a t_{max} of 0.29hrs

in the rat (Marier et al., 2002). In humans, Goldberg, Yan, and Soleas (2003) demonstrated a serum peak of aglycone resveratrol in humans approximately 0.5hrs after an oral dose of 25mg with a C_{max} of 8.5μg/L.

Resveratrol conjugates however, as explained above, are the predominant form of resveratrol identified in plasma and serum. Indeed, in the aforementioned Goldberg et al. (2003) investigation, the serum C_{max} of resveratrol aglycone at 8.5μg/L represented only, at most, 1.9% of the total concentration of resveratrol; the remainder comprising resveratrol conjugates. The glucuronidated conjugate appears to be the most abundant form of resveratrol (of aglycone and conjugates) identified in the plasma and serum of both animals and humans (Kuhnle et al., 2000; Wang et al., 2004 respectively). Marier et al. (2002), for example, demonstrated that glucuronidated resveratrol could reach mean levels of 105.2μmol/L (compared to the aglycone C_{max} of 6.57μmol/L), in rats, 0.42hrs after an oral dose of 50mg/kg. Boocock et al. (2007), conducting a phase-1 dose escalation pharmacokinetic study of resveratrol in healthy human volunteers after single doses of 0.5-, 1-, 2.5- or 5g pure resveratrol, demonstrated that two glucuronide conjugates attained a C_{max} of between 369.5- and 404.6 ng/mL at a t_{max} of 1.5- and 2hrs. This was in comparison to the aglycone C_{max} of 72.6ng/mL at a t_{max} of 0.8hrs. Conversely, it has been suggested that resveratrol sulfates are rarely detected in plasma (Abd El-Mohsen et al., 2006) with Wenzel et al. (2005) proposing this as evidence that sulfation via O- sulfotransferases is merely a secondary elimination pathway only supporting glucuronidation when a high dose of resveratrol is administered. This theory is difficult to support however as, in many studies which observe a distinct lack of the sulfated metabolite of resveratrol, relatively low doses are administered, i.e. 10-100μM (Kuhnle et al., 2000; Wang et al., 2004) and, as such, would not necessarily require this secondary elimination route.

Taken together, these results demonstrate that plasma concentrations of *trans*-resveratrol are very low after acute, oral consumption. However, the results from three preclinical chemoprevention studies suggest that extremely low daily doses of resveratrol (between 200μg/kg and 2mg/kg) are sufficient to produce peak plasma concentrations of aglycone resveratrol of ~20nM-2μM which was reported to exert beneficial chemopreventive effects (reported in Gescher & Steward, 2003). Research into repeated dosing/chronic consumption of resveratrol and the effects of this treatment regimen on bioavailability is lacking with, to the best of current knowledge, only Almeida et al. (2009) conducting a repeated-dose study in humans. This investigation was, however, limited by utilizing only a 48hr dosing period (with consumption of either 25-, 50-, 100- or 150mg resveratrol every 4hrs during this period) and did not observe a significant difference in the C_{max} between first and last

measurements of the 48hr period for any resveratrol dose. Theoretically though, chronic consumption of resveratrol (over a period of weeks rather than days) might represent a method to increase plasma levels due to cumulative increases over time. This may, in turn, affect the bioefficacy of resveratrol *in vivo*.

Conclusions

These investigations have demonstrated that resveratrol is well absorbed in both animals and humans but that extensive first pass metabolism severely reduces bioavailability of the aglycone. The aglycone form of resveratrol is largely considered to be the 'active' derivative but the issue of its bioavailability, and the potential bioactivity of its metabolites, still remains to be fully elucidated.

1.5 Other information

1.5.1 Safety of resveratrol

To date, few dose escalation and tolerance studies have been conducted to ascertain the safety and potential side effects of polyphenols in humans. Data from two pharmacokinetic/safety studies report that resveratrol is generally tolerated well at doses which far exceed those which would be consumed as part of the diet. In a dose-escalation study 40 participants (22 female, 18 male. 19-61yrs, mean age 32.5yrs) received four doses of resveratrol; 0.5-, 1-, 2.5- and 5g. Twenty three out of this sample, across all doses, reported some form of adverse event during the investigation. The most significant of these were two participants in the 1g condition who presented with raised bilirubin and aminotransferase levels although these were reported to be resolved within a week (Boocock et al., 2007). The remainder were considered to be mild and not serious.

Almeida et al. (2009) conducted a similar pharmacokinetic and safety profile of resveratrol in 40 (20 male, 20 female) healthy participants. Participants were assigned to one of five treatment conditions: placebo, 25-, 50-, 100- and 150mg resveratrol, with eight participants in each condition. Eighteen adverse events were reported with nine considered to be treatment related, the most common being headache with one participant in each of the 25-, 50- and 150mg conditions experiencing this. All events were considered to be mild.

These two investigations were the only reports of adverse events related to resveratrol which could be found at present. Bearing this lack of adverse events in mind, together with the fact that resveratrol is widely available off the shelf as a health food supplement with no anecdotal issues, resveratrol seems to be well tolerated. Where adverse events are reported, these tend to be mild in severity with short-term symptomatology.

1.5.2 Resveratrol and the blood brain barrier (BBB)

Most research into whether polyphenols can cross the BBB has been conducted with catechins. However, due to their structural similarity to resveratrol, tentative parallels can be drawn. EGCG, for example, has been reported to enter the mouse brain after oral administration (Suganuma et al., 1998) and in rat brain tissue after oral ingestion of

epicatechin (Abd El-Mohsen et al., 2006). In terms of mechanisms, this may be as a result of interaction with P-Glycoprotein (P-gp) efflux; with quercetin, catechin, epicatechin (EC) and epicatechin gallate (ECG) all demonstrating the capacity to stimulate P-gp (Critchfield, Welsh, Phang, & Chao Yeh, 1994; Mitsunaga et al., 2000; Wang, Barecki-Roach, & Johnson, 2002). Paradoxically, quercetin has also been reported to inhibit P-gp (Mitsunaga et al., 2000), as has epigallocatechin (ECG), catechin gallate (CG) and EGCG (Jodoin, Demeule, & Béliveau, 2002), suggesting that the relationship may be more complicated; perhaps dose-dependent.

With regards resveratrol specifically, few studies have investigated the potential for resveratrol to cross the BBB and, those which have, provide only indirect data. Wang et al. (2002) and Mokni et al. (2007), for example, both argue that neuroprotection in gerbils, after resveratrol administration, is proof of crossing the BBB. However, this indirect data fails to take into account the potential for resveratrol to be exerting these effects via indirect mechanisms. In terms of potential however, specific binding sites for polyphenols (specifically catechins and resveratrol) have been found to be broadly distributed in the rat brain, with the highest levels of labelling seen in the choroid plexus and subformical organ. The potency of neuroprotection is also well correlated (r=0.74) with binding affinity *in vitro* (Han, Bastianetto, Dumont, & Quirion, 2006).

1.6 Health effects and mechanisms of action of resveratrol

1.6.1 Chemopreventive and chemotherapeutic effects of resveratrol

With regards cancer prevention and chemotherapeutic effects, Kundu and Surh (Kundu & Surh, 2008) identify seven key areas in which resveratrol has shown the potential to influence relevant mechanisms and produce therapeutic outcomes. The first of these is by blocking carcinogen activation by inhibiting phase I enzymes; for instance cytochrome P450 enzymes in human liver cells (Chang, Lee, & Ko, 2000). The second is by boosting antioxidant capacity and inducing phase II carcinogen detoxifying enzymes; for instance by up-regulating activity of the endogenous antioxidant heme oxygenase-1 (HO-1) in human aortic smooth muscle cells (Juan, Cheng, Lin, Chu, & Lee, 2005). The third mechanism involves arresting cell proliferation by modulating cell cycle regulatory machinery; for instance by up-regulating expression of the p21 protein (which is involved in regulating cell cycle progression) *in vitro* (Kuo, Chiang, & Lin, 2002). The fourth is by inducing apoptosis of damaged or transformed cells; for instance by activating death receptor proteins such as CD95 (cluster of differentiation 95) as seen in human breast cancer cells (Clément, Hirpara, Chawdhury, & Pervaiz, 1998). The fifth mechanism involves turning off angiogenic switches and blocking neovascularisation (the formation of blood vessels) in tumour tissues; e.g. by inhibiting the expression of vascular endothelial growth factor (VEGF) as observed in human tongue cell carcinomas (Zhang et al., 2005). The sixth mechanism involves suppressing tumour invasion and metastasis (spread); for instance by reducing the expression and activity of proteins associated with cell proliferation and angiogenesis, such as matrix metalloproteinases- 2 and 9 (MMP-2 and MMP-9 respectively) (Hu, Guo, Zhang, & Tan, 2006). Finally, the seventh mechanism by which resveratrol may confer chemotherapeutic benefits is by sensitizing tumour cells for chemotherapy-induced apoptosis, alongside functioning synergistically with existing chemotherapy drugs. As an example, resveratrol has been observed to enhance the efficacy of the apoptotic drugs ‘bortezomib’ and ‘thalidomide’ *in vitro* (Bhardwaj et al., 2007) and this may be as a result of its ability to inhibit the multi-drug resistant protein (MDRP) efflux of chemotherapeutic drugs (Nabekura, Kamiyama, & Kitagawa, 2005).

1.6.2 Anti-viral/fungal/bacterial effects of resveratrol

Within the plant, resveratrol represents an active defence mechanism against invading microbial, fungal and bacterial attack (Harborne, 1993). Resveratrol has also received

attention for its potential to exert anti-fungal/viral/bacterial activity against human pathogens with some promising results.

With regards fungicidal effects of resveratrol, *in vitro* antifungal activity has been observed against the human oral and genital pathogenic yeast fungus *Candida albigans* at concentrations of 10-20μg/Ml (Jung et al., 2005). A later study by the same authors (Jung, Seu, & Lee, 2007) confirmed these effects and demonstrated that this fungicidal activity did not occur at the cost of the *ex vivo* human cells themselves. With regards mechanisms, resveratrol seems to be proffering fungicidal activity by disrupting key actions of fungicidal pathogenesis (e.g. the serum-induced mycelia forms) and arresting the cell cycle (Jung et al., 2007).

Resveratrol has also demonstrated anti-viral activity against the human *Varicella zoster* (chickenpox) virus, in a dose-dependent manner (Docherty, Sweet, Bailey, Faith, & Booth, 2006), and has been reported to target early-stage replication of the *Herpes simplex* virus types 1 and 2 (Docherty et al., 1999). Finally, there is also a small amount of evidence to suggest that resveratrol may offer protection against the HIV virus (Heredia, Davis, & Redfield, 2000), although a lack of research prevents any conclusions as to its efficacy here.

Antibacterial actions of resveratrol have also been observed; with Daroch et al. (2001) reporting on the ability of 16 Chilean red wines, with resveratrol identified as the main active component, to proffer antibacterial activity against six strains of the carcinogen *Helicobacter Pylori*. A crude *polygonum cuspidatum* (Japanese knotweed) extract has also been observed to inhibit five common food-borne bacteria (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli* and *Salmonella anatum*) as did the major bioactive components of this extract; including resveratrol (Shan, Cai, Brooks, & Corke, 2008).

1.6.3 Resveratrol and longevity

As discussed in section 1.2 of this chapter, polyphenols, including resveratrol, have long been appreciated within Ayurvedic medicine; with the literal translation of Ayurveda meaning ‘the science of life and longevity’. More recently, modern research has begun to uncover the longevity-enhancing capacity of resveratrol in a range of animal models; with demonstrations of improved health and survivability in a range of animal models.

These models include relatively simple organisms such as *saccharomyces cerevisae* (yeast) (Howitz et al., 2003) and *caenorhabditis elegans* (the nematode or 'roundworm') (Viswanathan, Kim, Berdichevsky, & Guarente, 2005) and more complex creatures like *nothobranchius furzeri* (the short-lived fish), whose average life-span was reportedly increased by 56% alongside improvements in motor function and delayed neuro-degeneration (Valenzano et al., 2006).

With regards underlying mechanisms, for the past decade resveratrol has been investigated for its ability to promote longevity by interacting with the sirtuin ('silent information regulator': SIRT) system; a class of proteins involved with multifarious biological processes, including mimicking the effects of caloric restriction. Caloric restriction (CR) is defined as a "reduction of energy intake in the absence of malnutrition" (Agarwal & Baur, 2011) and is associated with robust and well validated health-improving effects including attenuated neuro-degeneration, and disorders like diabetes and CVD (Agarwal & Baur, 2011). SIRT1 is also observed to deacetylate the peroxisome proliferator-activated receptor-g coactivator-1 alpha (PGC-1α) (Rodgers et al., 2005); a regulator of genes involved in energy metabolism, which may also underlie its life-extending properties.

Despite the popularity of the resveratrol-SIRT life-extending theory during the last decade, more recently, the role of SIRT here has been questioned. This is based on the modulation of this pathway having no efficacy in certain models (see Agarwal & Baur, 2011 for fuller review of this argument). A potential alternative to a purely SIRT-mediated pathway involves the mechanistic/mammalian target of rapamycin (mTOR) kinase. The role of mTOR complexes 1 and 2 is to integrate information from a number of cascades; specifically those relating to insulin and nutrient status (Lamming et al., 2012). The result of inhibition of this pathway, as above with SIRT, is the life-extension of a number of species; including the nematode (*Caenorhabditis elegans*), mouse and budding yeast (*Saccharomyces cervisiae*) (Harrison et al., 2009; Kaeberlein et al., 2005; Vellai et al., 2003). The authors of the latter paper suggest that TOR could be a conduit through which excess nutrient intake limits longevity; with its inhibition effectively mimicking caloric restriction in a similar manner to SIRT. Interestingly, SIRT has more recently been observed as a key regulator of the TOR pathway (Ghosh, McBurney, & Robbins, 2010) and research also suggests that resveratrol may directly facilitate life extension by inhibiting TOR directly (Brito et al., 2009).

Taken together, this research suggests that resveratrol can effectively extend the life-span of a number of vertebrate and invertebrate species. It appears as though resveratrol may be capable of influencing this directly; by inhibiting the activity of the

TOR pathway and/or indirectly; by interaction with SIRT. The lack of efficacy with resveratrol-SIRT mediated life extension in some models might suggest a species-specific effect or may be due to methodological differences between studies. It is likely, however, that other, unseen factors are also involved.

1.6.4 Cardioprotective effects of resveratrol

Resveratrol and other grape products are probably most notable for their effects relating to cardiovascular function and cardioprotection; hence the coining of the term 'French paradox' (Kopp, 1998). The cardiprotective effects of resveratrol, and indeed other grape products (which this section will also cover), can broadly be attributed to three main areas: anti-atherosclerotic effects; antioxidant and anti-inflammatory actions; and those actions relating to endothelial function and blood flow enhancement.

1.6.4.1 Anti-atherosclerotic effects of resveratrol

Atherosclerosis is typified by the accumulation of fatty deposits along the arterial wall and is a causal factor in endothelial dysfunction. Grape products have demonstrated protective properties here; chiefly by reducing cholesterol levels. In hamsters, for example, decreased atherosclerosis was observed after 10 weeks supplementation with Concord grape juice alongside a hypercholesterolemic diet (Vinson, Teufel, & Wu, 2001). Similarly, rabbits who were previously administered such a diet and subsequently supplemented with 225ml/day Concord grape juice for 48 days, also exhibited the same protective effects, which were related to concomitant reductions in serum cholesterol levels (Shanmuganayagam, Warner, Krueger, Reed, & Folts, 2007). These findings highlight the protective effects of grape products alongside a high fat diet (which supports the French paradox theory in animals) and the reversal of the deleterious effects of pre-existing CVD.

In humans, grape products also demonstrate the capacity to protect against atherosclerosis by reducing cholesterol levels. In pre- and post-menopausal women, for example, supplementation with 36g daily of grape powder for 4 weeks evinced reduced plasma triglycerides and cholesterol activity (Zern et al., 2005). More recently, a human clinical trial into the cardiprotective effects of resveratrol also observed a significant reduction in LDL levels in post-myocardial infarction patients after 10mg daily for 3 months (Magyar et al., 2012).

1.6.4.2 Antioxidant and anti-inflammatory effects of resveratrol

Resveratrol and grape products have potent antioxidant and anti-inflammatory actions which have the potential to offer both direct and indirect protection against CVD; i.e. by scavenging free-radicals and inhibiting inflammatory mediators directly (Donnelly et al., 2004; Jia et al., 2008) and by up-regulating endogenous antioxidant protection, e.g. by activating the Nrf2/antioxidant response element (ARE) pathway (Ungvari et al., 2010).

By preventing the oxidation of LDL cholesterol the release and build-up of fat along the arterial wall, and the ensuing inflammation, is prevented. This is observed in healthy adults after 2 weeks supplementation with 10mg/kg body weight/day of Concord grape juice (O'Byrne, Devaraj, Grundy, & Jialal, 2002) and decreased inflammatory markers have also been reported after 14-days supplementation of 7ml/kg body weight/day of this same juice product (Albers, Varghese, Vitseva, Vita, & Freedman, 2004).

1.6.4.3 Endothelial function and blood flow effects of resveratrol

Endothelial dysfunction contributes to CVD by preventing the release of chemicals which mediate vascular tone and the viscosity of blood; thus leading to impaired vasodilation and a reduction in blood flow capacity. Section 1.3.2 demonstrated how cocoa flavanols are able to interact with the natural vasodilatory response of the endothelium and, in turn, augment blood flow. Importantly, resveratrol also has the capacity to interact with this mechanism and is able to induce relaxation of the vasculature and smooth muscle *in vitro*/*ex vivo* and *in vivo*. Resveratrol appears to operate via two mechanisms here (although a third will be proposed) which are endothelial dependent and independent; i.e. involving an interaction with and without nitric oxide (NO) respectively.

1.6.4.3a Nitric oxide (NO)-dependent vasodilation

With regards the mechanisms underlying NO-dependent vasorelaxation, research utilizing red wine polyphenols, which include resveratrol, demonstrates that this effect seems to be mediated by an interaction with the existing cholinergic relaxation pathway at the calcium (Ca^{2+}) stage (see figure 1.2.). Specifically, this interaction manifests in stimulation of the Ca^{2+}-dependent release of NO via an increase in cytosolic free calcium from intracellular stores and by increasing cellular Ca^{2+} entry (Martin, Andriambeloson, Takeda, & Andriantsitohaina, 2002). The effects of this can be seen in the attenuation of red wine polyphenol-induced relaxation in rat thoracic aortic rings

by the absence of extracellular calcium (Andriambeloson, Stoclet, & Andriantsitohaina, 1999), and the increase in cytosolic free calcium in response to red wine polyphenols in bovine aortic endothelial cells (Martin et al., 2002). As a result of this interaction with NO, resveratrol (>3x10-5M) is able to induce endothelium-dependent vasorelaxation *ex vivo* in rat aortic rings (Chen & PaceAsciak, 1996). This is associated with a specific up-regulation in levels of the endothelial isoform of NO (eNOS) (Leikert et al., 2002) and the resultant peripheral vasodilation can alleviate hypertension in rats (Rush, Quadrilatero, Levy, & Ford, 2007) after chronic (28 day) consumption.

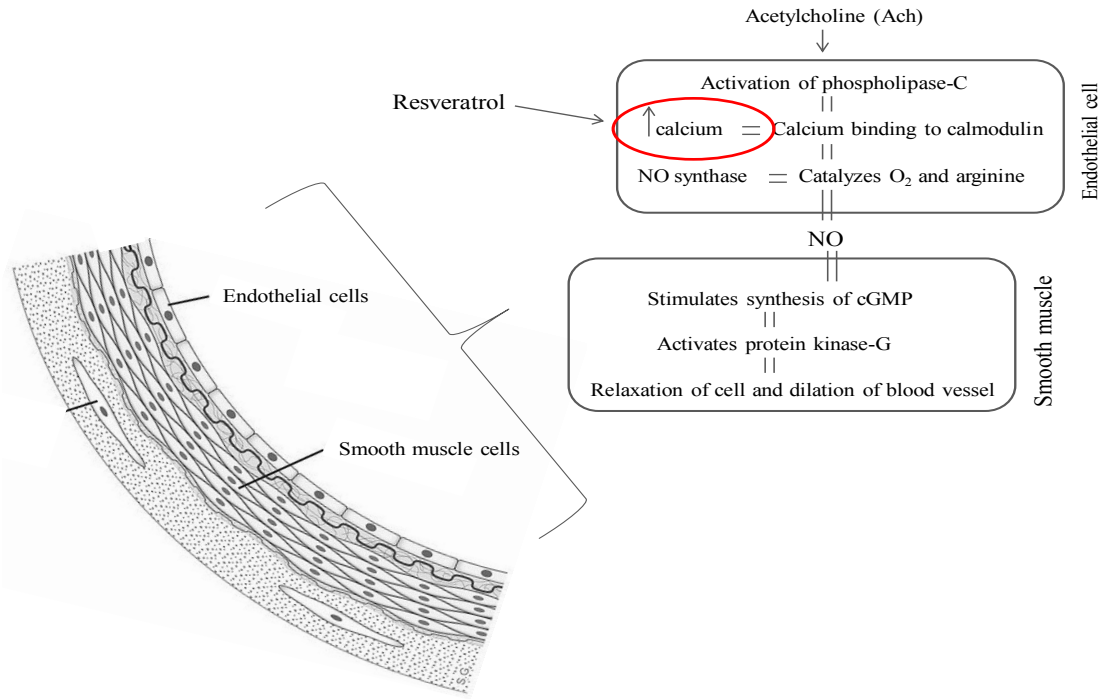


Figure 1.2. Resveratrol interaction with NO-dependent vasorelaxation. (Image of vascular smooth muscle cell adapted from Stijn Ghesquiere (Ghesquiere, 2005)). Nitric oxide (NO)-dependent vasorelaxation is instigated by cholinergic binding to a G-protein-coupled receptor on the endothelial cell. This activates the enzyme phospholipase-C which, in turn, increases intracellular calcium levels. Calcium then binds to the protein calmodulin which facilitates NO synthase. NO synthase then catalyses O_2 and the α-amino acid arginine which results in NO production. NO then diffuses into the smooth muscle where it stimulates synthesis of cyclic guanosine monophosphate (cGMP) leading to activation of protein kinase-G and, ultimately, relaxation of the cell and dilation of the blood vessel (see Dawson & Dawson, 1995 for review).

The above supports the proposition that resveratrol can augment peripheral blood flow in response to eNOS-induced vasodilation. However, resveratrol has also been observed to up-regulate the other vasorelaxatory isoform of NO; neuronal NOS (nNOS). Although both eNOS and nNOS are found in brain tissue (Toda & Okamura, 2003), it is nNOS that has been observed to make the greatest contribution to activity-dependent vasodilation in the neuronal vasculature (Ayata, Ma, Meng, Huang, & Moskowitz, 1996; Cholet, Seylaz, Lacombe, & Bonvento, 1997; Kitaura et al., 2007; Santizo, Baughman, & Pelligrino, 2000). Thus resveratrol should also be capable of augmenting CBF via nNOS mediated vasorelaxation. To date however, only indirect evidence from animal models exists; including demonstrations that resveratrol can up-regulate levels of eNOS and proffer neuroprotective effects in ischemic rats (Tsai et al., 2007) and attenuate the reduction in CBF during middle cerebral artery occlusion (MCAo) and reperfusion as a result of arterial vasodilation (Ritz, Ratajczak, et al., 2008).

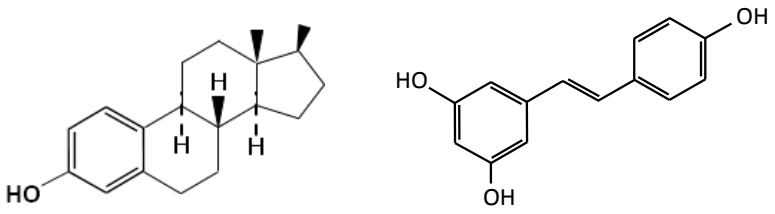
1.6.4.3b Nitric oxide (NO)-independent vasodilation

With regards NO-independent vasorelaxation, research shows that, at high doses, resveratrol (>6x10-5M) (Chen & PaceAsciak, 1996) is able to induce vasorelaxation in endothelium-denuded aortic rings which cannot be reversed by NO inhibitors. This suggests that mechanisms independent of NO are involved. Work by Novakovic et al. (Novakovic, Bukarica, et al., 2006; Novakovic, Gojkovic-Bukarica, et al., 2006) and Gojkovic-Bukarica et al. (2008) suggests the role of potassium (K^+) channels, located in the smooth muscle layer of the vasculature, in this phenomenon. Potassium channel-induced vasodilation is a relatively poorly understood process. However, early research demonstrated that local changes in extracellular K^+ levels are observed to occur during neuronal activity (Syková, Kříž, & Preis, 1983) and that vasorelaxation in cerebral blood vessels is elicited in response to increases in potassium concentrations in the hypothalamus of anesthetised rabbits (Cameron & Caronna, 1976).

1.6.4.3c Oestrogenic modulation of vasodilation by resveratrol

A third, and final, potential vasodilatory mechanism of resveratrol relates to its structural similarity to the hormone oestrogen (See figure 1.3). A small amount of literature suggests that polyphenols, specifically isoflavones like daidzein and genistein, may be able to exert estrogenic-like effects. Estradiol (E_2) is able to modulate vascular tone via rapid release of eNOS (Haynes et al., 2000) and supplementation with genistein (Teede et al., 2003) and a soy protein diet (Mahn et al., 2005) have been shown to increase eNOS expression and, in turn, improve endothelial function in humans and animals respectively. The structural similarity between resveratrol and

oestrogen, and its ability to bind to oestrogen receptors and transcribe oestrogen-responsive genes *ex vivo* (Gehm, McAndrews, Chien, & Jameson, 1997), suggests that resveratrol may too have potentially phytoestrogen properties; although research here is scarce.



17-β-Estradiol *Trans*-resveratrol

Figure 1.3. Chemical structure of oestrogen and *trans*-resveratrol. Structures from Kennedy, D.O. (In press). *Plants and the Human Brain*. Oxford University Press. New York.

1.6.4.3d Effects of vasodilation by resveratrol in humans

In humans, the acute consumption of a grape product (approximately equivalent to 1.25 cups fresh grapes) was observed to significantly improve brachial artery flow-mediated dilatation (FMD) 45- and 90 minutes post consumption and even more so after a 21 day regimen of consumption of ~2.5 cups per day. In a separate arm, the grape product was also reported to completely prevent the 50% reduction in FMD induced by a high fat meal at 45-, 90- and 180 minutes post consumption of the meal and grape product (Chaves et al., 2009). Supplementation with Concord grape juice is also reported to enhance vasodilation in adults at doses of 4- and 8ml/kg body weight/day after dosing regimens of 56 days and 2 weeks (Stein, Keevil, Wiebe, Aeschlimann, & Folts, 1999; Wiebe, Folts, & Stein, 2001). Finally, acute supplementation (1hr post oral consumption) of 30-, 90- and 270mg resveratrol alone was also observed to improve FMD in 19 overweight/obese individuals with mildly elevated blood pressure (Wong, Berry, Coates, Buckley, & Howe, 2012; Wong et al., 2011).

It is worth mentioning here that much of the above research has investigated the vasodilatory effects of 'grape products' rather than resveratrol alone. As such, it is not possible to categorically attribute these effects to resveratrol and indeed other grape polyphenols may be responsible and/or interacting synergistically. In the face of little research investigating the vascular/health effects of resveratrol alone, however, these findings can still provide insight into the potential effects of resveratrol.

1.7 Cognitive and mood effects of resveratrol

1.7.1 Cognitive/mood neuroprotection and enhancement by resveratrol in animals

1.7.1.1 Cognitive performance effects of resveratrol in animals

Resveratrol has demonstrated neuroprotective properties in a range of senescent animal models and this is associated with an attenuation of cognitive decline. Kumar et al. (2007), for example, assessed the potential for resveratrol to attenuate the cognitive decline observed in a rat model of Alzheimer's disease (AD). Supplementation with 10- and 20mg/kg/day of resveratrol for 25 days, beginning 4 days prior to, or after, AD insult, lead to a significant attenuation of cognitive impairment alongside significant reductions in markers of oxidative stress. These behavioural improvements included a significant reduction in time taken to reach the appropriate arm in the elevated plus maze, and a reduction in retention latency in the Morris Water Maze (MWM) test, with both doses of resveratrol at day 14 and 21 of the supplementation regimen. Both doses also significantly prevented the AD-induced increase in malondialdehyde (MDA) and nitrite levels, and depletion of glutathione (GSH), as well as attenuating the AD-induced reduction in acetylcholinesterase activity. Sharma and Gupta (2002) also report a preservation of cognitive function in a rat model of AD with the same doses of resveratrol (10- and 20mg/kg/day); demonstrating better acquisition and retention of memory on the elevated plus maze and passive avoidance tasks after 21 days. The findings were accompanied by a significant increase in levels of glutathione in the brain with both doses of resveratrol. Glutathione and superoxide dismutase were also increased in the brain tissue of senescence-accelerated mice after 8 weeks resveratrol supplementation, alongside improved learning and memory on the MWM (Liu, Zhang, Yang, & He, 2012).

As well as attenuation of cognitive decline in compromised models, animal data also demonstrates the potential for resveratrol to be beneficial in the preservation of cognitive function during healthy ageing. In healthy, adult grey mouse lemurs working and spatial memory were both increased in 200mg/kg/day resveratrol-supplemented animals, similar to that seen in a calorie-restricted condition (Dal-Pan, Pifferi, Marchal, Picq, & Aujard, 2011). In naturally ageing mice, Oomen et al. (2009) report that supplementation with 150µg resveratrol per day for one month enhanced memory acquisition in the Y-maze task. This was coupled with preserved microvascular density (which was 15% higher in the hippocampi of resveratrol-treated mice) and fewer microvascular abnormalities compared to untreated animals. This study suggests that the beneficial effects of resveratrol, in terms of preserving cognitive function, was as a result of maintaining cerebro-vascular health.

Taken together, resveratrol has demonstrated neurocognitive protection in both senescent and healthy-ageing rodents. However, that the former were more associated with improved markers of oxidative stress, and the latter those relating to improved CBF parameters, suggests that different mechanisms might be at work. It may be the case that antioxidant neuroprotection has a greater role in preserving cognitive function during neurodegeneration (disorders associated with increased oxidative stress and reduced endogenous antioxidant activity (Marcus et al., 1998)) and that improved cognition in healthy models is more a consequence of increased CBF parameters. However, as all above studies did not measure both oxidation and blood flow, it is not possible to support this argument fully.

1.7.1.2 Mood effects of resveratrol in animals

Resveratrol also possesses the capacity to augment mood via a number of mechanisms. In a paradigm assessing the behavioural and neurochemical anxiolytic properties of resveratrol, mice orally ingested either 10-, 20-, 40 or 80mg/kg *trans*-resveratrol and were subjected to two despair tests: forced swimming and tail suspension. The results with regards behaviour showed that the three largest doses significantly reduced immobility time in forced swimming but that only 40- and 80mg/kg were able to exert these effects in the tail suspension test. Neurochemical analysis revealed that both 40- and 80mg/kg were able to increase levels of 5-hydroxytryptamine (5-HT) in the hippocampus and that 80mg/kg was also able to augment levels of noradrenaline in the hippocampus and 5-HT in the hypothalamus. 80mg/kg was the only dose able to able to inhibit MAO-B activity but all doses, after a minimum 30 minutes post ingestion, were able to inhibit MAO-A activity (Xu, Wang, et al., 2010).

This selectivity for MAO-A, over MAO-B, by resveratrol has also been confirmed by other studies where the *cis*-isomer too is observed to preferentially inhibit the activity of the former over the latter (Yáñez, Fraiz, Cano, & Orallo, 2006). These results demonstrate that resveratrol is able to modulate the behavioural effects of stress, in rodents, after oral ingestion and that this might be via augmentation of levels of neurotransmitters relevant to mood. This assertion is supported by a further treatment condition in the aforementioned Xu et al. study in which rodents were pre-treated with a serotonergic antagonist which abolished the anti-immobility effects of resveratrol (Xu, Wang, et al., 2010).

1.7.2 Potential for cognitive effects of resveratrol in humans

To date, no intervention studies have been conducted to assess the potential cognition enhancing effects of resveratrol in humans. This is despite the plethora of *in vitro/ex vivo*/animal model research, described above, which suggests that resveratrol is capable of both preserving and improving aspects of cognitive performance, or influencing mechanisms which might facilitate cognition, in a range of circumstances. The evidence of cognitive enhancing effects of other structurally similar cocoa derived polyphenols in humans, outlined in section 1.3.3, lends additional support to this argument. Resveratrol has demonstrated similar mechanisms of action to those associated with cognitive enhancement by cocoa flavanols; namely vasodilation and improved CBF, and should, therefore, also be capable of improving cognitive function. Finally, resveratrol demonstrates direct cellular metabolic effects which might be anticipated to enhance cognitive processing; especially when coupled with enhanced CBF. These arguments for potential cognitive effects of resveratrol relating to both enhanced CBF and enhanced cellular metabolic processes will now be outlined.

1.7.2.1 Cerebral blood flow (CBF) enhancing effects of resveratrol and potential for improved cognitive performance

A continuous, oxygen-rich supply of blood is vital for general health. Poor circulation of blood and reduced oxygenation (hypoxia) is associated with a number of disorders including cardiovascular disease (Zeiher, Drexler, Saurbier, & Just, 1993; Zeiher, Drexler, Wollschlager, & Just, 1991) and stroke (Fieschi, Agnoli, Battistini, & Bozzao, 1966). Of importance here, blood flow and oxygenation are especially vital for neurocognitive performance; with research correlating reduced CBF and poorer cognitive function in humans (Celsis et al., 1997). Conversely, improvements to aspects of cognitive function can be seen following CBF-enhancing cocoa polyphenols (Desideri et al., 2012; Field et al., 2011; Scholey et al., 2010) and with supplementary inspiration of pure oxygen in deprived participants (Weiskopf et al., 2002) and in young, healthy participants (Moss, Scholey, & Wesnes, 1998; Scholey, Moss, Neave, & Wesnes, 1999).

These latter studies demonstrate that increased access to oxygen (Moss et al., 1998; Scholey et al., 1999) can improve cognitive performance in a group of young (mean age 24.5yrs) adult volunteers. The length of oxygen supplementation also related to performance on specific tasks with 1- and 3 minutes being most effective for immediate and delayed word recall and 30 seconds being most effective for tests of attention. Constant oxygen supplementation was observed to be less effective overall. Relatedly, supplementation of another metabolic substrate, glucose, has also demonstrated

efficacy in improving aspects of cognitive performance; specifically memory (Benton & Parker, 1998; Messier, 2004; Smith, Riby, Eekelen, & Foster, 2011). In an acute intervention study, Kennedy and Scholey (2000), for example, report that administration with 25g glucose can increase the number of subtractions on the cognitively demanding mental arithmetic task 'serial 7s' in healthy, young (mean age 20.4yrs) undergraduate students.

The enhancement in cognitive performance seen following supplementation of the metabolic substrates oxygen and glucose suggests that the increased provision in neural fuel might be driving this performance increase. This suggests that the increase in cognitive performance seen following supplementation of cocoa polyphenols might be related to the enhanced provision of blood-borne metabolic substrates via the increase in CBF. The ability of resveratrol to interact with the same vasodilatory mechanisms as cocoa polyphenols, and to induce vasodilatory effects, suggests that it too should be capable of improving CBF and, in turn, cognitive performance via this increased delivery of fuel.

1.7.2.2 Increased oxygen utilization by resveratrol and potential for improved cognitive performance

The enhanced delivery of metabolic substrates via augmented CBF offers the potential to increase cognitive performance; as outlined above. However, research also shows that resveratrol is capable of enhancing the utilisation of this fuel by influencing cellular oxygenation. This appears to be the product of interacting with mitochondrial phosphorylation and this is most likely facilitated indirectly by resveratrol's interaction with SIRT-1. As mentioned previously (section 1.6.3), SIRT-1 deacetylates PGC-1α (Rodgers et al., 2005), a gene which controls mitochondrial biogenesis and function, and the ability of resveratrol to interact with this mechanism has been attributed to a resultant increase in mitochondrial number (Baur et al., 2006) and function (Lagouge et al., 2006) observed in resveratrol-treated animals. With regards the latter study, 400mg/kg/day resveratrol, for a 15 week supplementation period, significantly enhanced mitochondrial function in mice. This was manifested as larger mitochondrial structures (both in size and content; the latter pertaining to increased mitochondrial DNA) in brown adipose tissue; enhanced mitochondrial enzymatic activity (as evidenced by increased citrate synthase activity in muscle homogenates); a significant increase in O_2 consumption and VO_2 max rate (which indicates an increase in oxidative type muscle fibres and/or increased oxidative capacity) and, as a result, an increase in running time and tolerance to cold.

In terms of the neurological effects of this interaction, resveratrol's modulation of mitochondrial function has been shown to proffer neuro-cognitive protection after stroke-induced (via middle cerebral artery occlusion (MCAo)) damage in rodent models. In rats administered resveratrol intravenously at 7-10g/kg, oxidation of lipids, proteins and cytochrome C were reduced, mitochondrial activity was restored and ATP content was increased. As a result, lesions in the striatum and overlying cortex were significantly decreased in resveratrol-treated rats, compared to MCAo alone. Ischemia induced behavioural dysfunctions, as assessed by the flexion test and spontaneous motor activity (which were severe in MCAo rats at 4hrs into the reperfusion phase), were also significantly reduced (Yousuf et al., 2009).

To date, no data on the oxygenation effects of resveratrol in humans has been reported. However, data from another red wine polyphenol, quercetin, provides tentative positive evidence that polyphenols are capable of interacting with mechanisms of oxygenation in humans. Here, McRae and Mefferd (MacRae & Mefferd, 2006) report that high-intensity cycling was significantly increased in 11 elite male athletes after consumption of quercetin, as part of an antioxidant drink, twice daily for 6 weeks.

.

1.8 Overall summary and conclusions

The aim of this thesis is to investigate the cognitive and cerebral blood flow (CBF) effects of the stilbene polyphenol resveratrol in healthy, young humans. The paucity of resveratrol research meant that this general introduction necessarily had to begin by providing evidence of the cognitive and CBF effects of other, structurally similar, polyphenols; in particular the cocoa flavanols that have attracted the lion's share of research interest in this area. Research then showed that resveratrol shares many of the underlying mechanisms relating to improved cognitive performance by cocoa flavanols; namely a potential to enhance CBF and, in turn, increase the provision of blood-borne metabolic substrates[1]. Research reveals that the supplementation of these substrates, i.e. oxygen and glucose, improves aspects of cognitive function in healthy, young humans. Resveratrol exhibits further cognitive-enhancing potential by interacting with mitochondrial function and increasing utilization of oxygen. Taken together, the hypothesis made here is that resveratrol should be anticipated to exert CBF and, in turn, cognitive enhancing effects in healthy, young humans. The following five experimental chapters aim to test this hypothesis.

[1] Note that the aim of this PhD is to investigate the effects of resveratrol on CBF and cognition; it is not the remit of this thesis to demonstrate the underlying mechanisms of resveratrol in this regard. Thus, whilst it is argued that the potential mechanisms sub-serving the CBF and cognitive effects of resveratrol are underpinned by NO, this cannot be supported here and may indeed be the result of other factors.

Chapter 2.

The cognitive and cerebral blood flow effects of 1000mg and 500mg *trans*-resveratrol in healthy, young humans: A pilot investigation.

2.1 Introduction

Resveratrol (3, 4', 5 trihydroxystilbene) is a polyphenolic secondary metabolite produced within plants in response to a range of environmental stressors. The protective role of resveratrol within the plant may also extend to the animals and humans who ingest it; with a wealth of associated health benefits. Of importance here, these positive health effects include cardio- and cerebro-vascular protection which is likely attributable to the antioxidant (Jia et al., 2008), anti-inflammatory (Donnelly et al., 2004), anti-atherosclerotic (Magyar et al., 2012) and vasodilatory (Chen & PaceAsciak, 1996; Novakovic, Bukarica, et al., 2006; Novakovic, Gojkovic-Bukarica, et al., 2006) effects of resveratrol.

This vasodilatory response has also been observed in other structurally similar polyphenols; such as cocoa flavanols (Fisher et al., 2003), and results in significant augmentation of cerebral blood flow (CBF) (Francis et al., 2006; Sorond et al., 2008). Reduced CBF has been correlated with poorer cognitive function in humans (Celsis et al., 1997) and, conversely, the administration of CBF-enhancing cocoa flavanols in humans has been reported to enhance aspects of cognitive performance. In healthy, young participants, for example, Field et al. (2011) report improved visual function (contrast sensitivity) and spatial cognition following consumption of 720mg cocoa flavanol chocolate; arguing that improved cerebral and retinal blood flow is likely the underlying mechanism. Scholey et al. (2010) also report that 994- and 520mg cocoa flavanols can improve performance on a mentally demanding mental arithmetic task; the rapid visual information processing (RVIP) task, in healthy, young adults although performance on another task, the serial 7 subtractions, was impaired by the higher dose. A later study, however, failed to find any cognition enhancing effects of 30-days consumption of 500- and 250mg cocoa polyphenols, although calmness and contentment were improved with the higher dose (Pase et al., 2013). Finally, in older adults improved visual attention and verbal fluency has been found in a sample suffering from age-related cognitive impairment following 8 weeks supplementation with 990- and 520mg cocoa flavanols (Desideri et al., 2012).

The above suggests that CBF-enhancing polyphenols may be capable of improving aspects of cognitive performance in young, healthy participants, as well as older adults,

following acute and chronic supplementation of a range of doses. Of importance here, resveratrol may share many of the underlying mechanisms of action attributed to cocoa flavanols and these include those relating to augmented CBF. Resveratrol is capable of interacting *in vitro* with nitric oxide (NO*)* which modulates vascular tone (Chen & PaceAsciak, 1996; Novakovic, Bukarica, et al., 2006; Novakovic, Gojkovic-Bukarica, et al., 2006) and vasodilatory effects of resveratrol have been reported in humans. To date improved brachial artery flow-mediated dilatation (FMD) has been observed after the acute and chronic consumption of grape products alone (Stein et al., 1999; Wiebe et al., 2001) and when consumed alongside a high-fat meal (Chaves et al., 2009), and resveratrol specifically has been observed to improve FMD in 19 overweight/obese individuals after acute supplementation (1hr post oral consumption) of 30-, 90- and 270mg (Wong et al., 2012; Wong et al., 2011).

However, whilst resveratrol is capable of peripheral vasodilation, research has yet to investigate whether resveratrol, like cocoa flavanols, can increase CBF and whether this could also extend to improved cognitive performance in humans. Research from animal models however, does provide evidence of neurocognitive protection and enhanced performance following supplementation with resveratrol. Sharma and Gupta (2002) and Kumar et al. (2007), for example, both demonstrated the efficacy of 10- and 20mg/kg/day resveratrol in attenuating the cognitive decline associated with a rodent model of Alzheimer's disease (AD) after 21- and 25-days supplementation respectively. Both studies observed better acquisition and retention of memory on the elevated plus maze, as well as the passive avoidance task (Sharma & Gupta) and Morris Water Maze (MWM) (Kumar et al.), and report these findings alongside significant attenuation of AD-induced oxidative stress. In healthy aged animals, 200mg/kg/day of resveratrol can increase working and spatial memory in the adult grey mouse lemur (Dal-Pan et al., 2011) and supplementation with 150µg resveratrol per day for one month enhanced memory acquisition in the Y-maze task in mice (Oomen et al., 2009).

Interestingly, whilst the neurocognitive protection afforded by resveratrol in compromised animals seems to be related to antioxidant actions, findings from Oomen et al. suggest that the cognitive enhancement observed in their healthy mice was actually the product of improved CBF parameters. Support for this argument comes from the preserved microvascular density (which was 15% higher in the hippocampi of resveratrol treated mice) and fewer microvascular abnormalities which were observed alongside cognitive enhancement in resveratrol-treated animals, as compared to controls. This suggests that resveratrol supplementation in animals can improve cognitive performance and that this may be the result of augmented CBF.

The mechanisms underlying CBF-enhancement of cognitive function are likely to include increased delivery of the blood-borne metabolic substrates oxygen and glucose, to the brain. In support of this, supplementation with these metabolic substrates alone is sufficient to enhance aspects of cognitive performance in healthy, young adults. Inspiration of pure oxygen for 1- and 3 minutes was observed to significantly improve immediate and delayed word recall and tests of attention were significantly enhanced by 30 seconds of inspired O_2 (Moss et al., 1998; Scholey et al., 1999) in a group of young (mean age 24.5yrs) adult volunteers. In another sample of young (mean age 20.4yrs) undergraduate students, the administration of 25g glucose improved performance on a cognitively demanding mental arithmetic task, the serial 7s subtractions; increasing the number of subtractions completed (Kennedy & Scholey, 2000). As such, if resveratrol were capable of augmenting CBF and, in turn, cognitive function, then the mechanisms underlying this are likely the provision of increased access to glucose and oxygen.

This increased access to oxygen has the potential be exploited further by active neural cells due to the effect of resveratrol on mitochondrial oxygen utilization. Resveratrol's interaction with the sirtuin ('silent information regulator': SIRT) system; a class of proteins involved with multifarious biological processes, facilitates a number of physiological effects. Of importance here, the SIRT-mediated deacetylation of Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α); a gene which controls mitochondrial biogenesis and function (Rodgers et al., 2005) has been reported to increase mitochondrial number (Baur et al., 2006) and function (Lagouge et al., 2006) in resveratrol-treated animals. The latter study demonstrated that, in mice, supplementation with 400mg/kg/day resveratrol, for 15-weeks, significantly increased mitochondrial structures in brown adipose tissue and enhanced mitochondrial enzymatic activity; as evidenced by increased citrate synthase activity in muscle homogenates. A significant increase in O_2 consumption and VO_2 max rate were also observed; which is indicative of an increase in oxidative-type muscle fibres and/or increased oxidative capacity. The culmination of these effects was to significantly increase running time; likely due to the increased utilization of oxygen by muscle cells, and a tolerance to cold.

In terms of neurocognitive effects, resveratrol's modulation of mitochondrial function has been shown to confer neurological protection after stroke-induced (via middle cerebral artery occlusion (MCAo)) damage in rodent models. In rats administered resveratrol intravenously at 10-7g/kg, oxidation of lipids, proteins and cytochrome C were reduced, mitochondrial activity was restored and ATP content was increased. As a result, lesions in the striatum and overlying cortex were significantly decreased in

resveratrol treated rats, compared to MCAo alone. Cerebral ischemia induced behavioural dysfunctions, as assessed by the flexion test and spontaneous motor activity (which were severe in MCAo rats at 4hrs into the reperfusion phase), were also significantly reduced (Yousuf et al., 2009).

To date, no data on the oxygenation effects of resveratrol in humans has been reported. However, data from another red wine polyphenol, quercetin, provides tentative positive evidence that polyphenols are capable of interacting with mechanisms of oxygenation in humans. Here, McRae and Mefferd (2006) report that high-intensity cycling was significantly increased in 11 elite male athletes after consumption of quercetin, as part of an antioxidant drink, twice daily for 6 weeks. Whilst this effect, like those demonstrated above in animal models, in also one facilitated by chronic polyphenol consumption, it would be interesting to investigate whether similar effects could be achieved after acute supplementation of resveratrol in humans.

To summarise, evidence demonstrates that polyphenols such as cocoa flavanols are capable of augmenting CBF and, in turn, cognitive performance in humans. This is potentially as a result of increased access to the metabolic substrates oxygen and glucose in cognitively active areas of the brain. The stilbene polyphenol resveratrol shares these CBF-enhancing mechanisms but research has yet to investigate whether it too is capable of increasing CBF and cognitive function in humans. In animal models however, data does seem to suggest that CBF augmentation and oxygen utilization is capable of enhancing cognitive function. Given the above, the hypothesis for the current study is that resveratrol may be able to modulate CBF in healthy, young humans and that this may, in turn, lead to enhanced cognitive function.

The current randomised, placebo-controlled, crossover pilot study aimed to test this hypothesis by measuring CBF in the prefrontal cortex after two doses of resveratrol (500- and 1000mg) while participants completed cognitive tasks which are predominantly sub-served by this brain region. This is the first study of its kind to assess the effects of resveratrol on cognitive performance and CBF in humans and one of the first to utilize the novel neuroimaging technique NIRS to assess the effects of a nutritional intervention in humans.

2.2 Method

2.2.1 Participants

This study recruited 24 healthy adults (10 males, 14 females; mean age 21.42yrs, range 18- 35yrs; 23 right handers, 1 left). All participants attended the laboratory after a 12hr overnight fast and reported that they met the inclusion criteria, i.e. to be in good health and free from social drugs (including alcohol), prescription medication, herbal extracts/food supplements, relevant food allergies, intolerances and digestive problems. All participants were non-smokers and did not consume excessive amounts of caffeine (>six cups of coffee or equivalent/d). In addition, participants who had suffered a head injury, neurological disorder or neuro-developmental disorder were excluded from participation, as were those who had uncorrected sight problems, were pregnant or seeking to become so. Data from two participants was excluded from analysis on the basis of failure to complete the study.

2.2.2 Treatments

Participants attended the laboratory on three active study days and received each of the three treatments in a counterbalanced order dictated by random allocation to a position on a Latin square. The three treatments comprised two capsules containing:

i) Placebo,

ii) 500mg pure *trans*-resveratrol, or

iii) 1000mg *trans*-resveratrol

The purity (99.02% pure) of the naturally extracted (i.e. non-synthetic) *trans*-resveratrol was confirmed by high-performance liquid chromatography. (Nb. the resveratrol utilized in all studies herein was derived from natural sources.) The treatments were administered in identical vegetarian soft-gel capsules, which were prepared and coded by a third party who had no further involvement in any aspect of the study. The research team were blind as to the nature of the individual treatments until after the initial statistical analysis when the aforementioned third party broke the sealed envelope containing the codes. Again, this was the procedure for all studies herein.

2.2.3 Cognitive tasks and mood

Cognitive demand battery (CDB)

The cognitive demand battery (CDB) is a collection of three tasks: 2 minutes each of Serial 3 and 7 subtractions and 5 minutes of Rapid Visual Information Processing (RVIP). This 9-minute battery has a well validated literature; demonstrating sensitivity to the effects of a number of interventions, e.g. ginkgo biloba and ginseng (Scholey & Kennedy, 2002), ginseng and glucose (Reay, Kennedy, & Scholey, 2006) and glucose and caffeine (Kennedy & Scholey, 2004). The rationale for utilizing these tasks to elicit cognitive demand is predicated on several assumptions: firstly, cognitively demanding tasks will require increased cognitive resources (evidenced by stronger neural activation in response to task difficulty (Hasegawa, Carpenter, & Just, 2002) and increased cerebral blood volume (CBV) as a direct result of increased workload (Son, Guhe, Gray, Yazici, & Schoelles, 2005)); secondly, there is a limit to the time and extent to which one can maintain performance on these tasks as the cognitive resources required dwindle over time; and, thirdly, by supplementing with purported cognitive enhancers, this reduction in cognitive performance may be attenuated/improved.

Serial subtractions (3s and 7s)

The serial 3s and 7s subtractions are completed consecutively with the procedure for both as follows: At the start of the 2 minute task a standard instruction screen informs the participant to count backwards in 3s or 7s as quickly and accurately as possible, using the keyboard's linear number keys to enter each response. Participants are instructed verbally at the outset that if they are to make a mistake they should carry on subtracting from the new incorrect number with subsequent responses scored as correct in relation to the new number. To begin, a random starting number between 800 and 999 is presented on the computer screen, which is cleared by the entry of the first response. Each three-digit response is represented on screen by an asterisk and pressing the enter key signals the end of each response and clears the three asterisks from the screen. Thus, participants are never aided by the previous number, nor the entry of the new number, existing on-screen. In terms of task outcomes, performance data for the subtraction tasks comprises number of correct and incorrect responses.

Rapid Visual Information Processing (RVIP)

The RVIP task requires the participant to monitor a continuous series of single digits for targets of three consecutive odd or even numbers. The white digits are presented on the black computer screen at the rate of 100 per minute; with eight correct target

strings in each minute presented in pseudo-random order. The participant responds to the detection of a target string by pressing the appropriate response button as quickly as possible. In terms of task outcomes, RVIP is scored for number of target strings correctly detected and the average reaction time (msec) for correct detections.

Visual analogue scales

Participants were required to rate how 'mentally fatigued' they felt and how 'difficult' they had found the tasks after each CDB repetition by placing a cross, with the mouse and cursor, on a 100mm on-screen line between the descriptors 'not at all' and 'extremely'. The VAS were scored as % along the line towards 'extremely'.

2.2.4 Near-Infrared Spectroscopy (NIRS)

Functional Near-Infrared Spectroscopy (NIRS) is a brain imaging technique that is predicated on the intrinsic optical absorption properties of oxygenated (oxy-Hb) and deoxygenated (deoxy-Hb) haemoglobin following the introduction of near-infrared light (photons) through the intact skull. NIRS has been used extensively as a technique for multiple-channel imaging of task related brain activity over relevant areas of the head (Schecklmann, Ehlis, Plichta, & Fallgatter, 2008), including in groups suffering from potential decrements in CBF (Schecklmann et al., 2007).

The continuous-wave NIRS machine utilized in this PhD (Oxymon system; Artinis Medical Systems B.V.) emits two nominal wavelengths of light (~765- and 855nm) and utilizes the scattering and absorption information of these photons to calculate relative concentration changes (μmol/L) in oxy-Hb, deoxy-Hb and total-Hb (the latter calculated by adding together oxy and deoxy) by means of a modified Beer-Lambert law (Obrig & Villringer, 2003). When assessed by NIRS, the increase in CBF in the surface layers of the cortex during localized neural activity is typically seen as an increase in the total concentration of haemoglobin (total-Hb) and comparative decrease in deoxy-Hb (Steinbrink et al., 2005) with both parameters corresponding strongly with the fMRI BOLD signal (Chul, Tak, Jang, Jung, & Jang, 2009; Huppert, Hoge, Diamond, Franceschini, & Boas, 2006; Steinbrink et al., 2005).

Functional magnetic resonance imaging is the most comparable neuroimaging tool to NIRS with both measuring the CBF response to preceding neural activation; the 'neurovascular coupling'. Neural activation instigates an increase in CBF which is primarily seen as increased concentrations of total and oxy-Hb. This CBF response is greater than the metabolic rate of oxygen extraction/utilization (deoxy-Hb) and, as such, the concentration of deoxy-Hb can be observed to decrease during cognitive performance (Hasegawa et al., 2002). This relative fall in deoxy-Hb is the product of

the local haemoglobin becoming more oxygenated and results in a slight increase in the magnetic signal; as haemoglobin is diamagnetic when oxygenated and paramagnetic when deoxygenated, which is known as the blood-oxygenation-level-dependent (BOLD) signal (Buxton, Uluda, Dubowitz, & Liu, 2004). As stated above, this BOLD signal is strongly correlated to that measured by NIRS; indeed the two techniques have been converged successfully in the past, and a relative rise in total- and oxy-Hb with a concomitant reduction in deoxy-Hb is the typical hemodynamic response to cognitive workload as assessed by NIRS (Tamura, Hoshi, & Okada, 1997).

To return to the use of NIRS in this thesis; a simple two emitter/optode pair (i.e. two channel) array was utilized throughout this programme of studies, with the channels positioned over the left and right prefrontal cortex using a standard optode holder headband. This separates the pairs from each other by a distance of 4cm (mitigating any cross-interference) and the transmitter and receiver from each other by the same distance. Each pair therefore collects data from an area of prefrontal cortex that includes the areas corresponding to the International 10-20 system Fp1 and Fp2 EEG positions. With regards spatial and temporal resolution, when set at a distance of 4cm apart, the transmitter and receiver are able to penetrate the photon light path to a depth of 0.5-2cm (Fukui, Ajichi, & Okada, 2003) thus reaching the capillaries of the prefrontal cortex (Haque, Musha, & Nakajima, 1998). The resulting 'banana-shaped' photon path (see figure 2.1.) is thus able to measure CBF changes in both hemispheres of the prefrontal cortex, but cannot be more specific about regions. However, as the region of interest in this programme of studies is merely the prefrontal cortex as a whole, this does not represent a methodological flaw here. The temporal resolution of NIRS is high and similar to that of fMRI, i.e. roughly less than one second (Hoshi, 2007).

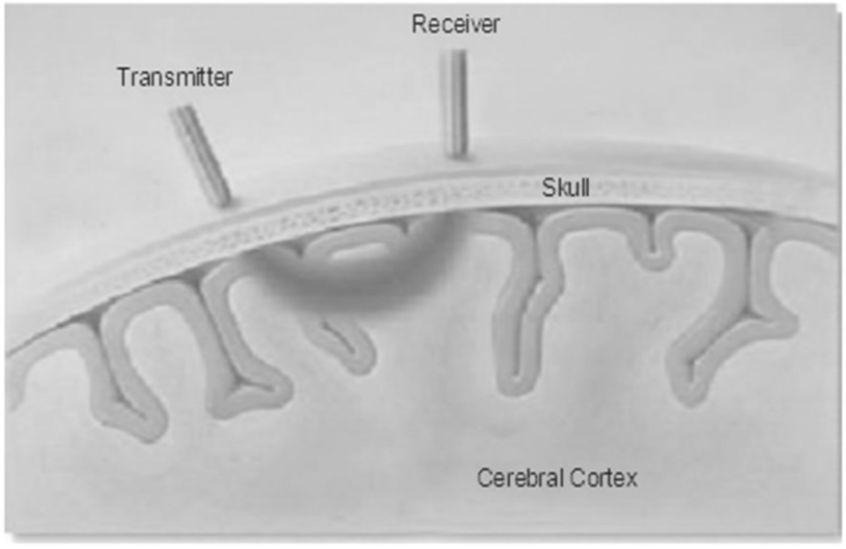


Figure 2.1. 'Banana-shaped' photon light path of Near-Infrared Spectroscopy (NIRS). (Image adapted from Bunce et al. (2006)).

To date, a small number of pharmacological intervention studies have used NIRS to infer localized brain activity (Kanamaru, Kikukawa, Miyamoto, & Hirafuji, 2008) and CBF and oxygenation (Bönöczk, Panczel, & Nagy, 2002) from changes in haemoglobin concentrations. Several studies utilising NIRS to investigate the CBF effects of nutritional interventions have also been conducted since the initial studies described in Chapters 2 and 3 of this thesis. These include studies demonstrating an increased haemodynamic response to task performance following docosahexaenoic acid (Jackson, Reay, Scholey, & Kennedy, 2012a, 2012b) and decreased CBF following a single dose of caffeine (Kennedy & Haskell, 2011) and the polyphenol epigallocatechin gallate (EGCG) (Wightman et al., 2012).

The rationale for utilizing NIRS technology to assess the potential CBF effects of resveratrol here is predicated on the aforementioned sensitivity to other pharmacological compounds with similar actions and the practicality of its use in this context, i.e. that the participant is able to move relatively freely, for a relatively long period of time, consume treatment and sit upright whilst utilizing a laptop to perform cognitive tasks. Factors which restrict the use in other neuroimaging techniques, including FMRI. In terms of expectations, the natural CBF response to neural activation and the anticipated interaction of resveratrol here provides three expected NIRS outcomes: The first is that, as the expression of nitric oxide (NO) and the ensuing vasodilation is activity-dependent, concentrations of total- and oxy-Hb will increase in the prefrontal cortex in response to frontally loaded tasks and that this would be accompanied by a comparative reduction in the concentration of deoxy-Hb (Herrmann, Ehlis, & Fallgatter, 2003). Secondly, as resveratrol is able to up-regulate NO levels (Leikert et al., 2002), it is hypothesized that this activity-driven increase in CBF (i.e. increased levels of oxy- and total-Hb) would be augmented further in response to resveratrol supplementation. Thirdly, if resveratrol exerts the mitochondrial effects seen previously (Lagouge et al., 2006), it would be anticipated that concentrations of deoxy-Hb would also be higher in response to resveratrol than placebo.

2.2.5 Procedure

Each participant was required to attend the laboratory on four occasions. The first of these was an initial screening/training morning, and this was followed within 14 days by the first active study morning. During the initial visit participants provided written informed consent and were screened with regards the study exclusion/inclusion criteria. Training was given on the Cognitive Demand Battery and the compliance requirements for the following visits were explained.

On the three active study mornings, which were conducted a minimum of 7 days apart, participants attended the laboratory between 8:00am and 10:00am in a fasted state and provided confirmation of continued compliance with the inclusion/exclusion requirements. To assess baseline cognitive performance participants performed two repetitions of the CDB (along with fatigue/difficulty visual analogue scales). The first of these was simply included to attenuate any 'on the day' practice effect. The second was used as the baseline against which post-dose performance would be measured. Following this, participants consumed their treatment for that day. After a 90 minute rest/absorption period (during which participants remained in a purpose built waiting room, within the lab, watching television or reading), participants returned for post-dose testing. After being prepared for NIRS recording, i.e. securing the NIRS headband and identifying a reliable trace, participants sat quietly for two-minutes; with this period of CBF measurement averaged to act as the 'baseline' for change from baseline statistical analysis. Participants then commenced the completion of six consecutive repetitions of the CDB (i.e. 54 minutes of continuous performance) with NIRS data being captured throughout. The timelines and running order of the testing session are shown in Figure 2.2.

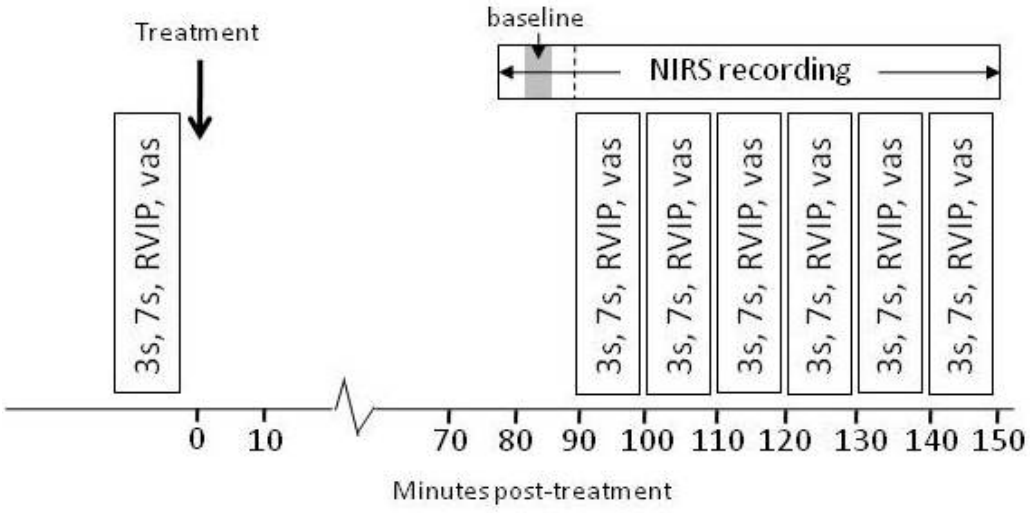


Figure 2.2. Chapter 2 study testing session timeline.

2.2.6 Statistics

The analyses of NIRS data were conducted with Minitab 15 for Windows (Minitab Inc, State College, PA) and behavioural data with SPSS 16.0 for Windows (SPSS Inc, Chicago, IL).

Near-Infrared Spectroscopy (NIRS) hemispheric differences analysis:

Prior to the primary analysis reported in this chapter a within subjects Analysis of Variance (ANOVA) was carried out with left/right optode included as a factor (hemisphere x treatment group x 2- (subtractions) or 2.5- (RVIP) min epoch) to examine any hemispheric differences in response. As there were no consistent treatment-related interactions involving this factor the data from the two channels were averaged across hemispheres for the analysis and figures reported below. (NB This was the procedure for all NIRS analyses in this thesis and, as no significantly consistent treatment-related hemispheric differences were observed in any chapter, all NIRS results represent an average of the two NIRS channels.)

Hemodynamic response to tasks analysis:

As this pilot study is the first in this thesis to utilize the CDB to inculcate increased cognitive demand, the analysis here also includes a within subjects ANOVA (task (subtractions, RVIP) x repetition (1 to 6) x treatment), using data averaged from the six cognitive task epochs, in order to ascertain if the CDB tasks are capable of inducing the typical CBF response to cognitive workload. This hemodynamic response is described in more detail in section 2.2.4 and typically manifests in increased concentrations of total- and oxy-Hb alongside concomitant reductions in levels of deoxy-Hb (e.g. Tamura et al., 1997). (NB This was the procedure for all NIRS analyses in this thesis and, as in this chapter, no significant differences in the hemodynamic response to tasks were observed.)

Main NIRS analysis:

Prior to any analyses (and pre un-blinding with regards treatment conditions), raw data was plotted for all participants to ascertain the existence of potential outliers. A standard deviation was calculated for this cohort and an *a priori* exclusion of 2 standard deviations from the mean applied to this data. This is a somewhat arbitrary measure but fits well with the data produced and includes a certain amount of flexibility to allow for individual differences in natural CBF levels. Note that this data clearing method was applied to all NIRS data in all studies within this thesis but means and standard deviations calculated for each individual study; thus allowing for potential cohort effects.

For the main NIRS analysis, data was first converted to 'change from baseline' (calculated from the resting period immediately before post-dose task completion) prior to conducting a within-subjects analysis of variance (ANOVA) (treatment group x 2 or 2.5 min epoch). *A priori* planned comparisons of data from each epoch were made between placebo and each of the resveratrol treatment groups (500mg resveratrol, 1000mg resveratrol) using t tests calculated with the Mean Squares Error from the ANOVA. For completeness, the results of the main ANOVA are reported in the results section, but a significant result on this ANOVA was not used as a prerequisite for carrying out and interpreting the planned comparisons (Keppel, 1991). However, in order to reduce the potential for Type I errors only those planned comparisons associated with a consistent pattern of significant effects are interpreted.

The rationale for this statistical approach is 4-fold: firstly, the research questions relevant to CBF in this chapter are driven by clear hypotheses (see section 1.8) which evince specific, focused questions of the data. As such, the specific *a priori*-derived questions concern only how performance in the resveratrol supplemented conditions differ to placebo, not how treatment conditions differ to each other. Secondly, the use of the prior F test as a protective mechanism against type I error can be regarded as over-conservative, unnecessary to the interpretation of planned comparisons and, finally and relatedly, that it can often be detrimental to their interpretation; where the F test can be non-significant despite a consistent pattern of significant planned comparisons (Rosenthal & Rosnow, 1985). Thirdly, this approach is validated by the exponentially reducing probability of significant differences occurring for one treatment at two or more consecutive time-points by chance. And fourth, and finally, an overly-conservative statistical approach has the potential to impede the research process, especially in a hitherto unexplored area of investigation, utilizing novel techniques, where new and subtle effects might be missed due to unnecessarily over-conservative statistical methods.

Behavioural data analysis:

Task performance data was analysed as change from pre-dose baseline for each individual task (Serial 3s, Serial 7s, RVIP, mental fatigue, difficulty) by within-subjects ANOVA (treatment x repetition), with Bonferroni corrected post-hoc comparisons conducted if a main effect of treatment or an interaction between treatment x repetition was observed. Prior to any analysis, baseline differences were investigated with regards these measures and any results only reported if significant. (NB this was the procedure with all chapters.)

2.3 Results

2.3.1 Near-Infrared Spectroscopy (NIRS) parameters (main NIRS analysis)

Total haemoglobin (total-Hb):

The omnibus ANOVA demonstrated no significant main effect of treatment [F(2,1012)= 1.37; p= .265] or an interaction between treatment x repetition [F(46,1012)= 1.05; p= .385]. Reference to the planned comparisons demonstrated that levels of total-Hb were significantly lower in the 500mg resveratrol condition (epochs 9-12, 18, 21 and 23 <.05; epochs 13-17, 19, 22 and 24 <.01; epoch 6 trend (.06)) and the 1000mg resveratrol condition (epoch 9 <.05; epochs 10 and 12-24 <.01; epochs 8 and 11 trends (.05 and .06 respectively)) as compared to placebo.

Oxygenated haemoglobin (oxy-Hb):

The omnibus ANOVA demonstrated no significant main effect of treatment [F(2,1012)= 1.37; p= .265] or an interaction between treatment x repetition [F(46,1012)= .89; p= .682]. Reference to the planned comparisons demonstrated that levels of oxy-Hb were significantly lower in the 500mg resveratrol condition (epochs 4, 10 and 11 <.05; epochs 6, 9 and 12-24 <.01; epochs 3 and 7 trends (.08 and .07 respectively)) and the 1000mg resveratrol condition (epochs 15 and 23 <.05; epochs 12, 13, 16-19 and 24 <.01; epochs 9, 10 and 20-22 trends (.06, .08, .05, .07 and .05 respectively)) as compared to placebo.

Deoxygenated haemoglobin (deoxy-Hb):

The omnibus ANOVA demonstrated no significant main effect of treatment [F(2,1012)= .99; p= .379] but a significant interaction between treatment x repetition was observed [F(46,1012)= 1.39; p= 0.046]. Reference to the planned comparisons demonstrated that levels of deoxy-Hb were significantly higher in the 500mg resveratrol (epoch 21 <.05; epoch 22 <.01; epoch 18 trend (.07)) as compared to placebo. Conversely, in the 1000mg resveratrol condition, levels of deoxy-Hb were significantly lower than placebo (epochs 14, 15, 21, 22 and 24 <.05; epochs 19, 20 and 23 <.01; epoch 10 trend (.08)).[2]

The results of total-, oxy-, and deoxy-Hb, for all three treatment conditions, across the entire post-dose task period, are presented in Figure 2.3.

.

[2] All t's (23) ≥2.01 to ≤2.91 for significant comparisons.

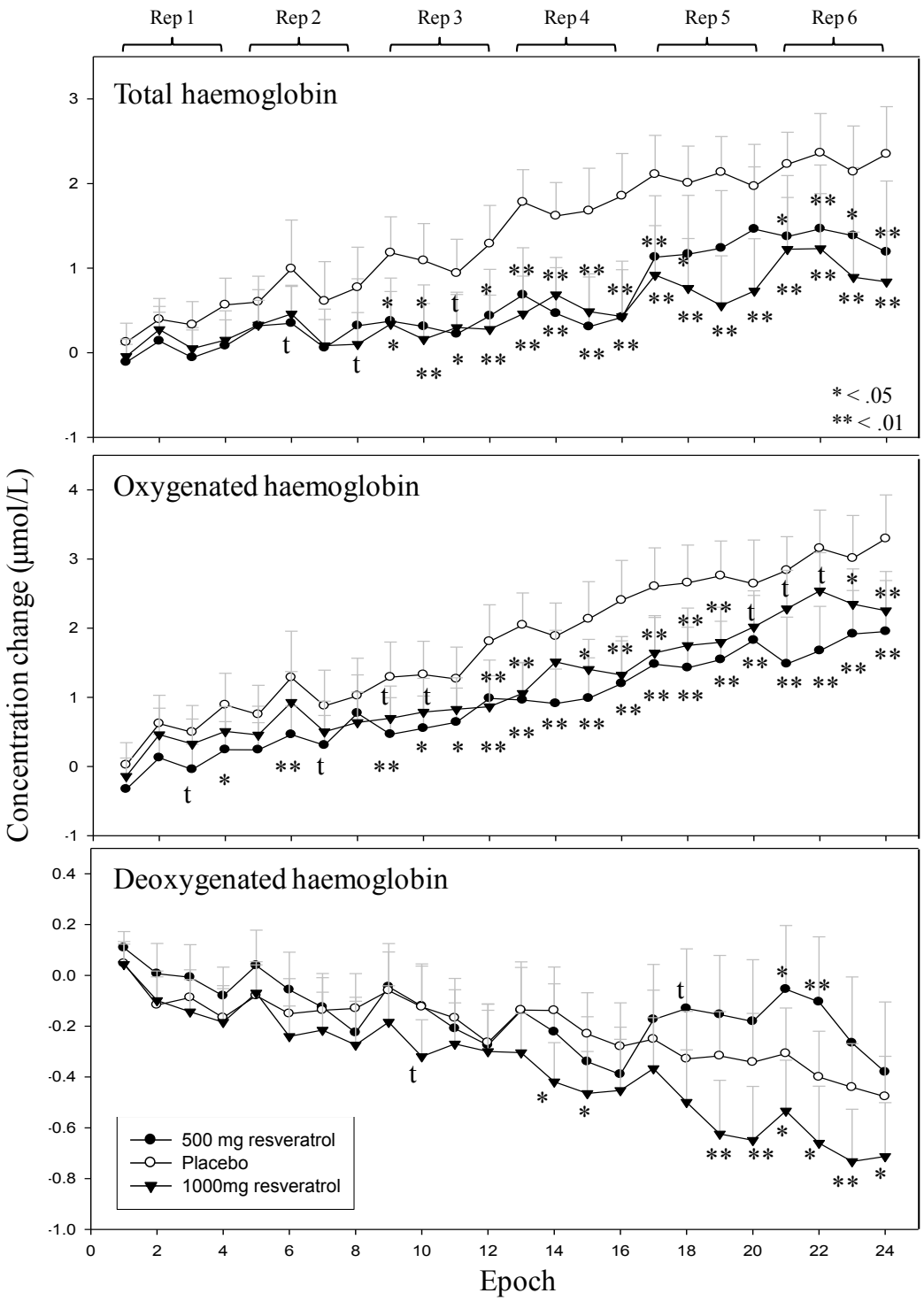


Figure 2.3. The acute effects of 1000- and 500mg *trans*-resveratrol on cerebral blood flow in the prefrontal cortex. Graph displays concentration change (µmol/L) levels of total, oxygenated and deoxygenated haemoglobin (with SEM error bars) during the six repetitions (rep) of the post-dose tasks in 22 healthy adults after placebo, 1000mg *trans*-resveratrol and 500mg *trans*-resveratrol. *p <.05, **p <.01 and t- trend p < 0.1.

2.3.2 Cognitive task performance and mood

The only significant findings on the omnibus ANOVAs were for a main effect of repetition, irrespective of treatment, with the 'RVIP%correct', 'mental fatigue' and 'difficulty' measures. No significant main effect of treatment or a treatment x repetition interaction was observed with either dose of resveratrol, for any task measure, on the omnibus ANOVAs. Thus, no post-hoc comparisons were performed.

Table 2.1 displays cognitive task performance and 'mental fatigue' and 'difficulty' ratings as well as F and P values from the omnibus ANOVA for each task.

Table 2.1. The effects of 1000- and 500mg *trans*-resveratrol on cognitive performance. Table displays raw baseline and change from baseline scores (with SEM values in brackets, underneath) for all 6 post-dose battery repetitions after placebo, 1000mg *trans*-resveratrol and 500mg *trans*-resveratrol for 22 healthy, young 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.

Measure	Treatment condition	Task battery repetition							ANOVA		
		Baseline	1	2	3	4	5	6	Effect	F	P
3s Correct (Number)	500mg resveratrol	42.79 *(3.51)*	0.96 *(1.13)*	3.00 *(1.41)*	1.04 *(1.39)*	0.98 *(0.47)*	1.00 *(1.40)*	0.29 *(1.38)*	T	.861	.430
	1000mg resveratrol	42.81 *(2.96)*	2.98 *(1.17)*	2.81 *(1.13)*	1.35 *(1.73)*	1.85 *(1.24)*	1.56 *(1.51)*	3.27 *(1.76)*	R	.962	.423
	Placebo	43.17 *(3.00)*	0.38 *(1.50)*	2.88 *(0.95)*	1.33 *(1.18)*	1.17 *(1.55)*	0.63 *(1.66)*	-0.42 *(1.99)*	T*R	.561	.845
3s Incorrect (Number)	500mg resveratrol	1.69 *(0.31)*	-0.23 *(0.33)*	0.56 *(0.43)*	0.27 *(0.40)*	0.98 *(0.47)*	0.65 *(0.42)*	0.19 *(0.34)*	T	1.042	.361
	1000mg resveratrol	1.27 *(0.21)*	0.65 *(0.37)*	0.44 *(0.41)*	1.10 *(0.43)*	0.60 *(0.38)*	1.10 *(0.51)*	0.85 *(0.61)*	R	1.232	.304
	Placebo	1.21 *(0.25)*	1.50 *(0.44)*	0.54 *(0.44)*	-0.13 *(0.32)*	0.42 *(0.36)*	1.54 *(0.59)*	2.13 *(1.19)*	T*R	1.967	.109

7s Correct (Number)	500mg resveratrol	27.54 *(2.72)*	2.33 *(0.97)*	2.83 *(0.97)*	1.04 *(1.31)*	1.88 *(0.90)*	2.08 *(1.05)*	2.33 *(0.78)*	T	.119	.888
	1000mg resveratrol	26.98 *(2.59)*	2.29 *(1.22)*	3.06 *(1.08)*	2.23 *(1.03)*	1.77 *(1.39)*	3.69 *(0.93)*	1.81 *(1.21)*	R	.690	.571
	Placebo	27.44 *(2.58)*	1.65 *(1.12)*	2.40 *(0.95)*	1.77 *(1.28)*	4.27 *(1.00)*	2.98 *(1.12)*	2.56 *(1.02)*	T*R	1.188	.300
7s Incorrect (Number)	500mg resveratrol	1.94 *(0.37)*	-0.56 *(0.36)*	-0.27 *(0.53)*	0.48 *(0.55)*	0.52 *(0.48)*	0.02 *(0.48)*	0.02 *(0.43)*	T	.237	.790
	1000mg resveratrol	2.04 *(0.33)*	0.29 *(0.45)*	-0.29 *(0.46)*	-0.21 *(0.43)*	0.92 *(0.86)*	0.50 *(0.38)*	0.75 *(0.56)*	R	.716	.612
	Placebo	2.15 *(0.29)*	0.44 *(0.38)*	0.35 *(0.40)*	0.52 *(0.55)*	-0.52 *(0.36)*	0.48 *(0.33)*	0.65 *(0.50)*	T*R	1.623	.176
RVIP (% correct)	500mg resveratrol	19.25 *(1.76)*	1.75 *(0.79)*	-0.92 *(0.94)*	-1.42 *(0.89)*	-2.79 *(1.05)*	-2.25 *(1.14)*	-2.71 *(1.01)*	T	.419	.660
	1000mg resveratrol	19.15 *(1.92)*	0.52 *(0.64)*	-0.90 *(0.93)*	-1.44 *(0.82)*	-2.48 *(0.82)*	-3.23 *(0.78)*	-1.40 *(0.86)*	R	11.814	.000**
	Placebo	18.31 *(1.72)*	1.27 *(0.62)*	-0.44 *(0.98)*	-1.60 *(0.79)*	-1.23 *(0.77)*	-1.15 *(0.82)*	-1.60 *(1.11)*	T*R	.945	.493
RVIP Reaction time (msec)	500mg resveratrol	468.10 *(5.49)*	3.27 *(5.43)*	-2.44 *(5.85)*	7.35 *(4.20)*	3.56 *(5.49)*	8.65 *(3.85)*	-6.90 *(5.67)*	T	.836	.440
	1000mg resveratrol	474.29 *(5.59)*	-13.58 *(5.19)*	-0.58 *(5.17)*	1.17 *(6.12)*	0.46 *(4.82)*	-6.92 *(6.31)*	-0.79 *(4.41)*	R	1.096	.336
	Placebo	477.17 *(4.84)*	5.50 *(6.40)*	-3.33 *(5.19)*	-2.33 *(5.24)*	-1.00 *(5.06)*	-9.42 *(5.65)*	-8.00 *(7.59)*	T*R	1.667	.143
Mental Fatigue	500mg resveratrol	42.17 *(4.20)*	-1.29 *(3.44)*	4.21 *(3.34)*	10.75 *(3.82)*	16.50 *(3.88)*	20.29 *(3.86)*	26.08 *(3.87)*	T	.330	.721
	1000mg resveratrol	39.35 *(5.40)*	-2.35 *(2.74)*	2.27 *(3.38)*	7.40 *(3.76)*	13.73 *(4.01)*	17.19 *(4.44)*	23.65 *(5.51)*	R	47.324	.000**
	Placebo	37.88 *(4.87)*	-1.25 *(1.73)*	3.04 *(1.88)*	11.92 *(2.36)*	16.29 *(3.12)*	21.54 *(3.34)*	27.08 *(3.87)*	T*R	.389	.830

Difficulty	500mg resveratrol	37.38 *(4.73)*	3.08 *(2.74)*	3.46 *(2.80)*	5.46 *(3.31)*	11.29 *(3.81)*	13.58 *(4.25)*	18.71 *(4.86)*	T	.466	.630
	1000mg resveratrol	36.29 *(5.13)*	-0.17 *(2.87)*	1.83 *(2.94)*	5.96 *(2.96)*	9.25 *(3.31)*	10.25 *(3.26)*	14.71 *(4.23)*	R	11.497	.001**
	Placebo	37.42 *(4.57)*	-1.38 *(2.26)*	0.00 *(2.98)*	5.67 *(3.11)*	6.88 *(3.29)*	10.00 *(4.06)*	14.04 *(5.02)*	T*R	.664	.629

2.4 Discussion

This pilot study was conducted to test two clear hypotheses: the first was that resveratrol would be able to augment cerebral blood flow (CBF) above and beyond the natural demand-driven changes induced by 'prefrontal' cognitive tasks and the second was that resveratrol would be able to increase performance on these tasks as a result of an increase in fuel provision to the prefrontal cortex. No data currently exists in this specific research area and so these hypotheses were predicated on findings from epidemiological, animal and *in vitro*/*ex vivo* studies as well as research into structurally similar polyphenols.

With regards increasing CBF, existing literature demonstrates that resveratrol is capable of interacting with the vasodilatory mediator nitric oxide (NO) *in vitro* (Chen & Pace-Asciak, 1996; Novakovic et al., 2006a and b) and of increasing vascular dilation in humans (Wong et al., 2011; Wong et al., 2012). These are potential mechanisms of action which are shared with structurally similar cocoa polyphenols and which, in the latter, are associated with improved CBF (Sorond et al., 2008; Francis et al., 2006) and cognitive performance (Field, Williams & Butler, 2011; Scholey et al., 2010; Desideri et al., 2012) in humans. Evidence from animal data also suggests that resveratrol is capable of preserving (Sharma & Gupta, 2002) and improving (Dal-Pan et al., 2011) cognitive function and that the underlying mechanisms might be related to improved CBF (Oomen et al., 2009). Improved cognitive performance in response to augmented CBF is likely the result of increased metabolic substrate delivery to active regions of the brain. In support of this, supplementation with oxygen and glucose alone has been observed to increase aspects of cognitive function in healthy humans (Moss, Scholey & Wesnes, 1998; Scholey et al., 1999). The effects of resveratrol on mitochondrial phosphorylation (Lagouge et al., 2006) might also be anticipated to drive cognitive performance by increasing utilization of the enhanced oxygen provision.

Taken together, these findings provide the hypothesis that resveratrol should be capable of augmenting NO-induced increases in CBF and that, as a result of this increased fuel delivery to cognitively active brain regions, and utilization by resveratrol-induced mitochondrial up-regulation, cognitive performance should be improved as a result.

In terms of the results of this pilot study, the analysis of hemodynamic data from the prefrontal cortex, as measured by near-infrared spectroscopy (NIRS), demonstrates a pattern of effects which is opposite to that anticipated. If the above hypotheses were supported then the NIRS results would depict greater total- and oxy-Hb concentrations

(i.e. increased CBF and oxygen demand respectively), in response to resveratrol supplementation, alongside higher levels of deoxy-Hb (representing oxygen extraction), relative to those concentrations evinced by placebo. The results here demonstrate that, during the 90-150 minutes post-dose recording period, both doses of resveratrol evinced a pattern of significantly lower levels of total- and oxy-Hb as compared to placebo. With regards deoxy-Hb, the pattern of effects was weaker, and significant differences between treatments only became apparent in the second half of the recording period and demonstrate significantly lower levels, as compared to placebo, in the 1000mg resveratrol condition. With regards the 500mg dose, the only significant difference in deoxy-Hb levels were observed for two out of the 24 epochs, recorded during the final task repetition (with a trend for another during recording of the fifth task repetition). In these instances the levels were higher than in the placebo condition (see figure 2.3.). This latter finding would support the hypothesis that resveratrol is capable of increasing oxygen extraction, and perhaps indicates that the time-frame for this to occur is towards the end of that utilized here. However, it could be argued that, due to the use of less conservative statistical comparisons, these 2/24 significant differences merely represent type I errors.

Overall however, the interpretation of this result, and indeed that of all three chromophores, is fundamentally hindered here by a methodological constraint in the way in which NIRS calculates haemoglobin levels. The continuous-wave (C-W) NIRS used here presents haemoglobin data in terms of concentration (μmol/L) change rather than in absolute levels. As such, it only generates data reflecting how haemoglobin levels are changing during the recording period. This provides an excellent measure of the haemodynamic response to task performance and any treatment related modulation of task related CBF, but no indication of quantitative differences that have taken place before the recording commences. In the current study we opted for a 90 minute absorption period and then 60 minutes of task performance. Given that NIRS has not been used for an extended recording period such as this previously, we decided that recording would have to start near the end of the 90 minute absorption period to reduce potential participant discomfort. The inherent problem in this approach is that if a gross increase in CBF levels had taken place in resveratrol-supplemented participants across the absorption period, then this group would have entered the post-dose recording period with higher CBF than placebo participants. The methodology employed here would fail to detect this. The pattern of results evinced here, that of a decreased haemodynamic response to task performance in the resveratrol conditions, could therefore be interpreted as being an artefact of an (unmeasured) increase in CBF in these groups during the absorption period. If the resveratrol-supplemented

participants had already experienced a global increase in CBF across the absorption period, it may be the case that a decreased haemodynamic response represents a reduced need for additional metabolic substrates during task performance.

This methodological constraint in interpreting the hemodynamic response to treatment also affects the interpretation of cognitive task performance and whether this was influenced by CBF. As it stands, no significant differences in cognitive task performance were observed between either dose of resveratrol and placebo. Taking the CBF results at face value, i.e. demonstrating that resveratrol was not an effective modulator of CBF and oxygenation, provides an apparent lack of efficacy of resveratrol in augmenting CBF and, therefore, arguably no modulation of cognitive task performance should have been anticipated either. However, if we take the view that the methodological constraints surrounding NIRS interpretation might be masking an effect of resveratrol which has already taken place prior to post-dose recording, then this complicates the interpretation of the lack of cognitive effects; as then, arguably, cognitive effects would have been anticipated as per the hypothesis. This argument, however, cannot be resolved with the data provided here. What is clear after conducting this pilot study is that in order to assess whether resveratrol is capable of altering CBF and, in turn, influencing cognitive performance, CBF would need to be recorded from the time of treatment administration and throughout the absorption period as well as the post-dose task period. This would provide a view of CBF changes from pre-treatment and would clarify the issue here regarding not being aware of hemodynamics during absorption and how this changes at the commencement of post-dose tasks

In summary, the results of this pilot investigation suggest that 500mg and 1000mg *trans*-resveratrol can modulate the haemodynamic CBF responses to task performance in the frontal cortex, but do not provide an indication of the gross CBF effects of resveratrol. The results here also demonstrate that neither dose is able to influence cognitive task performance. The conclusion drawn here is that the interpretation of both of these findings is hindered by the way in which NIRS was utilized in this paradigm; an issue which would have to be addressed by ensuing investigations.

Chapter 3.

The cognitive, cerebral blood flow and pharmacokinetic effects of 500mg and 250mg *trans*-resveratrol in healthy, young humans.

Kennedy, D.O., Wightman, E.L., Reay, J.L., Lietz, G., Okello, E.J., Wilde, A., & Haskell, C.F. (2010). Effects of resveratrol on cerebral blood flow variables and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation. *Am J Clin Nutr*, 91, 1590-1597.

3.1 Introduction

The results of chapter 2 suggest that resveratrol (at doses of 1000- and 500mg) was unable to modulate the haemodynamic cerebral blood flow (CBF) response to task performance in healthy, young humans. Cognitive performance was unaffected by either dose. The overall conclusion of chapter 2, however, was that this interpretation is muddied by using a methodology that failed to measure potential changes taking place across the absorption period where, hypothetically, a treatment-induced increase in hemodynamics might already have taken place. In order to redress this constraint, the current study will again assess the CBF and cognitive effects of resveratrol utilizing Near-Infrared Spectroscopy (NIRS), but will monitor hemodynamics across the entire testing session.

Due to the practicalities of NIRS measurement (namely that of the headband becoming uncomfortable after long periods of time and of participants requiring toilet breaks) the testing time-frame will necessarily have to be shorter than that utilized in the previous study. Thus the commencement of post-dose cognitive testing (which is reduced to comprise four repetitions of tasks as opposed to six) will be brought forward here to 45 minutes as opposed to 90 minutes. The main issue with this reduction in the absorption time of resveratrol is the potential to be premature in attempting to test it at its most efficacious (which is, arguably, when levels are most abundant in plasma). With this in mind, the literature investigating the bioavailability of resveratrol is very small and is subject to large variability due to the individual differences in pharmacokinetics; a factor which is exacerbated further by the small sample sizes which these studies typically utilise. Thus, trying to ascertain precisely when resveratrol is most abundant in circulation is very difficult; with previous literature varying markedly in their reports of the achievable peak plasma concentration of resveratrol (C_{max}) after oral consumption and the time taken to achieve this (t_{max}). However, the most comprehensive of the few currently existing resveratrol pharmacokinetic studies in humans reports that, after an oral 500mg dose, resveratrol can achieve a C_{max} of 72.6ng/mL after 0.8hrs (or 48-minutes) for the parent/aglycone form of resveratrol and between 369.5-404.6ng/mL

after 1.5-2hrs for the glucuronide metabolites and 1,135ng/mL after 1.5hrs for the sulfate metabolite.

With this in mind, it is likely that the current paradigm will commence post-dose cognitive testing at the point at which the parent form of resveratrol is most abundant in plasma, but not necessarily the metabolites. In order to clarify this, the current study will also include a measurement of blood plasma levels of resveratrol during key time-points relevant to the cognitive/NIRS assessment. This is chiefly to ascertain whether resveratrol is present and therefore potentially capable of influencing the parameters under investigation here, but also to add to the aforementioned paucity of literature on the pharmacokinetics of this polyphenol. Again, due to the constraints of NIRS measurements, this plasma analysis will necessarily have to be conducted in a separate cohort of individuals as the NIRS recording could be affected by repeated forearm blood sampling.

In terms of dose, the previous study found that both 500mg and 1000mg doses of resveratrol were associated with lower total and oxygenated haemoglobin than placebo but no real difference in treatment effects was found on this or any other measure. Therefore, in the face of little evidence to inform the dose/s of the current study, here 500mg will again be investigated alongside a lower dose of 250mg, rather than 1000mg, in order to extend the dose range downwards.

In summary, the current study aims to investigate the cognitive, CBF and pharmacokinetic effects of resveratrol in young, healthy humans and will attempt to redress some of the methodological constraints of chapter 2 by monitoring the hemodynamic effects or resveratrol from before the consumption of the resveratrol/placebo and across the entire testing session.

3.2 Method- Cognitive/NIRS assessment

3.2.1 Participants

This study recruited 24 healthy adults (4 males, 20 females; mean age 20.17yrs, range 18-25yrs; 21 right handed, 3 left handed). All participants attended the laboratory after a 12hr overnight fast and reported to meet the inclusion criteria, i.e. to be in good health and free from social drugs (including alcohol), prescription medication, herbal extracts/food supplements, relevant food allergies, intolerances and digestive problems. All participants were non-smokers and did not consume excessive amounts

of caffeine (>six cups of coffee or equivalent/d). In addition, participants who had suffered a head injury, neurological disorder or neuro-developmental disorder were excluded from participation, as were those who had uncorrected sight problems, were pregnant or seeking to become so. Data from two participants was excluded from analysis on the basis of failure to complete the study.

3.2.2 Treatments

During the three study visits participants received three single-dose treatments in an order dictated by random allocation to a counterbalancing (Latin Square) order. The three treatments comprised two capsules which were combined to give the following treatments:

i) Inert placebo,

ii) 250 mg *trans*-resveratrol or

iii) 500 mg *trans*-resveratrol

The natural, pure *trans*-resveratrol was purchased from Biotivia Bioceuticals (Austria). The purity of the extract (99.02%) had been confirmed by high-performance liquid chromatography (HPLC) for the manufacturer's certificate of analysis. The treatments were administered in identical size 0 vegetarian capsules, which were prepared and 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 a blind-data review was completed.

3.2.3 Cognitive tasks and mood

The cognitive demand battery (explained in more detail in section 2.2.3) was again utilized to assess cognitive function and induce cognitive demand.

3.2.4 Near-Infrared Spectroscopy (NIRS)

Cerebral blood flow (CBF) was monitored in the prefrontal cortex by NIRS. (See section 2.2.4 for further information on this technique.)

3.2.5 Procedure

Participants were required to attend the laboratory on four occasions. The first of these was an initial screening/training visit, and this was followed within 14 days by the first active study morning. During the initial visit participants provided written informed consent and were screened with regards the study exclusion/inclusion criteria. Training was given on the cognitive tasks and the compliance requirements for the following visit were explained.

On the three active study mornings, which were conducted 7 days apart, participants attended the laboratory between 8:00am and 10:00am, in a fasted state, and provided confirmation of continued compliance with the inclusion/exclusion requirements. Prior to taking their treatment for that day participants were fitted with the NIRS headband and completed a single repetition of each of the cognitive tasks in order to establish baseline performance. Following this, participants sat quietly for 5 minutes, with the last 3 minutes of this period utilised as the NIRS resting baseline measurement for change from baseline analysis. Participants then consumed their treatment for that day and sat quietly, watching one of a selection of non-arousing DVDs, during a 45 minutes 'absorption' period. They were then verbally instructed to start the post-dose period of task performance, and completed four consecutive repetitions of the CDB (i.e. 36 minutes of continuous performance). NIRS data was captured throughout. The timelines and running order of the testing session are shown in Figure 3.1.

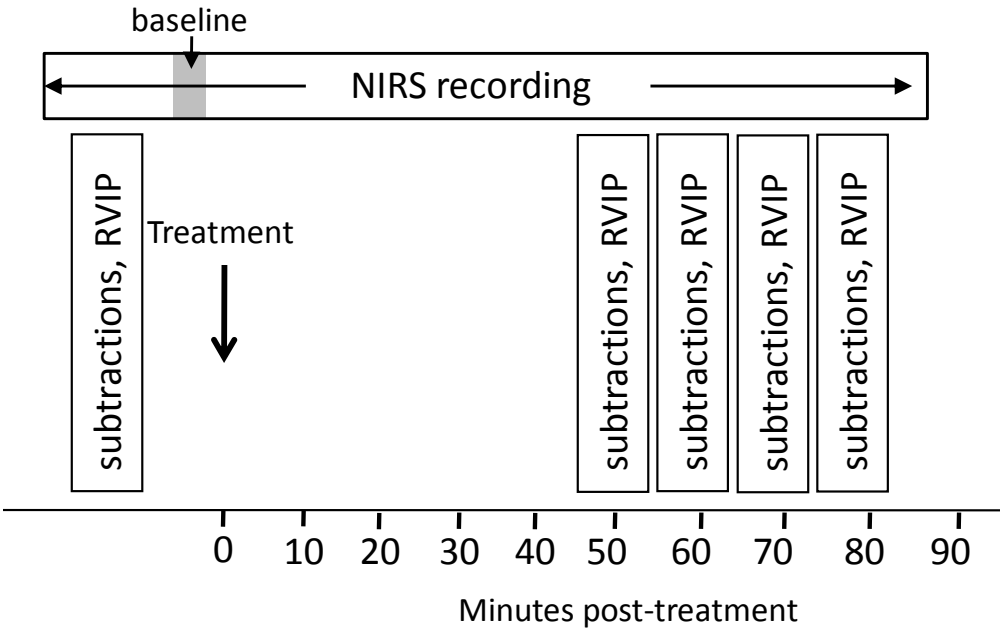


Figure 3.1. Chapter 3 study testing session timeline.

3.2.6 Statistics

The analyses of NIRS data were conducted with Minitab 15 for Windows (Minitab Inc, State College, PA) and behavioural data with SPSS 16.0 for Windows (SPSS Inc, Chicago, IL).

The analysis of NIRS data was first converted to 'change from baseline' (calculated from the resting period immediately prior to treatment consumption (rather than before post-dose task completion as per chapter 2)) prior to conducting a within-subjects analysis of variance (ANOVA) (treatment group x 4min epoch). *A priori* planned comparisons of data from each epoch were made between placebo and each of the resveratrol treatment groups (250mg resveratrol, 500mg resveratrol) using t tests calculated with the Mean Squares Error from the ANOVA (Keppel, 1991). (See section 2.2.6 for justification of this analysis plan.)

Task performance data was analysed as change from pre-dose baseline for each individual task (Serial 3s, Serial 7s, RVIP) by within-subjects ANOVA (treatment x repetition). Bonferroni corrected post-hoc comparisons were then conducted if a main effect of treatment and/or treatment x repetition interaction was observed here. Prior to any analysis, baseline differences were investigated with regards these measures and any results only reported if significant.

3.3 Method- Pharmacokinetic assessment

3.3.1 Participants

This aspect of the study recruited 9 healthy participants (mean age 24.8yrs, range 21-29yrs, all male). All participants either worked or studied at Northumbria University and had either attained, or were enrolled on, an undergraduate degree level course. All participants attended the laboratory having had nothing to eat or drink (except water) since the previous evening, and no resveratrol containing products for 24hrs. All participants reported themselves to be in good health and free from social drugs, alcohol, prescription medication and herbal extracts/food supplements. Participants with relevant food allergies or intolerances, who smoked tobacco, drank excessive amounts of caffeine (>six cups of coffee, or equivalent, per day) or took illicit social drugs were excluded. One participant was excluded from the analysis of bioavailability data due to failure to observe fasting requirements.

3.3.2 Treatments

Treatments were as per section 3.2.2. with the exception that participants in the bioavailability assessment did not take part in the placebo condition.

3.3.3 Procedure

Participants attended the laboratory at 8.30am on two separate occasions, receiving a different treatment on each occasion. Venous blood samples were collected using 4.7 ml monovettes (containing lithium heparin) before the days treatment was consumed and 45-, 90-, and 120 minutes post treatment administration. Samples were centrifuged at 2500rpm for 15min at 20^{o}C to yield plasma, which was then stored at -80^{o}C until analysis.

3.3.4 Treatment and analysis of plasma

Samples were prepared based on the method described previously for human plasma (Boocock et al., 2007). The HPLC system consisted of a Dionex GS50 pump, an AS50 autosampler and an AD25 Absorbance detector with UV detection carried out at 325nm. The HPLC system and detector was controlled by the Dionex Chromeleon software. The mobile phase consisted of A 5mM ammonium acetate containing 2% propan-2-ol and B methanol with 2% pro-pan-2-ol. Chromatographic separation was accomplished by injecting the samples on to a Synergi® 250mm x 4.6mm; 4µm C18 column. Temperature of the column was set at 40◦C with a flow rate of 1ml/min. A gradient elution was carried out as follows: 0 min, 0% B; 4 min 20% B; 7 min 20% B; 18 min 55% B ; 22 min 65% B; 95% B 24 mins, then equilibrating with 100% A for 6 min prior to the next injection. Identification of resveratrol conjugates was carried out by incubating serum samples with β-glucoronidase and sulfatase as described previously (Juan, Maijó, & Planas, 2010) and analysed by HPLC as described above. Quantification of resveratrol was carried out using standards ranging from 4 to 250ng/mL. However, resveratrol conjugate quantities were calculated based on the assumption that recovery characteristics and relationship between peak area ratios and concentrations were the same as those for resveratrol. Metabolite concentrations are therefore described as ‘‘resveratrol equivalents.’’

3.4 Results

3.4.1 Bioavailability

Analysis of plasma data demonstrated that resveratrol metabolites were present at the 45 minute post-dose time-point (which represents the time at which participants began the first of four post-dose task battery repetitions in the cognitive/NIRS aspect of the study) and that plasma levels peaked at the 90 minute sample time-point: thus demonstrating that resveratrol was bioavailable- indeed rising in concentration- during this post-dose task period (45 to 85 minutes post-dose). At the 120 minute post-dose sample time-point resveratrol levels began to decline for the majority of resveratrol forms at both doses.

The results also demonstrate, in conjunction with the small amount of previous literature, that resveratrol was predominantly available in metabolite form (and with higher sulfate than glucuronide metabolites) with the parent/aglycone form (just termed 'resveratrol') negligible or trace at all three sample time-points (5.65ng/mL and 14.4ng/mL for 250- and 500mg resveratrol respectively at 90-minutes post-dose). Concentrations of resveratrol also demonstrated a dose-response effect with 500mg evincing higher sulfate and glucuronide levels than 250mg (746.1ng/mL compared to 300.4ng/mL respectively for sulfate and 202.2ng/mL compared to 48.9ng/mL respectively for glucuronide).

Mean plasma concentrations of *trans*-resveratrol and its conjugates at pre-dose and 45-, 90-, and 120 minutes post-dose are shown in Figure 3.2.

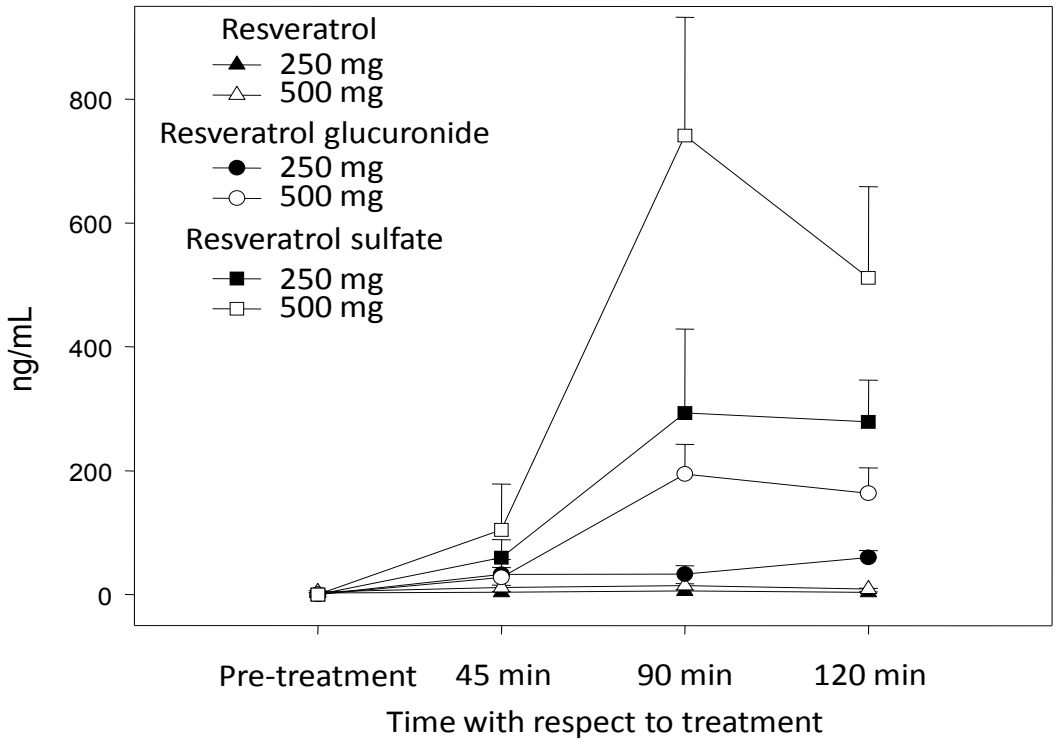


Figure 3.2. Bioavailability of resveratrol and metabolites after 250- and 500mg *trans*-resveratrol. Graph displays mean plasma concentrations (and SEM error bars) of *trans*-resveratrol and its conjugates after 250mg *trans*-resveratrol and 500mg *trans*-resveratrol at pre-dose and 45-, 90-, and 120 minutes post-dose in eight healthy males.

3.4.2 Near-Infrared Spectroscopy (NIRS) parameters

Total haemoglobin (total-Hb):

The omnibus ANOVA demonstrated no significant main effect of treatment [F(2, 1482)= 0.94; p= 0.401] but did find a significant interaction between treatment x epoch [F(78, 1482)= 2.12; p= <.001]. Reference to the planned comparisons showed that there were no significant differences in concentrations of total-Hb during the resting/absorption period prior to the start of the tasks, but thereafter the higher dose (500mg) resulted in significantly higher total-Hb during each task period epoch in comparison to placebo (all epochs P <.01[3] except 68-72 mins; P <.05[4]). Similar differences after the lower dose (250mg) were restricted to significantly higher total-Hb concentrations during the epochs spanning 46-49 min, 55-58 min, and 73-76 min (all P <.05)[5].

[3] t's (16) ≤2.87
[4] t(16)= 2.55
[5] t's (16)= 2.08, 2.38 and 2.47 respectively

Oxygenated haemoglobin (oxy-Hb):

The omnibus ANOVA demonstrated no significant main effect of treatment [F(2, 1482)= 0.42; p= 0.661] but did find a significant interaction between treatment x epoch [F(78, 1482)= 1.39; p= 0.015]. Planned comparisons revealed that only the higher, 500mg resveratrol dose was able to effect oxy-Hb levels and only during the post-dose task period (all epochs P <.05[6] except 46-49 and 73-76 mins; P <.01[7]). Epochs 50-54 evinced a trend (P= .08) and, at epochs 68-72 and 77-81, there was no significant difference between resveratrol and placebo.

Deoxygenated haemoglobin (deoxy-Hb):

The omnibus ANOVA demonstrated no significant main effect of treatment [F(2,1482)= 0.67; p= 0.520] nor a significant interaction between treatment x epoch [F(78, 1482)= 1.11; p= 0.246]. With regards deoxy-Hb, planned comparisons showed that both the 250mg and 500mg doses of resveratrol lead to significantly higher deoxy-Hb, in comparison to placebo. This was evident during the 21-25 min epoch for 500mg[8] and the last two 5 minute epochs of the resting/absorption period for 500mg[9] (P <.05) and 250mg resveratrol (36-40 min[10], P <.05, 41-45 min[11], P <.01). Both doses of resveratrol also resulted in higher deoxy-Hb during each epoch of task performance (all P <.01, except 500 mg/59-63 mins, P <.05)[12].

The mean data (± SEM) and the results of the planned comparisons for total-, oxy-, and deoxy-Hb are represented in Figure 3.3.

[6] t's (16) ≤2.39
[7] t's (16)= 1.69 and 3.11 respectively
[8] t(16)= 2.25
[9] t(16)= 2.14
[10] t(16)= 2.57
[11] t(16)= 3.20
[12] 250mg post-dose epoch t's (16) ≤3.77 and 500mg post-dose epoch t's (16) ≤2.52

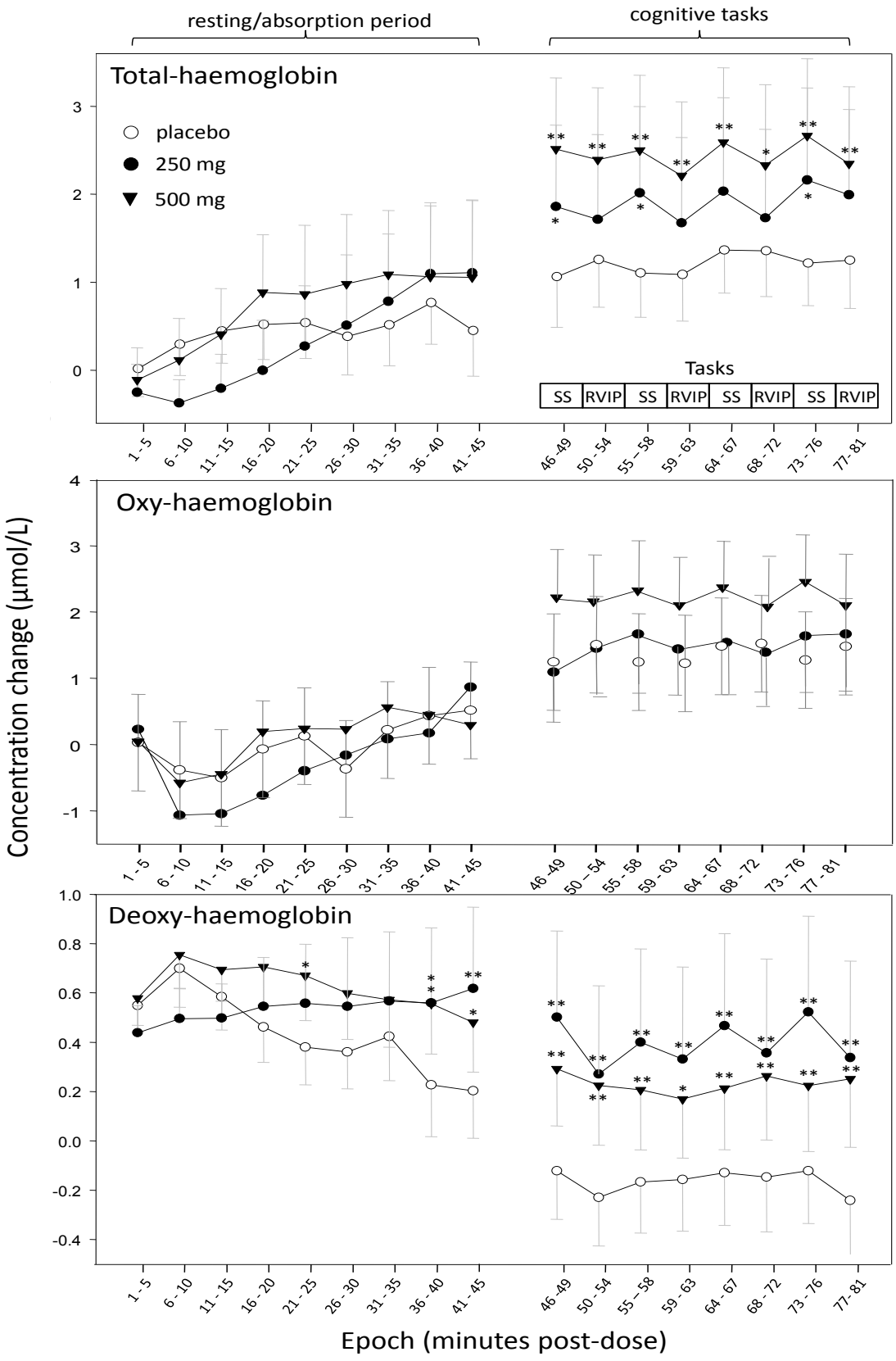


Figure 3.3. The acute effects of 250- and 500mg *trans*-resveratrol on cerebral blood flow in the prefrontal cortex. Graph displays concentration change (µmol/L) levels of total, oxygenated and deoxygenated haemoglobin (with SEM error bars) during a 45 minute absorption and 36 minute post-dose task period in 22 healthy adults after placebo, 250mg *trans*-resveratrol and 500mg *trans*-resveratrol. *<.05 and **<.01.

3.4.3 Cognitive task performance

The only significant finding on the omnibus ANOVAs was for a main effect of repetition, irrespective of treatment, with the 'RVIP%correct' measure. There were no significant, treatment related differences on any of the cognitive tasks. Thus, no post-hoc comparisons were performed (See table 3.1 for raw baseline and change from baseline values for all four post-dose battery repetitions. Table also includes ANOVA F and P values.)

Table 3.1. The effects of 250- and 500mg *trans*-resveratrol on cognitive performance. Table displays raw baseline and change from baseline scores (with SEM values in brackets) for all four post-dose battery repetitions after placebo, 250mg *trans*-resveratrol and 500mg *trans*-resveratrol for 22 healthy, young 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.

Measure	Treatment condition	Task battery repetition					ANOVA		
		Baseline	1	2	3	4	Effect	F	P
3s Correct (Number)	250mg resveratrol	37.91 *(2.76)*	0.91 *(1.01)*	1.14 *(1.92)*	-0.41 *(1.29)*	-1.09 *(1.86)*	T	.191	.827
	500mg resveratrol	38.91 *(3.37)*	-0.18 *(0.93)*	0.86 *(1.17)*	1.09 *(1.43)*	0.73 *(1.48)*	R	.804	.496
	Placebo	37.68 *(2.53)*	-0.36 *(1.19)*	1.82 *(0.79)*	0.64 *(1.04)*	1.86 *(1.37)*	T*R	.948	.463
3s Incorrect (Number)	250mg resveratrol	1.59 *(0.40)*	0.00 *(0.50)*	0.32 *(0.58)*	0.68 *(0.50)*	0.86 *(0.48)*	T	.422	.659
	500mg resveratrol	1.86 *(0.42)*	0.23 *(0.60)*	-0.23 *(0.48)*	-0.32 *(0.58)*	0.00 *(0.57)*	R	.194	.900
	Placebo	2.50 *(0.67)*	-0.05 *(0.48)*	-0.27 *(0.59)*	0.00 *(0.67)*	-0.36 *(0.80)*	T*R	.637	.700
7s Correct (Number)	250mg resveratrol	23.59 *(2.47)*	-0.27 *(0.48)*	0.50 *(1.04)*	-0.32 *(1.00)*	-0.14 *(0.88)*	T	2.33	.109
	500mg resveratrol	22.64 *(2.71)*	0.77 *(1.09)*	1.96 *(1.18)*	1.32 *(1.23)*	2.27 *(0.97)*	R	.935	.429
	Placebo	21.23 *(2.55)*	2.77 *(0.89)*	2.73 *(1.02)*	2.05 *(1.31)*	3.14 *(1.24)*	T*R	.370	.897

7s Incorrect (Number)	250mg resveratrol	1.82 *(0.48)*	0.77 *(0.45)*	0.50 *(0.39)*	0.73 *(0.40)*	0.91 *(0.44)*	T	3.36	.062
	500mg resveratrol	2.36 *(0.35)*	-0.68 *(0.39)*	0.14 *(0.50)*	0.27 *(0.56)*	0.86 *(0.66)*	R	2.51	.067
	Placebo	3.05 *(0.75)*	-1.05 *(0.67)*	-1.36 *(0.72)*	-0.59 *(0.78)*	-0.68 *(0.85)*	T*R	1.08	.380
RVIP (% correct)	250mg resveratrol	57.16 *(3.68)*	-5.57 *(3.04)*	-9.43 *(2.93)*	-10.23 *(3.12)*	-12.73 *(2.52)*	T	1.36	.268
	500mg resveratrol	56.14 *(3.43)*	-0.57 *(2.06)*	-5.46 *(2.57)*	-4.55 *(2.25)*	-8.41 *(2.65)*	R	14.71	<.001**
	Placebo	53.18 *(3.87)*	-2.39 *(2.54)*	-4.32 *(2.80)*	-8.98 *(3.29)*	-9.66 *(2.97)*	T*R	.535	.781
RVIP Incorrect (Number)	250mg resveratrol	4.23 *(1.03)*	-0.82 *(0.78)*	-0.14 *(0.65)*	-0.27 *(0.79)*	-0.86 *(0.79)*	T	.276	.693
	500mg resveratrol	4.18 *(0.86)*	-0.86 *(0.68)*	-0.96 *(0.93)*	2.50 *(2.82)*	0.18 *(1.17)*	R	2.15	.134
	Placebo	3.64 *(0.60)*	-1.18 *(0.38)*	-0.55 *(0.46)*	-0.32 *(0.49)*	1.09 *(0.77)*	T*R	1.25	.286
RVIP Reaction time (msec)	250mg resveratrol	492.11 *(7.58)*	12.81 *(7.24)*	9.38 *(9.68)*	23.00 *(12.48)*	18.75 *(9.58)*	T	1.16	.322
	500mg resveratrol	505.60 *(6.84)*	8.44 *(6.96)*	-3.39 *(11.80)*	6.49 *(10.29)*	-5.51 *(9.34)*	R	1.24	.302
	Placebo	501.65 *(7.62)*	1.39 *(6.14)*	15.75 *(7.44)*	20.61 *(8.12)*	21.63 *(9.84)*	T*R	1.58	.199

3.5 Discussion

The overall aim of this chapter was to investigate the CBF, cognitive and pharmacokinetic effects of resveratrol in healthy humans. A more specific aim of this chapter was to redress the methodological constraints of chapter 2, with regards assessment of hemodynamics with NIRS, which hindered the interpretation of the results. As such, this study was designed with the principal aim of recording CBF across the entire testing session. This necessarily meant that compromises had to be made with regards shortening the testing time-frame and conducting a pharmacokinetic study in a separate group of participants to ascertain that resveratrol was bioavailable during this time. The results here demonstrate that changing the testing paradigm to facilitate continuous NIRS recording did indeed lead to a better understanding of the CBF effects of resveratrol and, further, evinced results which are in line with the hypothesis.

Here it was demonstrated that 500mg resveratrol led to significantly higher levels of total- and oxy-Hb across the post-dose task period, in comparison to placebo. This effect was dose-related with 250mg evincing no significant effect on oxy-Hb levels and a significant effect on total-Hb only at a few post-dose epochs. In terms of utilization of this increased provision of oxygen, findings are also in line with the original hypothesis; demonstrating significantly higher levels of deoxy-Hb after both doses of resveratrol, compared to placebo, across the entire post-dose task period. Resveratrol-induced oxygen utilization has yet to be investigated in humans but these findings do support previous observations, in animal models, of the effect of resveratrol on mitochondrial phosphorylation. In these previous studies, increased mitochondrial number was reported (Baur et al., 2006) and the effect of enhanced mitochondrial enzymatic activity was observed in the increased citrate synthase activity in muscle homogenates and a significant increase in O_2 consumption and VO_2 max rate (Lagouge et al., 2006). In humans, data from an investigation with quercetin provides tentative positive evidence that polyphenols are capable of interacting with mechanisms of oxygenation in humans. Here McRae and Mefferd (2006) report that high-intensity cycling was significantly increased in 11 elite male athletes after consumption of quercetin, as part of an antioxidant drink, twice daily for 6 weeks. Whilst both above sources of support for the potential oxygenation effects of resveratrol are the product of chronic supplementation; i.e. 15 weeks of 400mg/kg/day resveratrol (Lagouge et al.) and 6 weeks of 300mg daily quercetin (as part of a ‘vitamin’ beverage) (MacRae & Mefferd), the effects of resveratrol in terms of increasing levels of deoxy-Hb in the current study

suggests that resveratrol may also be capable of acute augmentation of oxygen utilization.

What is particularly interesting in these results is that NIRS was sensitive enough to detect the natural neural-demand-induced increase in CBF (seen previously in the prefrontal cortex in response to increased workload Izzetoglu et al. (2004)); evidenced by the increase in total-Hb at the onset of post-dose cognitive tasks, irrespective of treatment, in the placebo condition (see figure 3.3). As stated above, resveratrol elicited a significantly greater CBF response to this workload-induced demand and, as such, provides support for the argument that resveratrol's mechanism of action here may be to amplify this natural response by interaction with the natural vasorelaxatory mediator NO. It is also noteworthy that NIRS appears to be sensitive to task-specific demands on fuel. This can be seen in the numerical rise in total CBF during the subtraction tasks and the ensuing decline in levels during the RVIP task in the treatment conditions. This pattern was also seen, to a lesser extent, for oxygen demand (oxy-Hb) but was more marked for oxygen utilization (deoxy-Hb); where the subtraction tasks also elicited a greater rise in oxygen use followed by an acute decline during the RVIP task. This oscillating pattern is consistent across the entire post-dose task period and serves to demonstrate both the applicability of NIRS as a sensitive technique in this field of research and again, due to the fact that this pattern was most marked in the treatment conditions, that resveratrol's mechanism of action is likely the amplification of the NO-mediated modulation of CBF in response to acute changes in neural activity.

At this point it would be tempting to try and compare the CBF results observed here to those evinced in chapter 2. However, two factors making direct parallels impossible: Firstly, as discussed previously, in chapter 2 the baseline for the NIRS recording was taken 90 minutes post-dose and the analysis only provides hemodynamic data after a potential treatment effect may already have taken place. In contrast, in the current chapter the baseline for the NIRS measurement was taken pre-dose and we therefore have a clearer picture of hemodynamic effects across the absorption period. Secondly, the time-frames of assessing post-dose effects of resveratrol are different; with chapter 2 commencing assessment of CBF and cognitive performance at 90 minutes post-treatment, i.e. the point at which that assessment ends in the current study. However, whilst direct comparisons cannot be made between chapter 2 and chapter 3 CBF results, the findings here might shed some light on the issue of interpreting the results from the previous chapter. The argument made was that a treatment effect might already have taken place in the resveratrol condition prior to the 90 minute post-dose assessment and, in effect, that this was masked by NIRS assuming that CBF was the

same in this and the placebo condition. As evidence, the current chapter demonstrates that resveratrol-induced hemodynamic changes can indeed take place before 90 minutes and could, therefore, have been expected in chapter 2. Further, levels of deoxy-Hb were demonstrating a treatment effect prior to any neural workload demands (i.e. at the final epochs of the absorption period, before post-dose cognitive tasks were inculcating a demand for fuel) which adds credence to the notion that CBF could have been affected in chapter 2, even during the absorption period.

With regards cognitive performance, in line with chapter 2, no significant effect of either treatment dose was evinced on any of the task measures. An argument could be made here that the reduction in post-dose testing commencement from 90 minutes (as per chapter 2) to 45 minutes might explain the failure to find an effect on cognitive performance. This was a concern when designing the current paradigm as, although one previous study investigating oral consumption of 500mg resveratrol demonstrated that the resveratrol aglycone is present at ~48 minutes post-dose, the metabolites weren't observed until >1.5hrs (Boocock et al., 2007). Thus it may have been possible that the window for assessing resveratrol as its most bioavailable/bioactive was missed by testing earlier than in the previous study. However, three counterarguments can be levied against this possibility.

Firstly, in chapter 2, neither 1000- nor 500mg resveratrol were able to influence cognition at a later time-frame than that measured here. Secondly, in the current study, resveratrol was able to evince CBF effects during this time period and so it would have been expected, as per the hypothesis, that cognitive enhancement would follow these CBF increases. And thirdly, the analysis of blood samples from a separate cohort demonstrates that resveratrol was present in plasma at the 45 minute sample time-point and indeed concentrations were rising across the 40 minute post-dose task period to t_{max} at the 95 minute time-point. At this point (corresponding to post-dose task completion in the cognitive/NIRS aspect of this study), overall, plasma concentrations began to decline (see figure 3.2).

In line with previous literature, however, whilst resveratrol was bioavailable during the post-dose task time-frame, concentrations were very low and predominantly comprised metabolites rather than the parent form. The levels observed here are broadly in line with the aforementioned Boocock et al. study after an oral dose of 500mg. This study reports an aglycone C_{max} of 72.6ng/mL; compared to 14.4ng/mL in this chapter. Levels of glucuronide metabolites were 369.5- 404.6ng/mL; versus 202.2ng/mL here and levels of the sulfate metabolite were 1,135ng/mL compared to 746.1ng/mL in this chapter. Whilst these concentrations are marginally lower than Boocock et al. (most

likely attributable to individual differences in absorption and metabolism) both studies demonstrate the poor bioavailability of resveratrol. Thus it could be argued that the lack of any cognitive effects seen here might be as a result of low plasma resveratrol levels, which may have been sufficient to evince augmentation of CBF but not to a level which could influence cognitive functioning.

In summary, the current study redressed the methodological constraints of chapter 2 and by monitoring CBF with NIRS across the entire testing session the results give a clear indication of the CBF effects of resveratrol. The results show that resveratrol is indeed able to enhance CBF in healthy, young participants and in particular during the post-dose task performance period. This study did not observe any effect of resveratrol on cognitive task performance and, whilst this is not due to a lack of exposure to resveratrol, it may be a symptom of poor bioavailability.

Chapter 4.

The cognitive, cerebral blood flow and pharmacokinetic effects of 250mg *trans*-resveratrol alone, and with 20mg piperine, in healthy, young humans.

Wightman, E.L., Reay, J.L., Haskell, C.F., Williamson, G., Dew, T.P., & Kennedy, D.O. (In press). Effects of resveratrol alone or in combination with piperine on cerebral blood flow parameters and cognitive performance in humans: a randomised, double-blind, placebo-controlled, crossover investigation. *Br J Nutr*

4.1 Introduction

The previous study found that 500mg *trans*-resveratrol (hereafter referred to as just 'resveratrol') was able to augment the natural activity-driven increase in cerebral blood flow (CBF) and oxygen utilization (deoxy-Hb) in the prefrontal cortex during tasks that activate this brain region, compared to placebo. In contrast, 250mg resveratrol was only able to evince significantly higher levels of total-Hb, and this effect was restricted to a few isolated epochs. With regards cognitive performance, the previous study found no significant effects of either dose of treatment on any of the task measures. The analysis of plasma data demonstrated that resveratrol, predominantly metabolites, was bioavailable during the post-dose task time-frame but that concentrations of the parent molecule were very low.

This phenomenon of very poor bioavailability of resveratrol, especially of the aglycone, is a well observed facet of the resveratrol literature (Boocock et al., 2007; Walle et al., 2004; Wenzel & Somoza, 2005). This fact represents something of an unaddressed paradox however, with resveratrol exerting a plethora of significant *in vitro*, *ex vivo* and *in vivo* effects despite this poor bioavailability. This fact might explain why neither chapter 2 or 3 observed any cognitive enhancing effects of 1000- and 500mg and 500- and 250mg resveratrol respectively. In the case of chapter 3, it is possible that plasma levels of resveratrol were sufficient here to influence CBF (as this was facilitated by interacting with the actions of NO) but not to augment cognitive function. Of course, due to methodological issues with chapter 2, differences in the time scale of cognitive testing and the fact that plasma resveratrol levels were not investigated there, direct comparisons between this and chapter 3 are not possible.

Therefore, the primary aim of the current study is to address the aforementioned paradox by investigating whether the bioavailability of resveratrol can be augmented, *in vivo*, and whether these augmented levels could inculcate significant effects on cognitive task performance. The primary factor limiting the bioavailability of resveratrol,

after oral administration, appears to be the high rate of first pass glucuronidation and the fact that the intestinal efflux pumps (specifically the Multi-drug resistance protein-3 (MRP3) pump) preferentially displace these glucuronidated metabolites into the blood stream whilst the Breast Cancer Resistance Protein (BCRP) pump effluxes the purported bioactive form of resveratrol- the aglycone- into the intestinal lumen (van de Wetering et al., 2008). Therefore, in order to increase the bioavailability of resveratrol, the current study attempted to inhibit glucuronidation by co-supplementing resveratrol with the pepper-derived alkaloid piperine.

Piperine has been observed to be a potent enhancer of the bioavailability of numerous compounds, including polyphenols, *in vivo*. For instance, piperine coadministration has increased the levels of epigallocatechin-3-gallate (EGCG) in rodents (Lambert, Hong, Kim, Mishin, & Yang, 2004), curcumin in rats and humans (Shoba et al., 1998), and beta-carotene following 14 days co-supplementation in humans (Badmaev, Majeed, & Norkus, 1999). A recent study by Johnson et al. (2011) also investigated the potential for piperine to enhance the bioavailability of resveratrol in mice. Following supplementation of 100mg/kg oral resveratrol with 10mg/kg piperine, exposure (as measured by AUC) to resveratrol was increased by 229%. The C_{max} of the parent form was increased to 1544% and to 184% for the glucuronide metabolite. This latter finding might shed some light on the underlying mechanisms of piperine-mediated increased bioavailability; with the authors arguing that the increased T_{max} of the glucuronide (from 0.25-0.5hrs) is evidence for inhibition of the UGT1A1 enzyme responsible for glucuronidation. This argument is also voiced by other authors (Reen et al., 1993; Singh, Dubey, & Atal, 1986) although further potential mechanisms have been forwarded, e.g. competition between resveratrol and piperine for membrane efflux pumps in the body and brain (a phenomenon seen when plant derived compounds are co-administered, for example polyphenols (van de Wetering et al., 2008)) and an enhancement of metabolism via thermogenic effects (Badmaev et al). None of these theories are validated however and, of the aforementioned bioenhancement studies, none have investigated whether increased bioavailability led to increased bioefficacy of the target compound. The current study assessed this and also took blood samples from a separate cohort of participants to ascertain whether piperine is indeed able to influence the pharmacokinetics of resveratrol *in vivo*.

A secondary aim of the current study was to investigate whether the null findings on cognitive task performance in the previous two chapters were as a result of the cognitive tasks not evincing sufficient cognitive demand. This is a particularly important consideration for the paradigms utilized in this programme of studies as the age range of participants utilized are all between 18-35yrs, and they were predominantly recruited

from the undergraduate student population; thus the study could be seen as taking place during a period of peak cognitive performance in an assumed highly educated sample (Rönnlund, Nyberg, Bäckman, & Nilsson, 2005) and a sample which often take part in multiple research projects of this type and may be sensitized to their cognitively demanding effects. As an example of the high baseline level of performance, prior to study enrolment participants must demonstrate understanding on the cognitive tasks in the training/screening session by meeting predefined 'norm' scores. These norms derive from averaged performance across all historic studies conducted by the Brain Performance and Nutrition Research Centre (BPNRC). For the tasks utilized in the current chapter participant accuracy must reach at least 18% for RVIP (with 60% the norm), and 68% for 3-Back (with 91% the norm). The number of correct serial 13 subtractions must reach a minimum of 2 (with the norm 17) and 1 for serial 17 subtractions (with a norm of 13). This stringent screening criteria is utilized as good clinical practice to ensure that all participants understand how to perform the cognitive tasks prior to commencement of the study; thus avoiding learning effects taking place during the study and potentially confusing results. However, it could be argued that this procedure also serves to exclude those with lower levels of performance and selectively chooses those with higher cognitive functioning (on the tasks utilized): functioning which can't be improved significantly further with nutritional supplementation. Thus, taking all of the above into account, the current study utilized a selection of the most 'cognitively demanding' and 'difficult' COMPASS tasks (based on the subjective perceptions of a sample of participants not included in this investigation- See appendix I for details) to ascertain if eliciting greater cognitive demand can inhibit performance to an extent that can be reversed by the fuel-enhancing and utilization effects of resveratrol.

The current randomised, double-blind, placebo-controlled, cross-over study therefore investigated the effects of 250mg resveratrol when administered alone, and when co-supplemented with 20mg piperine, on cognitive performance and CBF in the prefrontal cortex of healthy adults during cognitively demanding tasks which predominantly activate the prefrontal cortex. The previous study indicated that levels of oxygen utilization (as evidenced by a rise in deoxy-Hb) began to increase prior to post-dose task commencement, at the end of the absorption period, in both resveratrol dose conditions. This was a somewhat unanticipated finding as it might be expected that oxygen utilization is driven by increased neural activity and yet no cognitive demand was elicited in participants at this time. The current study, therefore, also investigates the idea that resveratrol may have been amplifying a natural, preparatory rise in the hemodynamic response which results from participants being aware that the post-dose

cognitive task period is due to commence. As such, a shortened absorption period (40 minutes as opposed to 45) is incorporated into this paradigm. If this idea holds, then resveratrol should be capable of hemodynamic effects earlier than 40 minutes post-dose, i.e. earlier than the argued '*preparatory rise*' in chapter 3.

This study also incorporates discrete measures of heart rate and blood pressure across the testing session. The rationale for these measures was to ascertain if the neural vasodilatory effects of resveratrol also extend to affecting peripheral vascular reactivity. Findings from cocoa flavanols suggest that polyphenols capable of affecting the vasculature and blood flow can also evince BP effects. Results from two systematic reviews/meta-analyses of randomised control trials, incorporating both acute and chronic flavanol supplementation, report a significant reduction in systolic- (Shrime et al., 2011) and diastolic BP, as well as mean arterial pressure (Hooper et al., 2012), in response to cocoa flavanols. Research into the effects of resveratrol on BP is lacking, especially considering the relatively larger related literature on the cardio-protective effects of resveratrol, with just a handful of papers in animal and human models to date. There is also a paucity of research here in healthy, young models, with animal research suggesting that lean rats experience no reduction in BP as compared to a 79.1% decrease seen in obese rats who had been supplemented with 10mg/kg body weight of resveratrol for 8 weeks (Rivera, Morón, Zarzuelo, & Galisteo, 2009). With regards humans, to date, the effects of resveratrol on BP have only been investigated in obese individuals; with this research demonstrating significantly lowered systolic BP and mean arterial pressure in 11 otherwise healthy males (mean age 52.5yrs) after a 30 day supplementation period of 150mg resveratrol daily (Timmers et al., 2011).

In terms of expectations, the similarities in the underlying mechanisms and subsequent effects between resveratrol and cocoa flavanols perhaps suggests that reduced BP should be anticipated here. However, the research in rodents and obese humans above could indicate that resveratrol is only an effective modulator of BP in those with already compromised vascular health. Taken together, the lack of human intervention trials, specifically in healthy participants, and following acute supplementation of resveratrol, provides a rationale for investigating BP effects here but precludes any clear hypothesis as to the anticipated effects.

The rationale for utilizing 250mg resveratrol, a dose which was largely ineffective at augmenting CBF or cognitive function in the previous study, was that, if piperine is capable of increasing the bioavailability and, in turn, the efficacy of resveratrol, then the combined resveratrol/piperine dose should be capable of potentiating the effects of a largely ineffective dose of resveratrol alone.

4.2 Method- Cognitive/NIRS assessment.

4.2.1 Participants

The sample here comprised 23 healthy adults (4 males, 19 females, mean age 21yrs, range 19-34yrs, SD 3.2yrs, all right handed). Data from one further participant was excluded from analysis due to data capture errors. All participants attended the laboratory after a 12hr overnight fast and reported that they met the inclusion criteria: i.e. to be in good health and free from social drugs (including alcohol), prescription medication, herbal extracts/food supplements, relevant food allergies, intolerances and digestive problems. All participants were non-smokers and did not consume excessive amounts of caffeine (>six cups of coffee or equivalent/d). In addition, participants who had suffered a head injury, neurological disorder or neuro-developmental disorder were excluded from participation, as were those who had uncorrected sight problems, were pregnant or seeking to become so.

4.2.2 Treatments

During the three study visits participants received three single-dose treatments in an order dictated by random allocation to a counterbalancing (Latin Square) order. The three treatments comprised two capsules which combined to give either:

i) Inert placebo,

ii) 250mg of *trans*-resveratrol or

iii) 250mg of *trans*-resveratrol plus 20mg piperine.

The treatments were administered in identical size 0 vegetarian 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. No member of the investigational team was aware of the contents of the capsules until a blind-data review was completed.

4.2.3 Cognitive tasks and mood

As per the previous two studies, cognitive performance was assessed, and cognitive demand induced, with Serial subtraction and RVIP tasks (explained in more detail in section 2.2.3). In this instance the Serial 3s task was replaced with more demanding Serial 13s and 17s tasks although the instructions are identical save the subtraction of a larger number. This study also incorporated the 3-Back version of the N-Back task and assessed a variety of mood parameters with visual analogue scales:

3-Back

This task requires participants to indicate whether the letter presented on screen was also present 3 letters back in the letter sequence. Participants must respond by pressing the 'yes' or 'no' button on the response box, to each letter, as quickly as they can. This task includes sufficient stimuli (letters) to last for at least 2 minutes although this is dependent on speed (i.e. slower reaction times will result in a lengthier task) and is scored for percentage accuracy and reaction time (msec).

Mood VAS

To assess mood, participants were required to rate how 'relaxed', 'alert', 'jittery', 'tired', 'tense' and 'mentally fatigued' they felt by placing a cross with the mouse and cursor on a 100mm on-screen line between the descriptors 'not at all' and 'extremely'. They also rated their 'overall mood' on a scale anchored by 'bad to 'very good' and their levels of 'headache' between 'not at all' and 'extremely'. All VAS were scored as % along the line denoting more of the relevant adjective.

4.2.4 Near-Infrared Spectroscopy (NIRS)

Again, CBF was monitored in the prefrontal cortex with NIRS (described as a technique in more detail in section 2.2.4).

4.2.5 Blood pressure (BP)

Blood pressure (BP) was assessed at discrete time-points during the testing session via a blood pressure cuff on the upper arm using a Boso-Medicus blood pressure monitor which gives readings of diastolic- and systolic BP (mmHg) and heart rate (BPM).

4.2.6 Procedure

Each participant was required to attend the laboratory on four occasions. The first of these was an initial screening/training visit during which participants provided written informed consent, were screened with regards the study inclusion/exclusion criteria, briefed with regards compliance requirements and given training in completing the cognitive tasks. This visit was followed within 14 days by the first of three active study mornings.

On each of the three active study mornings, which were conducted 2-14 days apart, participants attended the laboratory at 8:30am in a fasted state and provided confirmation of continued compliance with regards the inclusion/exclusion requirements. After a 5 minute seated resting period a blood pressure reading was taken (to assess for baseline differences) after which the NIRS headband was fitted. Participants then completed a series of mood VAS and two repetitions of baseline cognitive tasks in the following order: Serial 7s, RVIP, Serial 13s, N-Back, and Serial 17s, each 2 minutes long. Participants then rested for 10 minutes and provided a second blood pressure reading which acted as the 'baseline' measure for the change-from-baseline analysis on the ensuing two post-dose BP measurements. Treatment was then administered after which participants sat quietly, watching one of a selection of non-arousing DVDs, for a 40 minute 'absorption' period. Following this time a third blood pressure reading was taken after which participants completed four repetitions of the aforementioned tasks (38 minutes of post-dose task performance) in the same order and duration. After the post dose tasks were completed the same mood VAS were presented and the fourth and final blood pressure reading was taken. NIRS data was captured throughout. The timeline and running order of the testing session are shown in Figure 4.1.

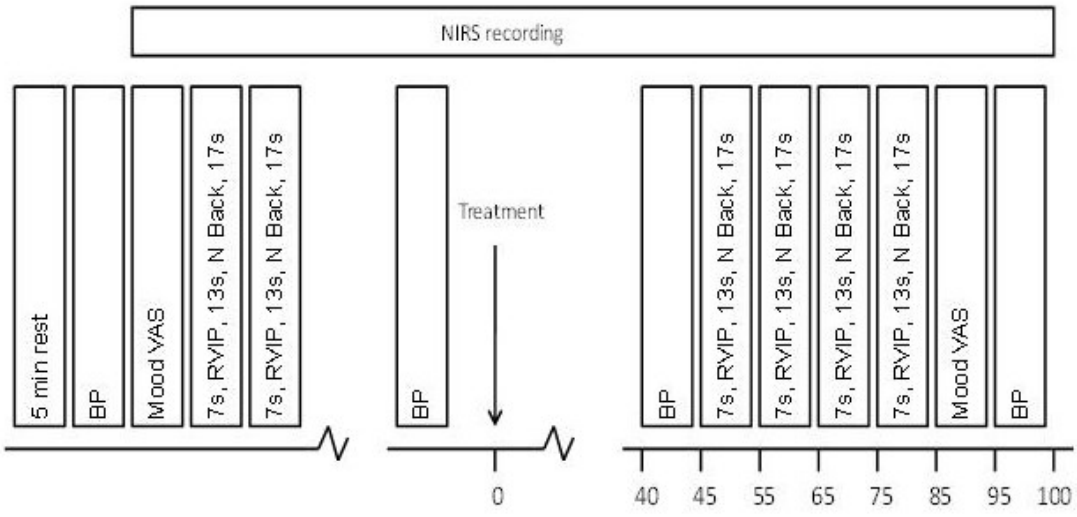


Figure 4.1. Chapter 4 study testing session timeline.

4.2.7 Statistics

Bioavailability analysis:

The analyses of plasma data was conducted with SPSS 16.0 for Windows (SPSS Inc, Chicago, IL), on raw post-dose means (not incorporating baseline as it was, as expected, 0 in all cases), utilizing within subjects analysis of variance (ANOVA) (treatment: 250mg resveratrol and 250mg resveratrol with piperine x time: 45-, 90- and 120 minutes post-dose) for each metabolite. Paired samples t tests were also utilized to compare AUC, C_{max} and T_{max}, between 250mg resveratrol and 250mg resveratrol with 20mg piperine, for each metabolite.

Near-Infrared Spectroscopy (NIRS) analysis:

NIRS data was analysed with Minitab 16 for Windows (Minitab Inc, State College, PA). For each variable (oxy-Hb, deoxy-Hb and total-Hb), data was converted to 'change from baseline' (calculated from the 10 minute pre-treatment resting period) and averaged across 2 minute epochs during the 40 minute 'rest/absorption' and 38 minute cognitive task performance period. The primary analysis of the averaged NIRS data was conducted by within-subjects ANOVA (treatment x 2min epoch) with *a priori* planned comparisons of data from each epoch being made between placebo and each of the resveratrol treatment groups (250mg resveratrol, 250mg resveratrol with 20mg piperine) using t tests calculated with the Mean Squares Error from the ANOVA (Keppel, 1991). (See section 2.2.6 for justification of this analysis plan.)

Cognitive task data analysis:

Task performance data (also analysed with SPSS 16.0) was analysed as change from pre-dose baseline for each individual task (Serial 7s, RVIP, Serial 13s, 3-back and Serial 17s) by within-subjects ANOVA (treatment x repetition (1, 2, 3 and 4)). Bonferroni corrected post-hoc comparisons were then conducted if a significant main effect of treatment and/or a treatment x repetition interaction was observed here. Prior to any analysis, baseline differences were investigated with regards these measures and any results only reported if significant. (NB. This was also the case for mood and BP analysis.)

Mood analysis:

Data for 'relaxed', 'alert', 'jittery', 'tired', 'tense' and 'mentally fatigued' mood VAS were analysed via a repeated measures ANOVA (time: pre-dose/pre-baseline tasks and post-dose/after post-dose tasks x treatment) with Bonferroni corrected post-hoc

comparisons then made if a significant main effect of treatment and/or treatment x time interaction was seen here.

Blood pressure (BP) analysis:

The two post-dose BP measures, taken at the beginning and end of post-dose cognitive tasks, were converted to change from baseline (from the pre-treatment BP readings) and analysed via a repeated measures ANOVA (treatment: 250mg resveratrol, 250mg resveratrol with 20mg piperine and placebo x time: pre-task and post-task) with post-hoc student t tests conducted if a significant main effect of treatment or an interaction between treatment x time was evinced here.

4.3 Method- Pharmacokinetic assessment.

4.3.1 Participants

This aspect of the study recruited six healthy (mean BMI 24.2, range 21.7-27.2, SD 2.38) participants, all male, with a mean age of 25.8yrs (range 23-29yrs). Inclusion/exclusion criteria were as per the cognitive and CBF assessment above. All participants either worked or were post-graduate students at Northumbria University.

4.3.2 Treatments

Treatments were as per the cognitive and CBF (section 4.2.2) assessment above with the exception that these participants did not take part in the placebo condition.

4.3.3 Procedure

On each study morning participants attended the laboratory at 8.30am in a 12hr fasted state. Venous blood samples were collected using 4.7ml monovettes (containing lithium heparin) before the day's treatment was consumed and then 45-, 90- and 120 minutes post treatment administration. During the time between sample-taking participants were permitted to leave the lab but confirmed that they had not eaten anything, engaged is strenuous activity or drank anything apart from water when returning for the next sample. Samples were centrifuged at 2500rpm for 15min at 20^{o}C to yield plasma, which was then stored at -80^{o}C until analysis.

4.3.4 Treatment and analysis of plasma

Preparation of Samples:

Samples were handled in low light conditions to reduce the scope for isomerisation. Plasma was defrosted at room temperature immediately before extraction, vortexed then sonicated for 5-minutes. A 200µL aliquot was mixed with 900µL of HPLC grade ethanol plus 0.1% formic acid (v/v), along with 100µL of naringenin internal standard (IS1; Extrasynthese, France) in ethanol (500ng/ml). Samples were vortexed, sonicated and then separated via micro-centrifuge at 17k R.C.F. for 10-minutes. The supernatant was removed and placed in an amber 1.5ml centrifuge tube (Eppendorf, UK). The pellet was re-extracted with 1.2ml of 83% aqueous ethanol (v/v) following the same protocol. Both extracts were evaporated to dryness under vacuum using a centrifugal evaporator (EZ2+, Genevac, UK), and frozen at -20^{o}C. On the day of analysis, a 70µL portion of ethanol was added to the secondary extract, which was vortexed and sonicated. A 50µL aliquot of this solution was then added to the primary extract, which following vortexing and sonication was mixed with 50µL taxifolin (IS2 at 2µg/ml; Extrasynthese, France) in 0.2% ascorbic acid solution. This solution was vortexed, separated by centrifugation and the supernatant placed in an amber vial and analyzed via LC-MS. Extractions were made in duplicate for each time point. To test extraction efficiency of this method, blank plasma was spiked with standards at 50nM, 500nM, 5µM and 10µM concentrations. Across this range, the average extraction efficiencies for *trans*-resveratrol (Cayman Chemicals, USA), the -3-0-sulfate, 4-0'-glucuronide and 3-0-glucuronide (Bertin Pharma, France) were 74%, 72%, 52% and 55%, respectively. IS1 and IS2 were extracted consistently at 82% and 100%, respectively.

LC-MS Analysis:

Analysis was conducted using a Shimadzu LC2010CHT HPLC, consisting integrated quaternary pump, degasser, chilled autosampler (8^{o}C), and column oven (30^{o}C), connected to an LCMS2020 single quad. A 10µL sample aliquot was separated on an XDB–C18 1.8µ 4.6 x 50mm column (Agilent, UK), running a binary gradient of LCMS grade water vs. acetonitrile, both containing 0.1% formic acid (v/v), running at 0.5ml/min. The gradient started at 5% acetonitrile, and moved to 10% at 5min, 40% at 20 minutes and 90% at 25 minutes. Following 4 minutes of washing, the column returned to 5% acetonitrile at 30 minutes and was re-equilibrated over 3 minutes. The MS ran with an interface temperature set to 350^{o}C, using nebuliser and drying gas flow rates of 1.5- and 15L/-minutes, respectively. The analysis was performed in negative SIM mode, following m/z of 403 (glucuronides), 307 (sulfates) 271 (naringenin IS1),

303 (taxifolin IS2) and 227 (aglycone resveratrol). A persistent formate adduct of aglycone resveratrol (m/z 273) was also followed as a qualifying ion. The limit of quantification (LOQ) for glucuronides was 16nM, 22nM for sulfates, and 145nM and 290nM for *cis*- and *trans*-aglycone resveratrol respectively. Peak areas were normalized to IS2 for quantification, whilst IS1 was used to judge individual sample extraction. The retention times of *cis*-isomer resveratrol conjugates were identified by subjecting commercially available *trans*-isomers (10 µg/ml in 50% aqueous ethanol, plus 0.1% ascorbic and 0.05% formic acids) to ultraviolet light (254nm) for 4hrs. *Cis*-isomer resveratrol conjugates were quantified as *trans*-isomer equivalents, and then summed with the corresponding *trans*-isomers.

4.4 Results

4.4.1 Bioavailability

No resveratrol (in any form) was found in baseline samples, indicating that all volunteers did not consume resveratrol before the study. The results of the treatment x time ANOVAs demonstrated only trends for main effects of time for the 4-*O*-glucuronide metabolite [$F(2,10)= 3.96$; $p= .054$] and 3-*O*-glucuronide [$F(2,10)= 3.62$; $p= .066$]. No main effects of treatment nor any treatment x time interactions were found.

Whilst average concentrations at C_{max} for resveratrol metabolites are lower following piperine co-supplementation compared to resveratrol alone, there was no statistically significant difference between treatments. Similarly, there was no significant difference for area under the curve values and T_{max} was not significantly changed between treatments.

No aglycone resveratrol was quantifiable in plasma at any time-point and resveratrol-3-*O*-sulfate was the predominant metabolite in all volunteers, contributing 59-81% of total metabolites. The 4'- and 3-*O*-glucuronide forms made roughly equal contributions to the remaining metabolites in circulation.

Mean plasma concentrations of *trans*-resveratrol metabolites at pre-treatment and at 45-, 90- and 120 minute post-dose time-points, for both treatment conditions, are shown in Figure 4.2 and table 4.1 where ANOVA F and P values and t tests are presented also.

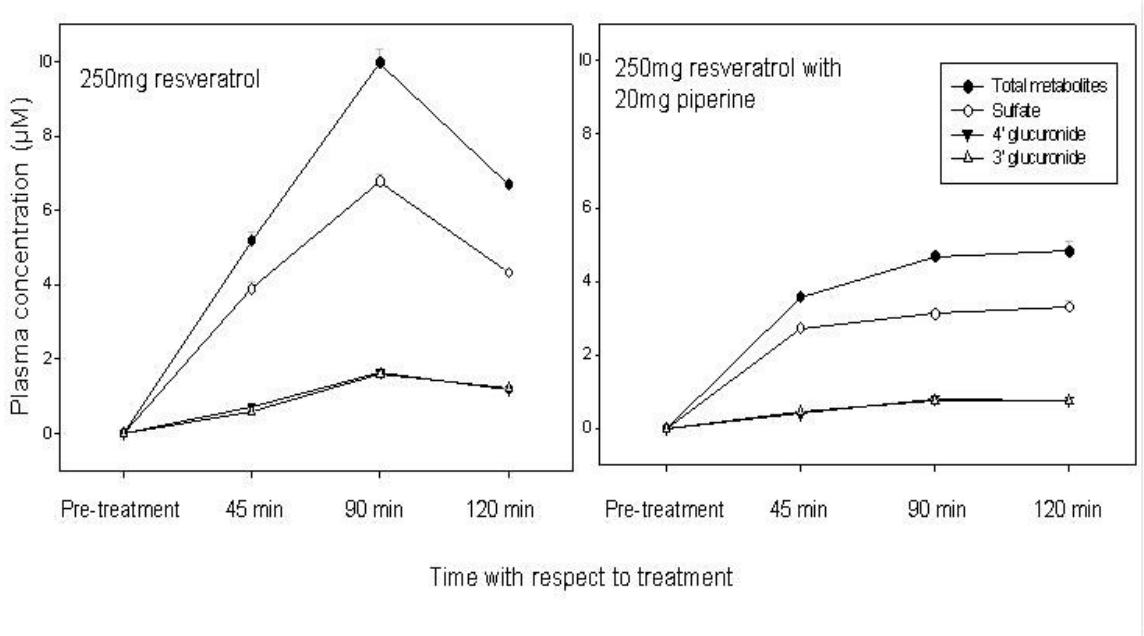


Figure 4.2. Bioavailability of resveratrol metabolites after 250mg *trans*-resveratrol alone and when co-supplemented with 20mg piperine. Graph displays mean plasma concentrations (µM) of *trans*-resveratrol metabolites at pre-treatment and at 45-, 90- and 120 minute post-dose time-points, after 250mg *trans*-resveratrol (left) and 250mg *trans*-resveratrol with 20mg piperine (right), in 6 healthy males. (Graph does contain SEM error bars but the low values (see table 4.1) make them difficult to distinguish beyond the treatment markers.)

Table 4.1. Mean plasma levels of resveratrol metabolites at baseline and 45-, 90- and 120 minutes post-dose after 250mg resveratrol and 250mg resveratrol with 20mg piperine. Table displays mean (with SEM in italics, in brackets) baseline and post-dose plasma levels of resveratrol metabolites in six healthy participants. Table also displays ANOVA F and P values for main effects of treatment (Tr) and time (Ti) as well as an interaction between the two (Tr*Ti) and the results of students t tests comparing T_{max}, C_{max} and AUC between treatments.

Resveratrol metabolite	Treatment	Time-point				ANOVA			t tests		
		Baseline	45-min PD	90-min PD	120-min PD	Effect	F	P	Effect	t	p
Total	250mg resveratrol	00.00 *(00.00)*	5.18 *(0.09)*	9.98 *(0.14)*	6.69 *(0.02)*	Tr	2.63	.166	T_{max}	1.18	.293
	250mg resveratrol with 20mg piperine	00.00 *(00.00)*	3.57 *(0.02)*	4.68 *(0.04)*	4.82 *(0.11)*	Ti	2.28	.153	C_{max}	1.40	.220
						Tr*Ti	1.03	.391	AUC	1.61	.168
3-*O*-sulfate	250mg resveratrol	00.00 *(00.00)*	3.90 *(0.07)*	6.78 *(0.09)*	4.32 *(0.03)*	Tr	2.74	.159	T_{max}	-.614	.566
	250mg resveratrol with 20mg piperine	00.00 *(00.00)*	2.73 *(0.02)*	3.12 *(0.03)*	3.29 *(0.07)*	Ti	1.65	.241	C_{max}	1.27	.259
						Tr*Ti	1.28	.321	AUC	1.56	.179
4-*O*-glucuronide	250mg resveratrol	00.00 *(00.00)*	0.71 *(0.02)*	1.62 *(0.03)*	1.17 *(0.42)*	Tr	1.67	.253	T_{max}	-.284	.788
	250mg resveratrol with 20mg piperine	00.00 *(00.00)*	0.39 *(0.004)*	0.80 *(0.008)*	0.76 *(0.02)*	Ti	3.96	.054 t	C_{max}	.933	.393
						Tr*Ti	.460	.644	AUC	1.42	.215
3-*O*-glucuronide	250mg resveratrol	00.00 *(00.00)*	0.57 *(0.01)*	1.58 *(0.03)*	1.19 *(0.42)*	Tr	2.09	.208	T_{max}	-.284	.788
	250mg resveratrol with 20mg piperine	00.00 *(00.00)*	0.45 *(0.004)*	0.77 *(0.01)*	0.77 *(0.02)*	Ti	3.62	.066 t	C_{max}	.943	.389
						Tr*Ti	.934	.425	AUC	1.55	.182

4.4.2 Near-Infrared Spectroscopy (NIRS) parameters

Total haemoglobin (total-Hb):

The omnibus ANOVA demonstrated no significant effect of treatment [F(2, 1482)= 0.94; p= 0.40] but did find a significant interaction between treatment x epoch [F(78, 1482)= 2.12; p= <.001]. Planned comparisons showed that, compared to placebo, the 250mg resveratrol treatment failed to elicit any modulation of total-Hb levels. However, following 250mg resveratrol combined with 20mg piperine, whilst there were no significant effects during the absorption period, levels of total-Hb were significantly higher than placebo for all task performance epochs, apart from 45, 51 and 79 (epochs 41, 49 and 61 <.05[13] and the remainder <.01[14]).

Oxygenated haemoglobin (oxy-Hb):

The omnibus ANOVA demonstrated no significant main effect of treatment [F(2, 1482)= 0.42; p= 0.66] or interaction effect between treatment x epoch [F(78, 1482)= 1.39; p= 0.02]. Reference to the planned comparisons shows that the pattern was similar to that seen with regards total-Hb, with no modulation seen following 250mg resveratrol, but significantly higher concentrations, as compared to placebo, of oxy-Hb seen following 250mg resveratrol with 20mg piperine (all epochs <.01[15], except 45, 49 and 51 which were <.05[16] and 79 which just failed to reach significance).

Deoxygenated haemoglobin (deoxy-Hb):

The omnibus ANOVA demonstrated no significant main effect of treatment [F(2, 1482)= 0.67; p= 0.52] or interaction effect between treatment x epoch [F(78, 1482)= 1.11; p= 0.25. With regards planned comparisons, again, no significant difference between 250mg resveratrol alone and placebo was observed but a consistent pattern of significant effects, which began to emerge during the end of the absorption phase, and continued throughout the post-dose task period, was evinced after the 250mg resveratrol with 20mg piperine dose. Here, levels of deoxy-Hb were significantly higher in comparison to placebo (during the absorption period epochs 27, 29, 33, 35 and 37 <.05[17] and 39 <.01; during post-dose task period all epochs <.01[18] apart from 77 which was <.05).

The mean data (± SEM) and the results of the planned comparisons for total-, oxy-, and deoxy-Hb are represented in Figure 4.3.

[13] t's (39) ≤2.08
[14] t's (39) ≤3.41
[15] t's (39) ≤2.63
[16] t's (39) ≤2.00
[17] t's (39) ≤1.96
[18] All <.01 t's (39) ≤2.87

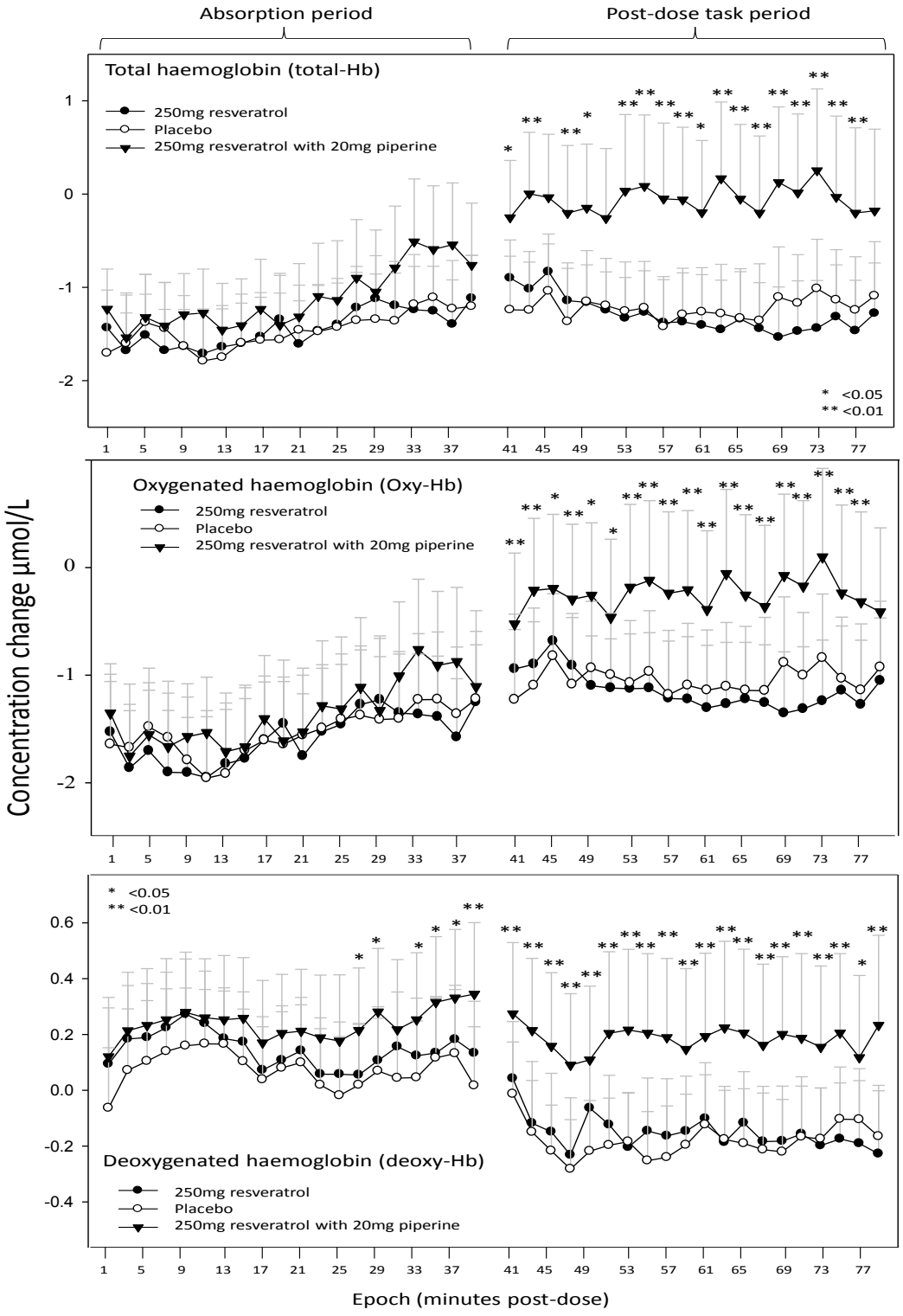


Figure 4.3. The acute effects of 250mg *trans*-resveratrol alone, and when co-supplemented with 20mg piperine, on cerebral blood flow in the prefrontal cortex. Graph displays concentration change (µmol/L) levels of total, oxygenated and deoxygenated haemoglobin (with SEM error bars) during a 40 minute absorption and 38 minute post-dose task period in 23 healthy adults after placebo, 250mg *trans*-resveratrol and 250mg *trans*-resveratrol combined with 20mg piperine. *<.05 and **<.01.

4.4.3 Cognitive task performance and mood

With regards cognitive task performance, significant main effects of repetition were observed on the 'NBack reaction time', 'RVIP%correct', '13s correct' and '13s incorrect' measures. No significant main effects of treatment or interaction effects between treatment x repetition were found.

With regards mood, a significant main effect of time was observed for the 'jittery', 'overall mood', 'relaxed', 'tense' and 'tired' factors. No main effect of treatment was evinced on any mood measure but a significant interaction between treatment x time was seen for the 'alert' rating [$F(2, 44)= 3.28$; $p= .047$]. However, further investigation with student's t tests failed to observe any significant differences between each treatment and placebo at any of the comparisons. (See figure 4.4 for 'alert' graph.)

See tables 4.2. and 4.3. for all task and mood variables respectively. (Tables also contain ANOVA F and P values.)

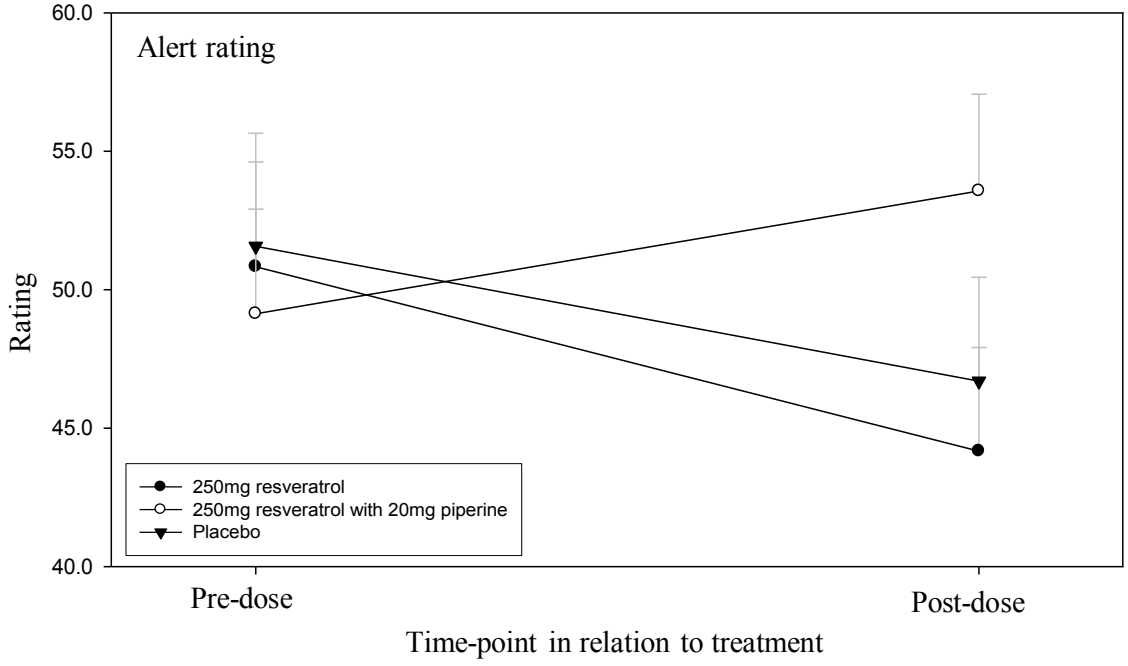


Figure 4.4. The effects of 250mg *trans*-resveratrol alone, and when co-supplemented with 20mg piperine, on 'alert' rating. Graph displays baseline and post-dose raw means (with SEM error bars) of ratings (% along 100mm VAS line) of alert for all three treatment conditions.

Table 4.2. The effects of 250mg *trans*-resveratrol alone, and when co-supplemented with 20mg piperine, on cognitive performance. Table displays raw baseline and change from baseline scores (with SEM values in brackets) for all four post-dose battery repetitions after placebo, 250mg *trans*-resveratrol and 250mg *trans*-resveratrol with 20mg piperine for 23 healthy, young 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.

Measure	Treatment condition	Task battery repetition					ANOVA		
		Baseline	1	2	3	4	Effect	F	P
7s Correct (Number)	250mg resveratrol	28.85 *(2.75)*	1.20 *(1.02)*	1.98 *(0.94)*	1.54 *(0.83)*	0.80 *(1.16)*	T	.252	.778
	250mg resveratrol with 20mg Piperine	28.83 *(2.59)*	1.52 *(0.85)*	-0.04 *(0.94)*	0.39 *(1.25)*	0.57 *(1.13)*	R	.487	.692
	Placebo	28.85 *(2.04)*	1.94 *(1.12)*	0.89 *(1.11)*	0.11 *(1.43)*	0.98 *(1.29)*	T*R	.675	.606
7s Incorrect (Number)	250mg resveratrol	1.87 *(0.30)*	0.35 *(0.51)*	-0.26 *(0.37)*	0.35 *(0.38)*	0.70 *(0.55)*	T	.517	.600
	250mg resveratrol with 20mg Piperine	1.67 *(0.23)*	0.11 *(0.39)*	0.67 *(0.38)*	1.33 *(0.52)*	1.07 *(0.49)*	R	2.09	.110
	Placebo	1.91 *(0.26)*	0.30 *(0.52)*	0.30 *(0.47)*	0.78 *(0.60)*	0.13 *(0.46)*	T*R	1.02	.416
13s Correct (Number)	250mg resveratrol	22.22 *(2.25)*	0.70 *(0.88)*	-0.78 *(0.90)*	0.22 *(0.87)*	-1.17 *(0.98)*	T	1.68	.199
	250mg resveratrol with 20mg Piperine	22.46 *(2.17)*	1.33 *(0.75)*	-1.15 *(1.26)*	-0.11 *(1.25)*	1.07 *(0.87)*	R	3.17	.030*
	Placebo	21.83 *(1.60)*	3.26 *(0.83)*	0.78 *(1.41)*	1.17 *(1.20)*	1.09 *(1.03)*	T*R	.644	.695
13s Incorrect (Number)	250mg resveratrol	2.04 *(0.23)*	0.13 *(0.40)*	2.04 *(1.00)*	0.65 *(0.51)*	1.17 *(0.47)*	T	.969	.388
	250mg resveratrol with 20mg Piperine	1.89 *(0.36)*	0.11 *(0.44)*	1.59 *(0.94)*	1.59 *(0.63)*	0.76 *(0.73)*	R	7.08	<.001**
	Placebo	2.39 *(0.36)*	-1.09 *(0.33)*	0.96 *(0.84)*	0.78 *(0.53)*	0.44 *(0.58)*	T*R	.445	.765

17s Correct (Number)	250mg resveratrol	17.22 *(1.68)*	1.39 *(0.71)*	1.48 *(0.81)*	2.35 *(0.75)*	1.09 *(1.13)*	T	.405	.670
	250mg resveratrol with 20mg Piperine	17.78 *(1.61)*	0.39 *(0.63)*	0.44 *(0.86)*	0.87 *(0.90)*	2.13 *(0.76)*	R	.502	.638
	Placebo	16.80 *(1.29)*	1.72 *(0.62)*	1.37 *(0.68)*	1.15 *(0.95)*	1.89 *(0.59)*	T*R	1.07	.383
17s Incorrect (Number)	250mg resveratrol	2.28 *(0.28)*	0.15 *(0.41)*	0.02 *(0.47)*	0.24 *(0.52)*	1.54 *(1.09)*	T	.719	.493
	250mg resveratrol with 20mg Piperine	2.17 *(0.29)*	0.30 *(0.42)*	0.30 *(0.50)*	0.57 *(0.42)*	0.52 *(0.37)*	R	1.41	.254
	Placebo	2.57 *(0.27)*	-0.30 *(0.37)*	-0.44 *(0.45)*	0.44 *(0.67)*	-0.04 *(0.36)*	T*R	.791	.578
N-Back Accuracy (%)	250mg resveratrol	93.38 *(1.17)*	-0.34 *(0.97)*	-1.02 *(1.05)*	-0.92 *(1.08)*	-0.05 *(1.00)*	T	.617	.544
	250mg resveratrol with 20mg Piperine	94.40 *(0.91)*	-2.03 *(1.02)*	-1.84 *(1.09)*	-0.29 *(0.89)*	-1.45 *(1.27)*	R	.274	.844
	Placebo	94.40 *(0.74)*	-1.26 *(1.08)*	-1.55 *(0.92)*	-2.61 *(1.13)*	01.45 *(0.93)*	T*R	.678	.599
N-Back Reaction Time (msec)	250mg resveratrol	1540.45 *(145.80)*	-291.04 *(48.75)*	-345.87 *(53.98)*	-312.95 *(52.58)*	-398.24 *(58.12)*	T	1.28	.288
	250mg resveratrol with 20mg Piperine	1476.26 *(189.03)*	-243.72 *(67.01)*	-287.30 *(77.69)*	-375.74 *(94.44)*	-292.16 *(70.96)*	R	3.93	.012*
	Placebo	1475.04 *(161.35)*	-194.12 *(34.69)*	-149.39 *(70.65)*	-264.79 *(81.89)*	-271.44 *(57.14)*	T*R	1.12	.347
RVIP correct (%)	250mg resveratrol	71.06 *(3.76)*	0.41 *(2.98)*	-4.48 *(2.44)*	-7.47 *(3.73)*	-7.76 *(2.58)*	T	1.17	.321
	250mg resveratrol with 20mg Piperine	65.81 *(4.00)*	3.76 *(2.32)*	1.31 *(3.39)*	-4.36 *(3.39)*	-1.68 *(3.51)*	R	7.58	<.001**
	Placebo	69.16 *(3.90)*	1.50 *(2.25)*	-7.38 *(3.65)*	-7.47 *(2.51)*	-6.66 *(3.40)*	T*R	.489	.816

RVIP Reaction Time (msec)	250mg resveratrol	494.24 *(8.87)*	5.10 *(5.73)*	0.61 *(8.76)*	1.18 *(7.86)*	3.90 *(9.57)*	T	.163	.765
	250mg resveratrol with 20mg Piperine	501.68 *(9.46)*	-7.17 *(8.07)*	2.06 *(10.99)*	-2.86 *(10.18)*	-4.06 *(9.98)*	R	.334	.800
	Placebo	499.22 *(0.13)*	-7.11 *(7.30)*	3.79 *(10.20)*	0.48 *(13.14)*	1.89 *(8.38)*	T*R	.246	.960

Table 4.3. The effects of 250mg *trans*-resveratrol alone, and when co-supplemented with 20mg piperine, on mood. Table displays raw baseline and change from baseline ratings (with SEM values in brackets) after placebo, 250mg *trans*-resveratrol and 250mg *trans*-resveratrol with 20mg piperine for 23 healthy, young adults. Table also displays ANOVA F and P values for main effects of treatment (T), repetition (R) and an interaction between the two (T*R) with *<.05, **<.01 and t= trend.

Measure	Treatment condition	Baseline	Post-dose	ANOVA		
				Effect	F	P
Alert	250mg resveratrol	50.83 *(3.79)*	-6.65 *(5.44)*	T	.767	.470
	250mg resveratrol with 20mg Piperine	49.13 *(3.78)*	4.43 *(4.07)*	R	.359	.555
	Placebo	51.57 *(4.08)*	-4.87 *(4.68)*	T*R	3.28	.047*
Jittery	250mg resveratrol	16.83 *(2.91)*	19.78 *(5.40)*	T	.532	.591
	250mg resveratrol with 20mg Piperine	18.61 *(3.33)*	20.48 *(4.95)*	R	25.79	<.001**
	Placebo	15.39 *(2.54)*	20.87 *(4.73)*	T*R	.022	.979
Mental Fatigue	250mg resveratrol	28.96 *(4.69)*	35.65 *(6.18)*	T	.839	.439
	250mg resveratrol with 20mg Piperine	27.48 *(4.86)*	32.48 *(5.93)*	R	45.47	<.001**
	Placebo	26.22 *(4.10)*	33.74 *(6.11)*	T*R	.147	.864
Overall Mood	250mg resveratrol	62.87 *(3.46)*	-16.13 *(4.48)*	T	2.66	.081 t
	250mg resveratrol with 20mg Piperine	64.48 *(3.04)*	-12.78 *(3.60)*	R	25.87	<.001**
	Placebo	67.35 *(2.71)*	-13.74 *(2.97)*	T*R	.321	.727
Relaxed	250mg resveratrol	62.91 *(2.67)*	-24.52 *(5.62)*	T	.566	.572
	250mg resveratrol with 20mg Piperine	60.35 *(3.29)*	-14.13 *(6.00)*	R	20.70	<.001**
	Placebo	62.52 *(1.98)*	-20.61 *(4.44)*	T*R	1.79	.179
Tense	250mg resveratrol	25.48 *(3.29)*	25.74 *(6.35)*	T	2.32	.110
	250mg resveratrol with 20mg Piperine	23.87 *(3.28)*	26.35 *(6.40)*	R	26.08	<.001**
	Placebo	19.83 *(3.02)*	25.30 *(5.37)*	T*R	.016	.984
Tired	250mg resveratrol	47.09 *(4.51)*	14.57 *(5.33)*	T	.405	.669
	250mg resveratrol with 20mg Piperine	50.74 *(5.05)*	4.04 *(3.92)*	R	5.96	.023*
	Placebo	45.57 *(4.42)*	11.52 *(6.39)*	T*R	1.72	.191

4.4.4 Blood pressure

The results of the repeated measures ANOVAs demonstrated a significant main effect of time for systolic BP [F(1, 22)= 9.61; p= .005] and a trend for a main effect of time for pulse rate [F(1, 22)= 3.38; p= .080]. This analysis also revealed a significant main effect of treatment for diastolic BP [F(1.6, 34.5)= 3.68; p= .045] where levels, overall, were higher in the 250mg resveratrol with 20mg piperine condition, as compared to placebo. However, post-hoc students t tests revealed only trends for higher diastolic BP at both post-dose BP measurements (p= .068 and .055 respectively).

(See table 4.4. for blood pressure data and ANOVA F and P values.)

Table 4.4. The effects of 250mg *trans*-resveratrol alone, and when co-supplemented with 20mg piperine, on blood pressure. Table displays raw baseline (immediately prior to treatment) means (with SEM in brackets) and change from baseline means for the two post-dose BP measures (PD 1: immediately prior to post-dose tasks and PD 2: immediately after post-dose tasks) for 250mg *trans*-resveratrol, 250mg *trans*-resveratrol with 20mg piperine and placebo. Table also displays ANOVA F and P values for main effect of treatment (Tr) and time (Ti) and an interaction effect between the two (Tr*Ti) with *<.05, **<.01 and t=trend.

Measure	Treatment condition	Task battery repetition			ANOVA		
		Baseline	PD 1	PD 2	Effect	F	P
Systolic Blood Pressure (mmHg)	250mg resveratrol	112 *(1.98)*	2.35 *(1.77)*	4.87 *(1.21)*	Tr	.621	.542
	250mg resveratrol with 20mg Piperine	114.17 *(1.98)*	1.39 *(1.26)*	4.90 *(1.72)*	Ti	9.61	.005**
	Placebo	113.22 *(2.31)*	-0.04 *(1.78)*	3.39 *(2.13)*	Tr*Ti	.089	.915
Diastolic Blood Pressure (mmHg)	250mg resveratrol	75.65 *(1.66)*	2.57 *(0.90)*	4.17 *(0.96)*	Tr	3.68	.045*
	250mg resveratrol with 20mg Piperine	75.09 *(1.62)*	4.83 *(1.38)*	4.70 *(1.65)*	Ti	.628	.437
	Placebo	76.91 *(2.48)*	-0.17 *(2.08)*	0.65 *(1.77)*	Tr*Ti	.258	.724
Pulse Rate (BPM)	250mg resveratrol	68.43 *(2.48)*	-0.83 *(1.07)*	-2.26 *(1.51)*	Tr	1.77	.192
	250mg resveratrol with 20mg Piperine	67.91 *(2.14)*	0.35 *(1.87)*	-3.74 *(3.78)*	Ti	3.38	.080 t
	Placebo	70.87 *(2.29)*	-3.78 *(1.63)*	-6.87 *(1.63)*	Tr*Ti	.368	.584

4.5 Discussion

The overall aim of this study was to investigate the cognitive and cerebral blood flow (CBF) effects of resveratrol in healthy, young humans. Whilst chapter 3 demonstrated evidence of the effects of resveratrol with regards CBF, both chapters 2 and 3 failed to observe any effect of resveratrol on cognitive task performance. The argument was made that the poor bioavailability of resveratrol observed in chapter 3, in line with the small amount of existing literature into the pharmacokinetics of resveratrol, may have been sufficient to elicit CBF effects but that the resulting hemodynamic response was not sufficient to improve cognitive function. As such, the specific aim of this chapter was to attempt to alter the natural bioavailability of resveratrol, via the co-supplementation of piperine, to ascertain whether augmented plasma levels would bolster the CBF effects of resveratrol such that cognitive performance would also be improved.

In terms of the effects of resveratrol on the hemodynamic response to cognitive tasks, the current study does indeed demonstrate that the bio-enhancer piperine is able to increase the efficacy of the polyphenol resveratrol, when co-supplemented, in healthy humans. The results demonstrate that; whereas 250mg orally administered resveratrol alone had no significant effects on overall CBF (total-Hb and oxy-Hb) during cognitive task demands, co-administration of the same dose of resveratrol with 20mg piperine resulted in significantly higher total- and oxy-Hb for the duration of the 38 minute post-dose task period. With regards levels of deoxy-Hb, as in chapter 3, this investigation also demonstrated the increased oxygen utilization evinced by resveratrol, across the entire post-dose task period, although, as above, only when co-supplemented with piperine. In the case of all three NIRS chromophores, the levels evinced by 250mg resveratrol alone were similar to those in the placebo condition and, broadly, mirror the lack of efficacy of this dose on CBF (specifically total- and oxy-Hb) in chapter 3.

A further area of interest in the hemodynamic effects of resveratrol was the so-named '*preparatory rise*' in levels of deoxy-Hb witnessed at the end of the absorption period in chapter 3. The argument was made that this may have represented a pre-emptive hemodynamic response in anticipation of imminent increased cognitive workload; as the post-dose cognitive task period was due to begin. In order to test this theory here, the absorption period here was shortened from 45- to 40 minutes with the hypothesis that if this natural pre-emptive rise was being amplified by resveratrol, that this would be apparent by significantly higher deoxy-Hb, in response to resveratrol, before the 40 minute post-treatment epoch and sooner than that seen in chapter 3. Indeed this was the case, in the 250mg resveratrol with 20mg piperine condition, with significantly

higher levels of deoxy-Hb, as compared to placebo, evident from epoch 27 of the absorption period; a full 13 minutes prior to the commencement of post-dose tasks and earlier than that evinced by resveratrol in chapter 3; where the absorption period and, therefore, arguably the anticipation of cognitive workload, occurred later. Why this preparatory rise should exist is purely hypothetical. It could merely represent a co-incidental artefact of the natural changes in deoxy-Hb levels. It could be indicative of the effects of resveratrol occurring sooner than one might anticipate; that it occurred even sooner here (as compared to chapter 3) could merely be a cohort effect rather than a product of reducing the absorption period. And finally, this pre-emptive increase in fuel use could present a natural rise in readiness for the impending increase in cognitive workload in the post-dose task period. That this was only seen in the co-supplemented condition might be suggestive of piperine merely enhancing the effect of resveratrol; a dose which was ineffective in amplifying this natural response in itself.

This chapter was the first in this thesis to investigate the potential for resveratrol to affect the peripheral vasculature by monitoring BP and heart rate pre- and post-dose. The rationale for this was based on the reduction in these parameters seen following cocoa flavanol consumption in humans (Hooper et al., 2012) and in obese rats (Rivera, Morón, Zarzuelo, & Galisteo, 2009) and humans (Timmers et al., 2011) following resveratrol and is likely the product of vasodilatory effects. The results here demonstrate a significant main effect of treatment with levels of diastolic BP significantly higher in the 250mg resveratrol with 20mg piperine condition, as compared to placebo. These results are at odds with the above research and presents two potential explanations. Firstly, resveratrol may not be associated with reduced blood pressure in young, healthy participants. That this effect wasn't present in the resveratrol alone condition might be indicative of piperine enhancing the efficacy of resveratrol; as with the CBF results in this chapter. Secondly, as this increase in diastolic BP wasn't seen in the resveratrol alone condition, this finding could be the product of some unknown mechanism of piperine. The lack of research into the vascular effects of piperine, and indeed of resveratrol, in healthy, young humans makes this argument difficult to disentangle at present but the ensuing chapter will aim to do so.

In terms of cognitive performance, the results of this chapter support both previous chapters in finding no significant effect of resveratrol, nor resveratrol co-supplemented with piperine, on any aspect of cognitive function. This chapter did find a significant treatment x time interaction for the 'alert' mood variable as assessed by VAS. However, despite the direction of effects (depicted in figure 4.4) indicating that the piperine co-

supplemented group were more alert at the post-dose rating than placebo, further investigation failed to find any significant differences between treatment groups.

With regards plasma levels of resveratrol, the current study investigated the capacity of piperine to enhance concentrations of resveratrol and whether this, in turn, might enhance the efficacy of resveratrol with regards cognitive performance and CBF. Considering the above results, i.e. specifically such significant CBF effects existing in only the co-supplemented condition, it might have been expected that plasma levels had indeed been augmented by piperine. However, no significant differences were found in plasma levels of resveratrol metabolites between treatment conditions and, considering the pattern of effects, piperine appears actually to be inhibiting plasma levels of resveratrol and extending the rate of elimination (evidenced by the failure of levels of metabolites to begin returning to baseline, as in the resveratrol alone condition, in the co-supplemented plasma samples) (see figure 4.2). However, this lack of enhanced bioavailability of resveratrol by piperine does raise the question of how piperine seemingly increases the efficacy of resveratrol with regards CBF. This really provides two possibilities; either that piperine is able to exert CBF effects independently of resveratrol or, alternatively, it potentiates the effects of resveratrol.

Taking the first of those possibilities then, it is notable that only one study (Vaibhav et al., 2012) exists to suggest that piperine is capable of potentially conferring similar effects to resveratrol with regards CBF; finding that piperine was able to interact with the vasodilatory mediator nitric oxide (NO). However, piperine was only observed to augment the inducible NO synthase isoform (iNOS) which is stimulated in response to immunological stimuli (Nathan, 1997) and is not associated with cerebral vaso-relaxation and increased blood flow. No data exists to suggest that piperine is capable of affecting oxygenation. In light of a lack of evidence to suggest that piperine has any influence on parameters relevant to CBF, and in the face of a lack of significant modulation of CBF in the resveratrol condition alone (a finding similar to that seen in chapter 3 with the same dose) it seems more likely that piperine is increasing the efficacy of resveratrol by potentiating its vasorelaxatory properties. In support of this, resveratrol has been found to be a vasorelaxatory mediator (Wong et al., 2011) and, at a higher dose (500mg), can consistently increase CBF in healthy humans (chapter 3).

Of the potential remaining mechanisms to explain the efficacy enhancing effects of piperine, one possibility is that piperine is able to enhance the activity of resveratrol, the neuronal vasculature, and/or some other factor relevant to CBF via thermogenic properties. As evidence of piperines' heat-proffering properties, specifically in neural tissue, Reanmongkol et al. (Reanmongkol, Janthasoot, Wattanatorn, Dhumma-

Upakorn, & Chudapongse, 1988) report on the ability of piperine to stimulate activity of ATPase (but inhibition of oxidative phosphorylation) which produces heat as a by-product (Clapham & Arch, 2006). Thermogenic increases in tissue activity have previously been proposed as an explanation for piperine-mediated increases in plasma beta-carotene levels in humans (Badmaev et al., 1999) via an increase in the absorption rate of the intestinal epithelium and, as a mechanism, could exist without piperine evincing an overall increase in resveratrol bioavailability: a phenomenon observed previously (Badmaev et al., 1999; Lambert et al., 2004; Shoba et al., 1998) but not replicated here.

In conclusion, this study reports that piperine co-supplementation enhances the efficacy of 250mg resveratrol with regards CBF effects but that neither resveratrol alone, nor co-supplemented with piperine is able to influence cognitive function. This lack of cognitive enhancement is in line with the previous two chapters. This chapter also raised the potential for resveratrol to increase BP but that this was only seen in the co-supplemented condition may indicate that this was a piperine-mediated effect. The analysis of plasma levels of resveratrol demonstrated no significant differences between treatments which suggests that the above CBF effects are not due to a piperine-induced increase in plasma levels of resveratrol but rather an amplification in the efficacy of resveratrol at target tissue, perhaps via thermogenic effects. The interpretation of the above findings is somewhat hindered by the lack of a piperine-only condition and, whilst there was no prior justification in doing so; due to the lack of evidence of piperines efficacy in the domains of interest here, certainly further research with a piperine-only investigation would be advantageous and likely add clarity.

Chapter 5.

The chronic (28-day) effects of 500mg *trans*-resveratrol on cognitive performance, cerebral blood flow, blood pressure, subjective mood, sleep quality and health and pharmacokinetics in healthy, young humans.

5.1 Introduction

The previous chapter demonstrated that, when co-supplemented with piperine, a hitherto ineffective dose of resveratrol i.e. 250mg (chapter 3), demonstrated significant modulation of cerebral blood flow (CBF) in the prefrontal cortex during cognitively demanding tasks; thus increasing the efficacy of this dose. Co-supplementation also resulted in significantly higher diastolic BP. No significant effect of resveratrol alone was seen on CBF parameters nor on BP or heart rate and cognitive function was unaffected in both treatment conditions. This lack of effect is supported by chapters 2 and 3. One factor which may have had an impact on the potential cognitive enhancing effects of resveratrol is the acute methodology employed thus far. Whilst this paradigm might be sufficient to facilitate resveratrol's interaction with the vasodilatory mediator nitric oxide (NO), resulting in an amplification of the natural CBF response, this might not be to the degree necessary to influence cognitive performance. Chapter 4 aimed to address this by attempting to augment the bioavailability of resveratrol, via co-supplementation with piperine. The hypothesis here being that the increased bioavailability of resveratrol might enhance the previously seen CBF augmentation to a degree which might affect cognitive task performance. However, whilst observing increased efficacy on CBF parameters, piperine demonstrated no enhancement of plasma resveratrol levels, nor cognitive function.

The adoption of single dose methodology across the preceding studies leaves the question of the chronic effects of resveratrol, and the potential for sustained consumption to benefit cognitive function, entirely open. The current study therefore aimed to investigate the cognitive and CBF effects of *chronic* supplementation of resveratrol in healthy, human participants. Here a 500mg dose was utilized as this dose has hitherto proven effective, at least in modulating CBF, when administered alone (chapter 3). Given the lack of any human data a supplementation period of 28 days was chosen as a somewhat arbitrary time-frame due to the exploratory nature of this study. However, it may represent an ecologically valid dosing regimen; with the manufacturer of the resveratrol supplement used here (Transmax™ by Biotivia™)

recommending consumption of 1x 500mg capsule daily, with sufficient capsules for a 1 month supplementation period.

Given the constraints surrounding NIRS measurement of CBF outlined in the discussion of chapter 2, and the inability to avoid incorporating a break in the recording of hemodynamic changes across the supplementation period, this chapter will also utilize a second *quantitative* measure of CBF. Trans-Cranial Doppler (TCD) uses sonographic technology to provide information on cerebral blood flow velocity (CBFV) which, in turn, can be taken as a proxy measure for CBF. This method lends itself to repeated, discrete, measurements of CBF over periods of time and has been converged with NIRS successfully previously (Ide, Horn, & Secher, 1999). Utilized together in the current study, the aim is for NIRS to provide data reflecting acute concentration changes in CBF following consumption of the resveratrol/placebo on day 1 and day 28 and for TCD to add a measure of quantitative changes in CBFV within day 1 and day 28, as well as providing information on the chronic change in CBF that might have taken place across the supplementation period.

A secondary aim of this study was to capitalize on the chronic aspect of this study to expand the range of measures assessed; given the lack of information on the effects of resveratrol in humans. The rationale for this is predicated on the wide-ranging effects that resveratrol is able to exert and the evidence, predominantly from *in vitro* and animal data, that resveratrol may be able to affect mood, sleep and general health.

With regards mood, a number of animal studies suggest that resveratrol may have beneficial anxiolytic effects which may be as a result of modulation of monoamine neurotransmitters. Xu et al. (2010), for example, observed reduced immobility in mice during despair tasks with 10-, 20-, 40- and 80mg/kg *trans*-resveratrol administered by intestinal gavage. These results were similar to those evinced by imipramine and fluoxetine and all treatments increased levels of monoamine neurotransmitters; namely serotonin (5-HT), noradrenaline and dopamine, in the frontal cortex. At doses of 40- and 80mg/kg resveratrol also increased 5-HT levels in the hippocampus and, at 80mg/kg it increased levels of noradrenaline. This modulation was attributed to the ability of resveratrol to inhibit MAO-A (and MAO-B in the case of 80mg/kg), an effect that was seen ~30 minutes after administration.

With regards sleep, resveratrol is able to activate PGC-1α, a key molecule in the integration of the mammalian clock and energy metabolism (Liu, Li, Liu, Borjigin, & Lin, 2007). Oike and Kobori (2008) therefore investigated whether resveratrol was able to regulate circadian clock genes in cultured rat-1 fibroplast cells. Results showed that rat-

1 cells cultured with 100μM resveratrol for 8hrs had significantly increased mRNA expression of representative clock genes Per1, Per2 and Bmal1 (Arntl). Per1 and Bmal1 mRNA were gradually up-regulated up to 4hrs after resveratrol addition and then decreased until 14hrs. Expression of Per2 was up-regulated in the first 8hrs and also decreased until 14hrs.

Taken together, the wealth of research reporting potential positive health effects associated with resveratrol (see Smoliga, Baur, & Hausenblas, 2011 for review) and the small amount of research above which suggests that resveratrol may also be able to influence aspects of mood and sleep, gives cause to investigate these factors here.

Finally, a third aim of the current study was to ascertain whether chronic consumption of resveratrol could lead to an additive increase in bioavailability. As discussed in chapter 3, the bioavailability of resveratrol after acute, bolus administration is regarded as very poor and may underlie the lack of efficacy with regards CBF-induced improvements to cognitive task performance. However, a small amount of research indicates that repeated dosing may lead to cumulative levels of plasma resveratrol. This data comes from three preclinical chemopreventive efficacy papers which report that relatively low daily doses of resveratrol (between 200μg/kg and 2mg/kg) are sufficient to produce peak plasma concentrations of aglycone resveratrol of ~20nM-2μM and in turn exert beneficial chemopreventive effects (results reported in Gescher & Steward, 2003).

The current randomised, double-blind, placebo-controlled, between subjects study therefore investigated the effects of 28 day supplementation with 500mg resveratrol on cognitive performance and CBF (utilizing NIRS and TCD) as well as BP, and subjective mood, sleep quality and health. This study also assessed whether the bioavailability of resveratrol can be augmented with chronic dosing.

5.2 Method

5.2.1 Participants

All participants (see table 5.1. for participant demographics) reported themselves to be in good health and free from social drugs, prescription medication and herbal extracts/food supplements. Participants confirmed that they would abstain from all of the above for the duration of the study and that any changes in medication or health status would be reported to the researcher if/when they occurred. Participants who had suffered a head injury, neurological disorder or neuro-developmental disorder were

excluded from participation, as were those who did not have English as their first language (or were not equivalent to a native English speaker- due to the complex task instructions) had any relevant food allergies or intolerances, digestive problems, smoked tobacco, drank excessive amounts of caffeine (>six cups per day as assessed by a caffeine consumption questionnaire), took illicit social drugs, were pregnant, seeking to become so, or breast feeding.

A sub-sample of seven of these participants also provided blood samples during their testing days (see testing session timeline figure 5.1 for details). This group comprised six females and one male with a mean age of 19.43yrs (range 18-21). Data from a further participant was excluded from analysis due to failure to adhere to the study protocol.

Table 5.1. Participant demographics for all measures.

Measure (Number of participants in analysis/recruited)	F/M	Mean age (Age range)	R/L	P/R
Overall recruited (N=60)	51/9	20.52 (18-29)	53/7	30/30
Cognitive performance (N=41)	36/5	20.00 (18-27)	33/6	19/22
NIRS (N=46)	39/7	20.45 (18-29)	39/7	24/22
TCD (N=46)	40/6	20.08 (18-29)	40/6	21/25
BP (N=24)[19]	21/3	20.75 (18-29)	21/3	15/9
GHQ (N=53)	45/8	20.17 (18-29)	46/7	28/25
POMS (N=54)	46/8	20.07 (18-29)	47/7	28/26
PSQI (N=53)	45/8	20.15 (18-29)	47/6	28/25
Food consumption (N=55)	47/8	20.15 (18-29)	48/7	29/26
Treatment guess (N=57)	49/8	20.25 (18-29)	50/7	28/29

Footnote. Table displays number of participants included in the analysis for each measure. Reasons for all 60 participants (apart from BP where 30 participants were intended to provide data failing to provide data for analysis included technical issues with equipment, non-compliance with the study protocol and/or data which lay outside of 'normal' performance ranges.

[19] Only a sub-section (N=30) of participants took part in the BP measure.

5.2.2 Treatments

Over the course of this 28 day supplementation study, participants received either 500mg pure *trans*-resveratrol (Transmax™ by Biotivia™ with guaranteed purity 98%. This product also contains 10mg piperine per capsule), or an inert placebo (methyl cellulose), once daily; with the treatment allocation dictated by Latin square. Participants were instructed to consume their daily capsule in the morning and preferably with breakfast.

Participants consumed their first and last capsule of treatment during the two lab visits and were instructed to self-supplement every day in the interim. (Participants kept a treatment log during this time; noting down the time of capsule consumption every day.) A treatment pot containing 32 capsules was given to each participant at the end of visit 1- enough for 28 days of supplementation plus extra in case of loss/continued supplementation due to unforeseen circumstances/and to verify compliance.

All treatments were administered in identical green vegetarian capsules with the Biotivia™ logo and were presented in identical white treatment pots with only the participant number to identify them. All treatments were produced by Biotivia™, prepared by the lead investigator and 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 a blind-data review was completed.

5.2.3 Cognitive tasks and behavioural questionnaires

The cognitive tasks utilized in this study comprise: 2 minutes each of serial 7s, 13s and 17s subtractions (see section 2.2.3 for description of the subtraction task procedure) and a 2 minute version of the RVIP task (also described in more detail in section 2.2.3) and the 3-Back version of the N-Back task (see section 4.2.3 for description).

Food consumption questionnaire

A food consumption questionnaire (see appendix II) was utilized to collect information on the general diet of participants (e.g. 'How many portions of fruit and vegetables did you eat on an average day in the past week?') and specifically polyphenol/resveratrol consumption (e.g. 'In the entire previous week, on how many occasions have you eaten a portion of berries or grapes?'). The questionnaire consisted of 13 questions with several also relating to compliance (e.g. 'Was treatment consumed with breakfast and/or before 9:30am every day in the past week?') and medication ('Have you

consumed any medication in the past week? If so, please state the medication, dose, when taken and for what reason.').

General Health Questionnaire (GHQ)

The GHQ (Goldberg, 1978) utilized in the current study was the 28 item scaled version which assesses somatic symptoms, anxiety and insomnia, social dysfunction and severe depression. The 28 items are scored from 0-3 with participants indicating the frequency or extent to which they have experienced a number of issues, such as 'Have you recently been having hot or cold spells?', in the previous week. The items combine to assess the four aforementioned sub-scales and the total possible score (when these four sub-scales are collated) ranges from 0-84.

Profile Of Mood States (POMS)

The POMS is a well validated questionnaire of mood states, and their fluctuations, both in the clinical and research setting (McNair, Lorr, & Droppleman, 1971). Participants rated 65 adjectives (e.g. unhappy, considerate), in terms of how much they had felt each one in the past week, utilizing a 5 point scale from 'not at all' to 'extremely'. Scores from these 65 items (which includes seven dummy adjectives) are combined to give six global scores of 'tension', 'depression', 'anger', 'fatigue', 'confusion' and 'vigour'. A total mood disturbance score can also be calculated by adding the scores from the first five of these global scores and subtracting 'vigour'.

Pittsburgh Sleep Quality Inventory (PSQI)

The PSQI is a well validated subjective measure of the quality and pattern of sleep (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). The current study tailored this questionnaire to assess sleep during the past 'week' rather than 'monthly' as per the original. The PSQI assesses seven factors: subjective sleep quality; sleep latency; sleep duration; habitual sleep efficiency; sleep disturbances; use of sleep medication and daytime dysfunction, via a 0-3 point scale where participants rate whether they have experienced a number of issues (e.g. 'During the past week, how often have you had trouble sleeping because you have had bad dreams?') from 'not during the past week' to 'three or more times in the past week'. A global sleep score is created by totalling the seven sub-factor scores.

5.2.4 Near-Infrared Spectroscopy (NIRS)

Again, CBF was monitored in the prefrontal cortex with NIRS (described as a technique in more detail in section 2.2.4).

5.2.5 Trans-Cranial Doppler (TCD)

Trans-cranial Doppler (TCD) sonography is a non-invasive method of measuring cerebral blood flow velocity (CBFV) from the basal intracerebral vessels through the intact skull (Markus, 2000). Pulses of ultrasound penetrate the skull at a number of 'acoustic windows', which include: temporal, orbital, foraminal and submandibular, insonating vessels at particular depths, with the returning 'echo' displayed as a Doppler waveform (Nicoletto & Burkman, 2009). The mean velocity, peak systolic velocity, diastolic velocity, and pulsatility index (all cm/sec) of the insonated vessel are provided; indicating the speed of the flow of blood and the variability of blood velocity.

TCD has been utilized to investigate blood flow abnormalities in a number of haematological; e.g. stroke risk in sickle cell patients (Adams et al., 1997), and vascular; e.g. cerebrovascular reactivity in degenerative and vascular dementia (Vicenzini et al., 2007), disorders. It has also been used as a tool to investigate the relationship between brain activity in response to cognitive tasks and blood flow velocity in healthy participants; demonstrating increased CBFV as a result of increased task complexity (Harders, Laborde, Droste, & Rastogi, 1989).

TCD has proven sensitivity to the CBFV effects of a number of nutritional interventions, e.g. caffeine; revealing that withdrawal can significantly increase mean-, systolic-, and diastolic velocity (Jones, Herning, Cadet, & Griffiths, 2000) and drugs; showing that cocaine abusers display significantly lower mean-, systolic-, and diastolic velocity than controls, and that blood flow velocity can be increased after a month of cocaine abstinence (Herning, King, Better, & Cadet, 1999). TCD has yet to be applied to the investigation of the CBFV effects of resveratrol but relatedly, it has been applied to the CBFV effects of cocoa flavanols. Sorond et al. (2008) investigated the CBF response to a high (900mg) and low (36mg) cocoa flavanol drink in a cohort of healthy older (mean age 72 ± 6yrs) adults. Mean blood flow velocity (MBFV) was significantly increased after 1- and 2 weeks daily supplementation of the higher dose in a sub-sample of 13 participants and, in a sub-sample of 21 participants, a greater number experienced increased MBFV in the high flavanol condition, as compared to the low flavanol group, after 1 weeks supplementation. The similarities between cocoa flavanols and resveratrol relating to vasodilation and CBF have been outlined previously (see

sections 1.3, 1.6.4.3, 1.7.2.1). The CBF effects of resveratrol reported already in this thesis lends support to the hypothesis made here that resveratrol would share the same CBF outcomes of these mechanisms as cocoa polyphenols. As such, the hypothesis made here is that resveratrol should also evince increased CBFV; as measured by TCD.

In terms of how TCD was utilized in this study, CBFV was measured with participants sitting in a reclined position in a quiet room. The trans-temporal acoustic window was utilized for assessment of the right middle cerebral artery (MCA) using pulsed TCD (Digi-Lite^{TM}, RIMED) with a 2MHz probe held in place by a light, mounted head frame. This machine provides mean velocity, peak systolic velocity, diastolic velocity, and pulsatility index information every 30 seconds. For each of the four aforementioned variables, these values are averaged to give just one value for each five minute recording time point (i.e. pre-dose and post-dose) for statistical analysis.

The rationale for utilizing TCD here is to compliment the concentration change data of haemoglobin in the prefrontal cortex, provided by NIRS, with quantitative hemodynamic information from the MCA. The hemodynamic response in the MCA is strongly correlated with the CBF response in the frontal cortex; evidenced by Rollnik et al (2002) who report decreased MCA flow velocity in response to repeated trans-cranial magnetic stimulation (rTMS) in the right dorsolateral prefrontal cortex of 38 healthy human volunteers. Conversely, an increase in MCA mean blood velocity is observed to correlate with a concomitant increase in oxy-Hb in the frontal cortex (as assessed by NIRS) in 12 healthy cyclists during sub-maximal exercise (Ide et al., 1999). An issue with CW NIRS, previously mentioned in section 2.4, is that the data it provides is a concentration change in haemoglobin levels based on the assumptions of the modified Beer-Lambert law rather than an absolute quantity. As such, by converging operations with TCD, it is hoped that the hemodynamic effects of resveratrol can be understood more clearly via the quantitative data it provides. In terms of expectations, based on the Sorond et al. (2008) and Ide et al. (1999) findings above, it would be expected that any resveratrol-induced increases in CBF in the prefrontal cortex would be mirrored by increased CBFV in the MCA.

5.2.6 Blood pressure (BP)

Blood pressure was monitored in a sub-sample of participants at discrete time-points during the testing session (see procedure) with the rationale described in more detail in the introduction of chapter 4.

5.2.7 Procedure

This investigation required participants to attend the laboratory for an initial training/ screening session and then on two separate occasions, 28 days apart, for laboratory-based testing sessions. Participants were required to consume one capsule of their allotted treatment per day in the interim.

The procedure on Day 1 and day 28 was the same: Upon arrival participants completed four questionnaires: a food consumption questionnaire; the GHQ; POMS; and the PQSI. All questionnaires were answered in relation to the previous seven days and completed every seven days during the supplementation period. After filling in the questionnaires, participants then gave a blood pressure reading or an intravenous blood sample (in only a sub-sample of seven participants- see above section on demographics for more information on this group) which was immediately followed by a 5 minute rest. A 5 minute recording of cerebral perfusion in the middle cerebral artery was then taken with TCD. The NIRS headband was then positioned onto the forehead of the participant to monitor CBF in the prefrontal cortex throughout the session. Once a reliable trace was identified participants commenced 20 minutes of baseline cognitive tasks. A 10 minute rest (which acted as the NIRS statistical baseline for CFB calculations) then followed during which participants watched a non-arousing DVD. After 10 minutes participants consumed the first capsule of their 28 day provision and continued to watch the DVD for a further 40 minute absorption period. After this period a blood pressure reading was taken in those who did not provide a blood sample previously and 36 minutes of post-dose tasks commenced. After task completion a further blood pressure reading was taken from the relevant participants and was followed by a short break before the second TCD recording was conducted. Following the TCD recording participants were either free to leave the lab or provided a final blood sample if they were part of the aforementioned sub-section of participants. At the end of the final visit participants were asked to guess which treatment they believed they had been taking across the 28 days. The acute testing session procedure and chronic timeline of the study are shown in figure 5.1.

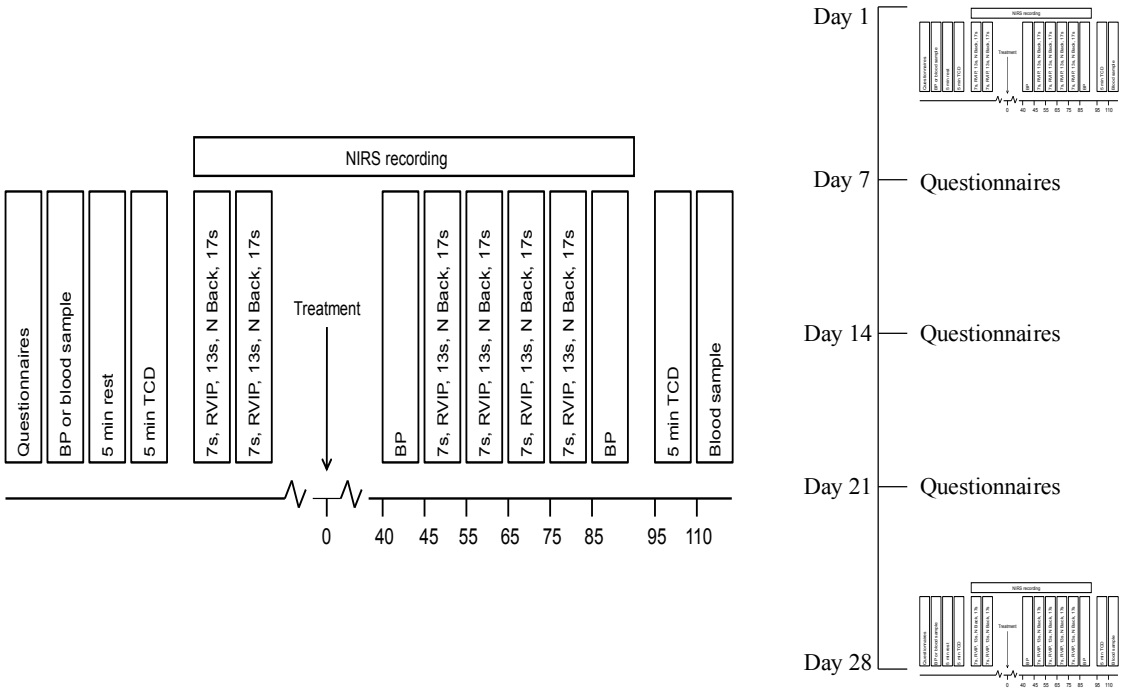


Figure 5.1. Chapter 5 study testing session timeline. Figure displays acute (right) testing session procedure and chronic (left) timeline of the overall study.

5.2.8 Treatment and analysis of plasma

The treatment and analysis of plasma data was as per chapter 4 (section 4.3.4).

5.2.9 Statistics

The analyses of TCD, plasma, questionnaire, behavioural and treatment guess data were conducted with IBM SPSS Statistics 19.0 for Windows (SPSS Inc, Chicago, IL). NIRS data was analysed with Minitab 16 for Windows (Minitab Inc, State College, PA).

Questionnaire data analysis:

Questionnaire data (GHQ, POMS and PSQI) for each of the four post-dose weekly completions was analysed as change from baseline (the questionnaire scores obtained on day-1 prior to treatment) for each individual variable/sub-component by a mixed (Day: 7 (week 1), 14 (week 2), 21 (week 3), 28 (week 4) by Treatment: 500mg resveratrol and placebo) ANOVA with Bonferroni corrected post-hoc students t tests conducted if a significant main and/or interaction effect was evinced here.

Treatment guess analysis:

Treatment guess data was analysed by Chi-Square.

Trans-cranial Doppler (TCD):

The raw data for each of the four TCD variables (Mean Velocity, Peak Systolic Velocity, Diastolic Velocity and Pulsatility Index) were analysed by a mixed (Treatment (x2): 500mg resveratrol and placebo, by time (x4): baseline day 1, post-dose day 1, pre-dose day 28 and post-dose day 28) ANOVA.

Plasma analysis:

The raw data for each of the four forms of plasma resveratrol (resveratrol-3-sulfate, resveratrol-4-glucuronide, resveratrol-3-glucuronide and 'total metabolites'; which is the sum of the three metabolites) was analysed via ANOVA with time as a factor (x4= baseline day 1, post-dose day 1, pre-dose day 28 and post-dose day 28).

Cognitive task data and Blood Pressure (BP) analysis:

The cognitive task and BP measures produce data that can be analysed to assess both acute (potential treatment effects within day 1), pure-chronic (chronic treatment-related effects which have taken place across the 28 day supplementation period but prior to taking the day 28 treatment) and superimposed acute/chronic (the difference in 'acute' effects between day 1 and day 28) effects of resveratrol. In order to adequately analyse the 'acute', 'pure chronic' and 'superimposed acute/chronic' effects of the treatments 2 ANOVAs were conducted:

1. Pure chronic effects:

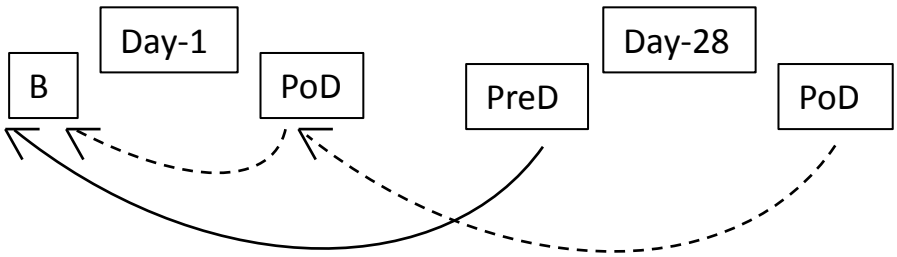
To ascertain if any pure chronic effects of resveratrol supplementation had taken place, pre-dose data on day 28 was converted to change from day 1 pre-dose baseline and analysed via ANOVA to compare performance between treatments.

2. Acute, chronic and superimposed effects:

To ascertain if any acute and/or superimposed chronic effects of resveratrol supplementation had taken place, data was converted to change from day 1 baseline and analysed via a repeated measures ANOVA (treatment: 500mg resveratrol and placebo x repetition: 1, 2, 3, 4 for cognitive data and 1, 2 for BP x day: day 1 and day 28).

Utilizing both of these ANOVAs will tease apart acute effects restricted to day 1 (treatment x day interactions with significant effects restricted to day 1), acute effects across both day 1 and day 28 (evidenced by a main effect of treatment and/or a treatment x repetition interaction) and a superimposed acute/chronic effect (treatment x day interaction with significant effects restricted to day 28 [interpreted with reference to the pure chronic ANOVA results]). If any such main and/or interaction effects are

observed then Bonferroni corrected post-hoc students t tests will be conducted to assess where these differences lie. This analysis plan has proven sensitivity in detecting the acute and chronic effects of ginseng in healthy, human participants previously (Reay, Scholey, & Kennedy, 2010). See figure 5.2 for diagram of statistical analysis.



1. Analysis (pure chronic) filled line:
PreD on day-28 minus B on day-1 followed by ANOVA (treatment x repetition) to ascertain any pre-treatment (pure chronic) effect on day-28.

2. Analysis (acute, chronic and superimposed) dashed lines:
PoD on day-1 and day-28 minus day-1 B followed by treatment x repetition x day ANOVA.
Acute effects: Main effect of treatment and/or treatment x repetition interaction restricted to day-1 only.
Chronic effects: Main effect of treatment and/or treatment x repetition interaction restricted to day-28 only.
Pure chronic effect: If a significant chronic effect and analysis 1. evinces significant pure chronic effect then= superimposed effect of treatment.

Figure 5.2. Diagram outlining statistical analysis for cognitive task and blood pressure data.

Near-Infrared Spectroscopy analysis:

NIRS data was converted to 'change from baseline' (calculated from the 10 minute pre-treatment resting period) and averaged across 2 minute epochs during the 40 minute 'rest/absorption' and 36 minute cognitive task performance period. Analysis of variance (ANOVA) (treatment group x 2min epoch x day) was conducted on this data with planned comparisons of data from each epoch being made between placebo and 500mg resveratrol using t tests calculated with the Mean Squares Error from the ANOVA (Keppel, 1991). (See section 2.2.6 for justification of this analysis plan.)

5.3 Results

5.3.1 Compliance and treatment guess

Average compliance was 101.4% with a range of 92.9%-114.3%.

Chi-Square revealed no significant difference between treatment guesses in the 2 treatment groups: χ^2= .766; df= 1; p= .381. (See table 5.2. for treatment guess table.)

Table 5.2. Treatment guess table. Table displays the number of guesses by participants in the 500mg *trans*-resveratrol and placebo condition regarding which treatment they believed they had been receiving across the 28 day supplementation period.

		Group		Total
		500mg Resveratrol	Placebo	
Guess	500mg Resveratrol	5	8	13
	Placebo	23	21	44

5.3.2 Near-Infrared Spectroscopy (NIRS) parameters

Total haemoglobin (total-Hb):

The primary omnibus ANOVA demonstrated no significant main or interaction effects for levels of total-Hb apart from a main effect of epoch [F(39, 1716)= 8.36; p <.001]. Planned comparisons revealed that, on day 1, levels were significantly higher after resveratrol, compared to placebo, at epochs 35 (<.01) and 37 (<.05) of the absorption period and epochs 75 (<.05) and 77 (<.01)[20] of the post-dose task period. No significant differences were found between resveratrol and placebo on day 28 and there was no treatment x day interaction.

Oxygenated haemoglobin (oxy-Hb):

The primary omnibus ANOVA demonstrated no significant main or interaction effects for levels of oxy-Hb apart from a main effect of epoch [F(39, 1716)= 10.41; p <.001]. Planned comparisons revealed that, on day 1 levels were significantly higher in the resveratrol condition, compared to placebo, at epochs 23 (<.01), 27 (<.05), 33 (<.01), 37 (<.01) and 39 (<.05) of the absorption period and epochs 41 (<.05), 43 (<.01), 53 (<.01), 61-67 (all <.01 except epoch 65 <.05), 71 (<.01), 75 (<.01) and 77 (<.01)[21] of

[20] Epoch 35 t(39)= 3.03. Epoch 37 t(39)= 2.67. Epoch 75 t(39)= 2.67. Epoch 77 t(39)= 2.84

[21] All <.05 t's (39) ≤2.17 and <.01 t's (39) ≤2.61

the post-dose task period. No significant differences were found between resveratrol and placebo on day 28 and there was no treatment x day interaction.

Deoxygenated haemoglobin (deoxy-Hb):

The primary omnibus ANOVA demonstrated a trend for a main effect of day [F(1, 1716)= 3.48; p= .07] and a significant main effect of epoch [F(3, 1716)= 6.45; p <.001] and interaction between epoch x day [F(39, 1716)= 4.03; p <.001]. Planned comparisons revealed that, on day 1, levels were significantly higher in the placebo condition, compared to resveratrol, at epochs 27 (<.01), 29 (<.05) and 35 (<.01) of the absorption period and epochs 43 (<.01), 51 (<.01), 53 (<.01), 61-71 (all <.01) and 75-79 (all <.01)[22] of the post-dose task period. No significant differences were found between resveratrol and placebo on day 28 and there was no treatment x day interaction.

Mean levels of total-, oxy- and deoxy-Hb for placebo and resveratrol, across day 1 and day 28, shown in figures 5.3, 5.4 and 5.5.

[22] <.05 t(39)= 2.73 and all <.01 t's(39) ≤2.85

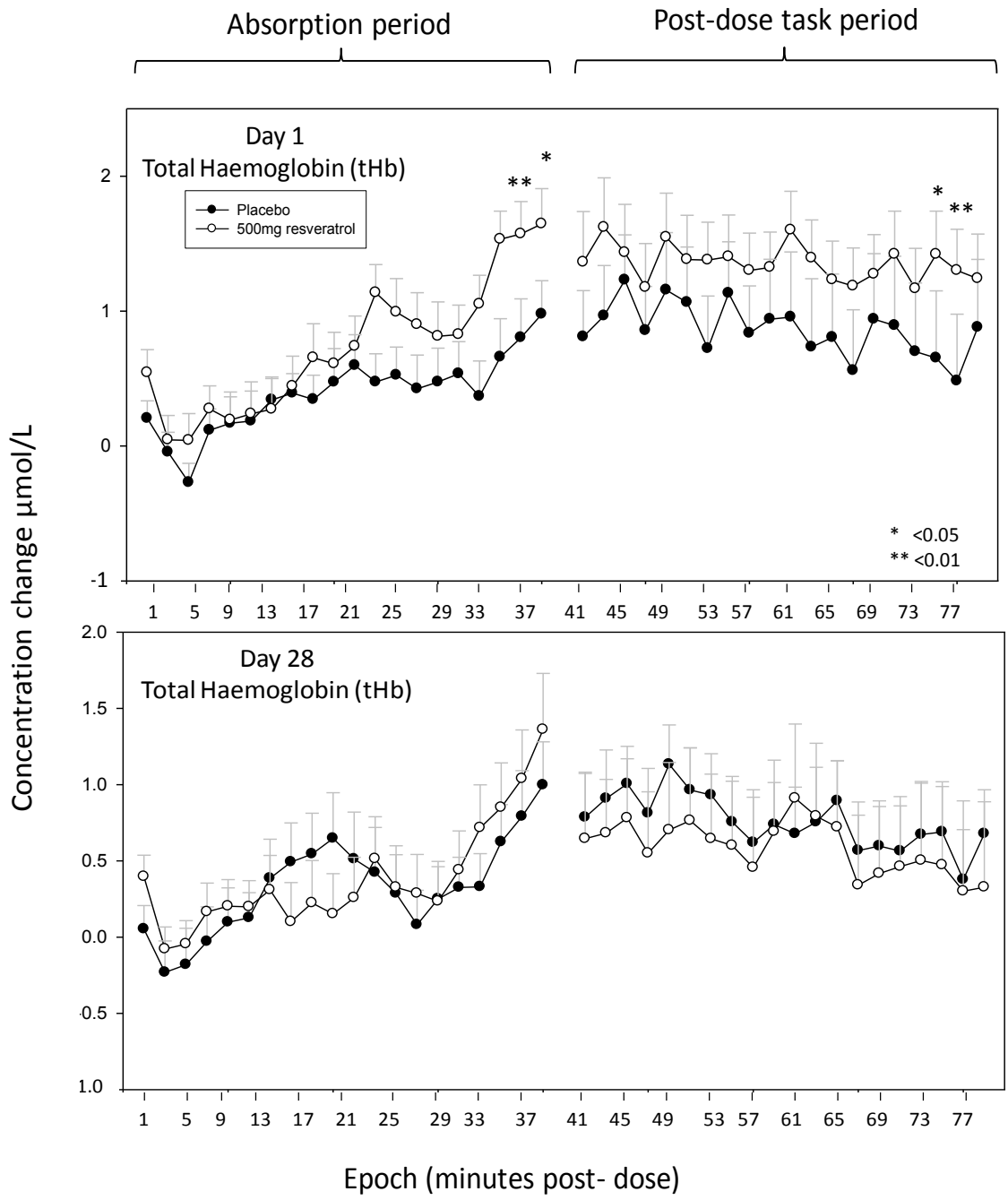


Figure 5.3. The effects of 500mg *trans*-resveratrol, compared to placebo, on total haemoglobin levels on day 1 and day 28 of the supplementation period. Graph displays concentration change (µmol/L) levels of oxygenated haemoglobin (with SEM error bars) on day 1 and day 28 during a 40 minute absorption and 38 minute post-dose task period in 46 healthy adults after taking placebo and 500mg *trans*-resveratrol. *<.05 and **<.01.

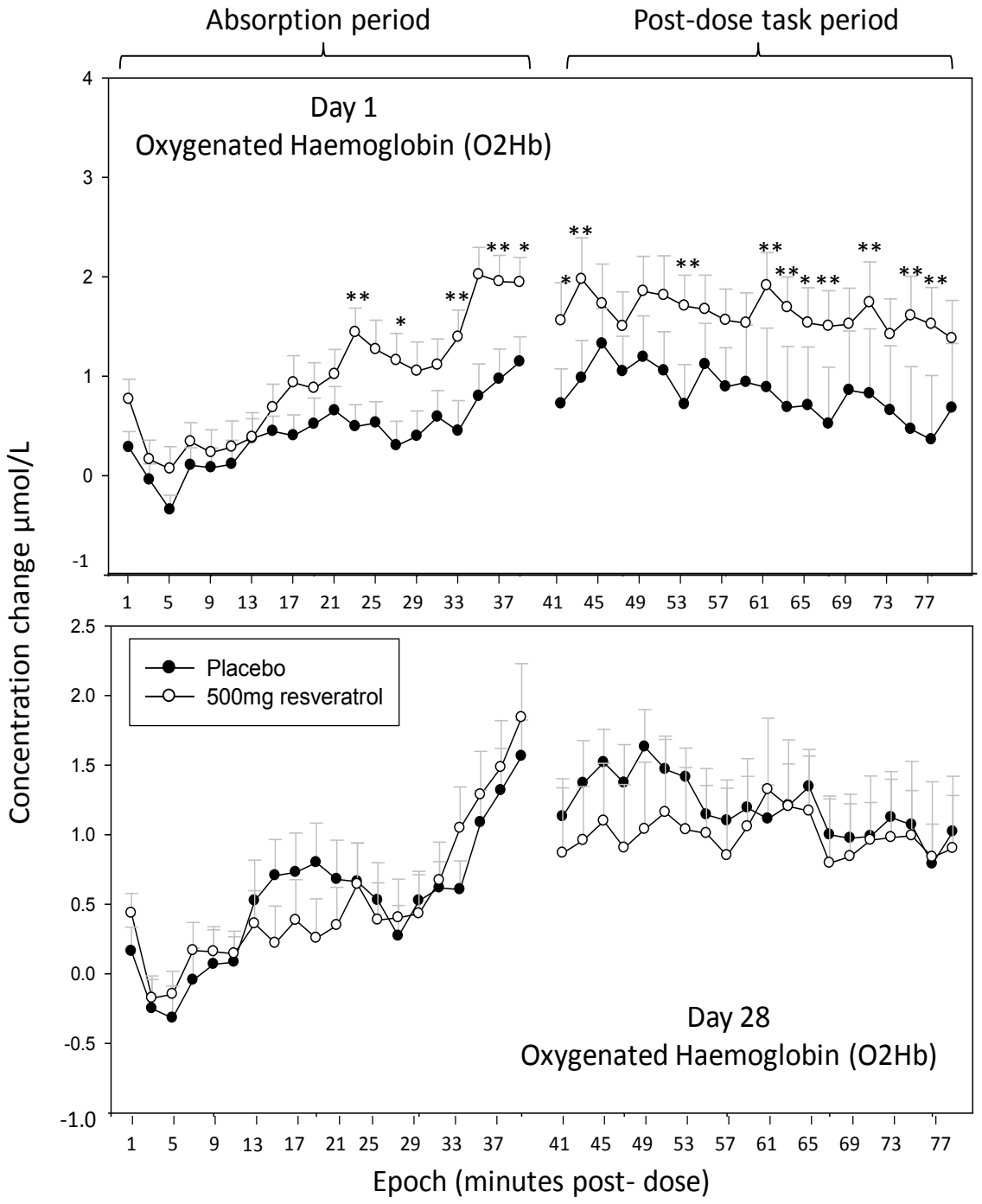


Figure 5.4. The effects of 500mg *trans*-resveratrol, compared to placebo, on oxygenated haemoglobin acutely, and after a 28 day supplementation period. Graph displays concentration change (µmol/L) levels of oxygenated haemoglobin (with SEM error bars) on day 1 and day 28 during a 40 minute absorption and 38 minute post-dose task period in 46 healthy adults after placebo and 500mg *trans*-resveratrol. *<.05 and **<.01.

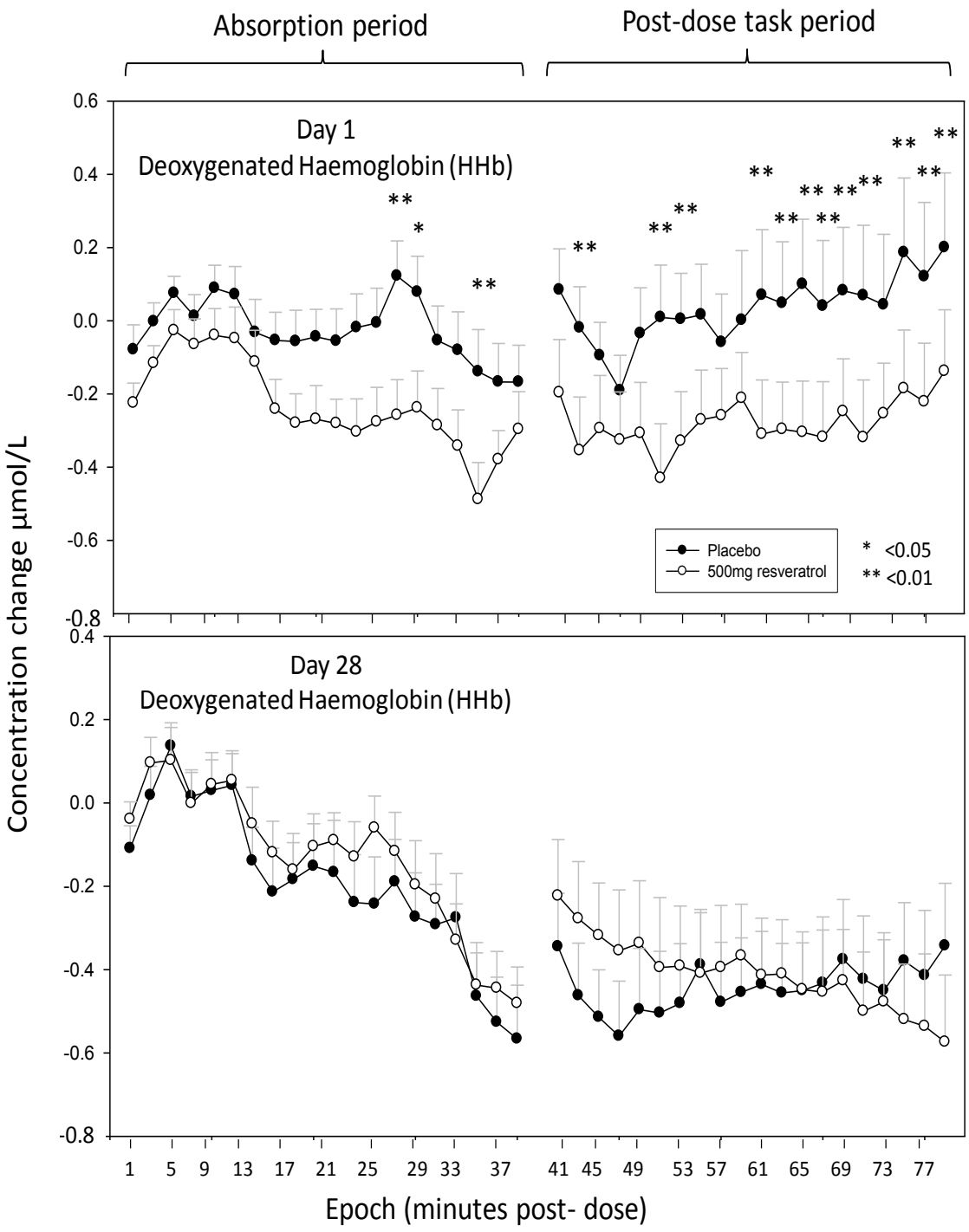


Figure 5.5. The effects of 500mg *trans*-resveratrol, compared to placebo, on deoxygenated haemoglobin acutely, and after a 28 day supplementation period. Graph displays concentration change (µmol/L) levels of oxygenated haemoglobin (with SEM error bars) on day 1 and day 28 during a 40 minute absorption and 38 minute post-dose task period in 46 healthy adults after placebo and 500mg *trans*-resveratrol. *<.05 and **<.01.

5.3.3 Trans-Cranial Doppler (TCD) parameters

No significant main effects of time or treatment were observed, nor an interaction between the two, on any of the four TCD parameters (Mean velocity; Peak systolic velocity; Diastolic velocity; and Pulsatility index). (See table 5.3. for TCD data table and ANOVA F and P values.)

Table 5.3. The effects of 500mg *trans*-resveratrol, compared to placebo, on cerebral blood volume in the middle cerebral artery acutely, and after a 28 day supplementation period. Table displays raw mean values (cm/sec), and SEM in brackets, for the baseline and post-dose recordings on day 1 and day 28 for all four TCD variables: Mean Velocity; Peak Systolic Velocity; Diastolic Velocity and Pulsatility Index after 500mg *trans*-resveratrol and placebo. Table also displays ANOVA F and P values for main effects of time (Ti), treatment (Tr) and an interaction between the two (Ti*Tr).

Measure	Treatment condition	Day 1		Day 28		ANOVA		
		Baseline	95mins PD	Baseline	95mins PD	Effect	F	P
Mean Velocity (cm/sec)	500mg resveratrol	33.42 *(3.27)*	32.35 *(4.17)*	36.54 *(2.55)*	35.38 *(4.04)*	Ti	.361	.782
	Placebo	37.73 *(3.39)*	36.87 *(4.68)*	38.63 *(3.84)*	37.21 *(3.75)*	Tr	.808	.377
						Ti*Tr	.117	.950
Peak Systolic Velocity (cm/sec)	500mg resveratrol	56.22 *(5.22)*	52.71 *(7.04)*	61.42 *(4.15)*	58.96 *(6.82)*	Ti	.476	.700
	Placebo	63.43 *(4.77)*	62.34 *(7.35)*	65.26 *(5.97)*	61.59 *(6.00)*	Tr	1.03	.321
						Ti*Tr	.211	.888
Diastolic Velocity (cm/sec)	500mg resveratrol	23.00 *(2.47)*	22.00 *(3.08)*	25.12 *(2.08)*	24.05 *(3.54)*	Ti	.443	.723
	Placebo	26.23 *(2.85)*	24.59 *(3.96)*	26.83 *(3.31)*	25.02 *(3.32)*	Tr	.548	.466
						Ti*Tr	.091	.965
Pulsatility Index (cm/sec)	500mg resveratrol	1.05 *(0.10)*	.927 *(0.11)*	.964 *(0.11)*	1.01 *(0.14)*	Ti	.557	.645
	Placebo	1.08 *(0.09)*	1.07 *(0.08)*	1.04 *(0.08)*	.951 *(0.13)*	Tr	.229	.637
						Ti*Tr	.662	.578

5.3.4 Cognitive task performance

1. Pure chronic ANOVA

The results of the ANOVA on day 28 pre-dose data (converted to change from day 1 baseline) comparing performance between 500mg resveratrol and placebo, demonstrated a significant treatment effect for 'NBack % correct' only [F(1,40)= 8.60; p= .006] with a higher mean number of correct NBack answers, overall, in the resveratrol condition as compared to placebo.

2. Acute, chronic and superimposed ANOVA

The results of the treatment x repetition x day ANOVA for each task variable are as follows:

7s correct: Main effect of day [F(1,39)= 12.95; p=.001] and a main effect of repetition [F(3, 117)= 7.83; p= <.001].

7s incorrect: Main effect of treatment [F(1, 39)= 6.40; p= .016] (with the mean for number of serial 7s incorrect responses for placebo, overall, higher than the mean for 500mg resveratrol) and of repetition [F(3, 117)= 5.23; p= .001] and an interaction between day x repetition [F(3, 117)= .854; p= .007] and between day x repetition x treatment [F(3, 117)= .260; p= .034]. Further investigation into this latter interaction with Bonferroni corrected students post-hoc t tests revealed a significant difference on day 1 at repetition 4 (<.01)[23] and trends for differences on day 1 at repetition 2 (p= .073) and on day-28 at repetition 3 (p= .070). The mean number of incorrect responses was lower in the 500mg resveratrol in all 3 cases. (See figure 5.6 for graph.)

13s correct: The only significant finding was a main effect of day [F(1, 39)= 16.39; p= <.001].

13s incorrect: The only significant finding was a main effect of repetition [F(3, 117)= 2.79; p= .044].

17s correct: The ANOVA showed there was an interaction between day x repetition [F(3, 117)= 5.25; p= .002] and between day x treatment x repetition [F(3, 117)= 3.45; p= .019]. Further investigation with students post-hoc t tests revealed significant differences on day 28 at repetition 1 (<.05) and repetition 3 (<.05)[24] with the mean number of serial 17s correct completions higher in the placebo condition in both cases. (See figure 5.7 for graph.)

17s incorrect: The ANOVA showed a main effect of treatment [F(1, 39)= 5.79; p= .021] (with the mean number of 17s subtraction incorrect responses, overall, higher in the placebo condition as compared to 500mg resveratrol) and of repetition [F(3, 117)= 6.25; p= .001] as well as an interaction between repetition x treatment [F(3, 117)= 3.55;

[23] t(18)= 3.17

[24] t's (18)= 2.20 and 2.25 respectively.

p= .017] and a trend for an interaction between day x repetition [F(3, 117)= 2.33; p= .078]. With regards the repetition x treatment interaction, post-hoc students t tests revealed only one significant comparison between treatments at the 4th repetition on day 28. Here the mean number of incorrect responses was higher (<.005)[25] in the placebo condition. (See figure 5.8 for graph.)

RVIP % correct: The only significant finding was a main effect of repetition [F(3, 117)= 8.66; p= <.001].

RVIP RT: The only significant finding was a main effect of repetition [F(3, 117)= 3.07; p= .039].

NBack % correct: No significant main or interaction effects.

NBack RT: The only significant finding was a main effect of repetition [F(3, 117)= 3.47; p= .018].

(See table 5.4 for cognitive task data table and 5.5 for ANOVA F and P values.)

[25] t(18)= 3.47

Table 5.4. The effects of 500mg *trans*-resveratrol, compared to placebo, on cognitive performance acutely, and after a 28 day supplementation period.
Table displays raw baseline scores and change from baseline values (with SEM in italics, in brackets, underneath) for all four post-dose battery repetitions for day 1 and 28 after 500mg *trans*-resveratrol and placebo.

Measure	Treatment condition	Day 1					Day 28				
		Baseline	1	2	3	4	Baseline	1	2	3	4
7s Correct (Number)	500mg resveratrol	23.50 *(1.52)*	3.36 *(0.97)*	1.23 *(0.95)*	0.68 *(1.06)*	-0.27 *(1.12)*	26.66 *(1.68)*	2.25 *(0.82)*	0.48 *(0.97)*	-0.75 *(1.13)*	-0.07 *(0.96)*
	Placebo	23.53 *(1.69)*	2.68 *(1.04)*	-0.16 *(1.02)*	0.79 *(1.14)*	1.84 *(1.20)*	25.92 *(1.74)*	2.08 *(0.89)*	0.34 *(1.05)*	-0.45 *(1.22)*	0.03 *(1.04)*
7s Incorrect (Number)	500mg resveratrol	2.11 *(0.37)*	-0.52 *(0.38)*	-0.11 *(0.32)*	0.93 *(0.35)*	-0.11 *(0.39)*	1.25 *(0.30)*	0.02 *(0.37)*	0.16 *(0.44)*	0.39 *(0.46)*	0.61 *(0.39)*
	Placebo	1.89 *(0.27)*	0.11 *(0.41)*	0.74 *(0.34)*	1.00 *(0.37)*	1.37 *(0.42)*	2.53 *(0.38)*	0.56 *(0.40)*	0.84 *(0.47)*	1.79 *(0.50)*	1.00 *(0.42)*
13s Correct (Number)	500mg resveratrol	18.98 *(1.18)*	0.71 *(0.91)*	0.57 *(0.82)*	-0.43 *(1.09)*	0.02 *(0.87)*	20.48 *(1.27)*	0.84 *(0.66)*	-0.02 *(1.10)*	0.98 *(0.85)*	0.80 *(0.94)*
	Placebo	18.45 *(1.31)*	2.45 *(0.98)*	0.61 *(0.88)*	-0.45 *(1.17)*	1.71 *(0.94)*	21.05 *(1.32)*	2.16 *(0.71)*	0.58 *(1.19)*	0.26 *(0.92)*	-0.26 *(1.02)*
13s Incorrect (Number)	500mg resveratrol	1.84 *(0.32)*	-0.07 *(0.36)*	0.66 *(0.55)*	0.66 *(0.63)*	0.48 *(0.57)*	1.73 *(0.26)*	0.16 *(0.40)*	0.71 *(0.58)*	0.61 *(0.51)*	0.57 *(0.59)*
	Placebo	2.13 *(0.33)*	0.27 *(0.39)*	0.87 *(0.59)*	1.40 *(0.67)*	1.13 *(0.62)*	2.11 *(0.38)*	-0.08 *(0.43)*	1.03 *(0.62)*	0.50 *(0.54)*	1.40 *(0.64)*
17s Correct (Number)	500mg resveratrol	14.41 *(1.02)*	1.46 *(0.60)*	2.05 *(0.69)*	0.96 *(0.70)*	2.36 *(0.70)*	14.91 *(0.96)*	1.71 *(0.69)*	1.39 *(0.85)*	2.07 *(0.69)*	0.80 *(0.72)*
	Placebo	14.87 *(1.29)*	0.76 *(0.64)*	2.29 *(0.75)*	0.76 *(0.75)*	3.18 *(0.75)*	16.74 *(1.17)*	2.97 *(0.74)*	2.34 *(0.92)*	2.82 *(0.75)*	2.66 *(0.78)*
17s Incorrect (Number)	500mg resveratrol	2.52 *(0.45)*	-0.57 *(0.35)*	-0.52 *(0.49)*	-0.02 *(0.46)*	-0.43 *(0.50)*	2.55 *(0.51)*	-0.50 *(0.39)*	0.50 *(0.43)*	0.18 *(0.30)*	1.32 *(0.54)*
	Placebo	2.24 *(0.42)*	0.08 *(0.38)*	0.40 *(0.53)*	1.24 *(0.50)*	0.87 *(0.54)*	2.24 *(0.29)*	-1.13 *(0.42)*	-0.18 *(0.42)*	-0.24 *(0.33)*	0.03 *(0.58)*

RVIP Correct (%)	500mg resveratrol	68.47 *(3.86)*	-0.57 *(3.09)*	-8.24 *(3.09)*	-2.84 *(3.27)*	-11.65 *(3.06)*	66.19 *(3.87)*	3.13 *(2.71)*	-0.28 *(3.47)*	-4.83 *(3.59)*	-6.95 *(3.71)*
	Placebo	72.04 *(4.70)*	-3.95 *(3.33)*	-3.62 *(3.67)*	-9.54 *(3.52)*	-11.18 *(3.29)*	66.48 *(3.72)*	0.63 *(2.91)*	-1.31 *(3.73)*	-0.36 *(3.87)*	-4.64 *(3.99)*
RVIP Reaction Time (ms)	500mg resveratrol	478.65 *(8.33)*	-5.50 *(7.79)*	-20.81 *(14.87)*	-6.89 *(11.83)*	-11.82 *(6.02)*	489.05 *(9.12)*	-11.82 *(6.02)*	8.03 *(10.71)*	19.65 *(9.51)*	-0.35 *(8.03)*
	Placebo	470.09 *(9.76)*	0.01 *(8.38)*	-6.09 *(16.00)*	25.96 *(12.73)*	23.55 *(15.25)*	475.93 *(9.22)*	-10.80 *(6.48)*	-2.19 *(11.52)*	-0.38 *(10.23)*	-6.15 *(8.64)*
N-Back Correct (%)	500mg resveratrol	69.60 *(5.52)*	-0.61 *(3.66)*	0.41 *(3.77)*	0.81 *(2.54)*	-0.10 *(3.70)*	75.25 *(5.43)*	1.62 *(1.18)*	0.51 *(1.61)*	-1.72 *(1.47)*	0.51 *(1.32)*
	Placebo	84.56 *(2.12)*	1.05 *(3.94)*	1.29 *(4.06)*	2.69 *(2.73)*	0.35 *(3.98)*	87.13 *(1.91)*	-0.82 *(1.26)*	-1.40 *(1.73)*	-2.69 *(1.58)*	-1.52 *(1.42)*
N-Back Reaction Time (ms)	500mg resveratrol	621.48 *(66.91)*	-95.58 *(35.85)*	-115.75 *(38.72)*	-116.47 *(31.09)*	-130.25 *(42.44)*	586.40 *(64.11)*	-45.68 *(15.90)*	-52.04 *(16.72)*	-65.59 *(27.85)*	-89.91 *(28.44)*
	Placebo	737.98 *(65.73)*	13.42 *(38.58)*	-23.55 *(41.66)*	-86.11 *(33.45)*	-55.78 *(45.67)*	676.08 *(59.31)*	-46.05 *(17.11)*	-57.44 *(17.99)*	-44.08 *(29.97)*	-50.32 *(30.60)*

Table 5.5. Analysis Of Variance data table. Table displays the F and P values for both ANOVAs conducted on cognitive task data. The number 1. 'Pure chronic' ANOVA displays the results of a main effect of treatment at day 28 pre-dose (where the values were changed from day 1 baseline). The number 2. 'Acute, chronic and superimposed' ANOVA displays main effects of day (D), treatment (T), an interaction between the two (D*T), a main effect of repetition (R), an interaction between repetition and treatment (R*T), an interaction between day and repetition (D*R) and an interaction between day, repetition and treatment (D*R*T). *<.05, **<.01 and t= trend.

Measure	Treatment condition	ANOVAs				
		1. Pure chronic ANOVA		2. Acute, chronic and superimposed ANOVA		
		F	P	Effect	F	P
7s Correct (Number)	500mg resveratrol	.711	.404	D	12.95	.001**
				T	.036	.851
				D*T	.108	.745
	Placebo			R	7.83	<.001**
				R*T	1.90	.133
				D*R	.483	.695
				D*R*T	1.51	.216
7s Incorrect (Number)	500mg resveratrol	.428	.517	D	.173	.680
				T	6.40	.016*
				D*T	.275	.603
	Placebo			R	5.23	.001**
				R*T	1.15	.330
				D*R	.854	.007**
				D*R*T	.260	.034*
13s Correct (Number)	500mg resveratrol	1.32	.257	D	16.39	.708
				T	2.07	.442
				D*T	.455	.870
	Placebo			R	1.88	.044*
				R*T	.055	.419
				D*R	.964	.665
				D*R*T	2.02	.456
13s Incorrect (Number)	500mg resveratrol	.052	.821	D	.142	.708
				T	.604	.442
				D*T	.027	.870
	Placebo			R	2.79	.044*
				R*T	.950	.419
				D*R	.483	.665
				D*R*T	.876	.456
17s Correct (Number)	500mg resveratrol	1.26	.269	D	15.39	<.001**
				T	.784	.381
				D*T	2.79	.103
	Placebo			R	.871	.458
				R*T	1.03	.380
				D*R	5.25	.002**
				D*R*T	3.45	.019*

17s Incorrect (Number)	500mg resveratrol	.165	.687	D	.080	.779
				T	5.79	.021*
				D*T	.013	.911
	Placebo			R	6.25	.001**
				R*T	3.55	.017*
				D*R	2.33	.078 t
				D*R*T	1.76	.158
RVIP Correct (%)	500mg resveratrol	.092	.763	D	.177	.676
				T	.016	.901
				D*T	.143	.707
	Placebo			R	8.66	<.001**
				R*T	2.18	.094
				D*R	.196	.899
				D*R*T	.507	.678
RVIP Reaction Time (ms)	500mg resveratrol	.473	.495	D	1.53	.224
				T	1.81	.186
				D*T	1.01	.320
	Placebo			R	3.07	.039*
				R*T	.856	.451
				D*R	1.64	.193
				D*R*T	.763	.495
N-Back Correct (%)	500mg resveratrol	8.60	.006**	D	1.73	.196
				T	.102	.751
				D*T	1.22	.276
	Placebo			R	.253	.859
				R*T	.257	.842
				D*R	2.20	.092
				D*R*T	.267	.849
N-Back Reaction Time (ms)	500mg resveratrol	2.65	.112	D	1.01	.321
				T	.601	.443
				D*T	2.51	.121
	Placebo			R	3.47	.018*
				R*T	1.16	.330
				D*R	1.55	.205
				D*R*T	2.02	.114

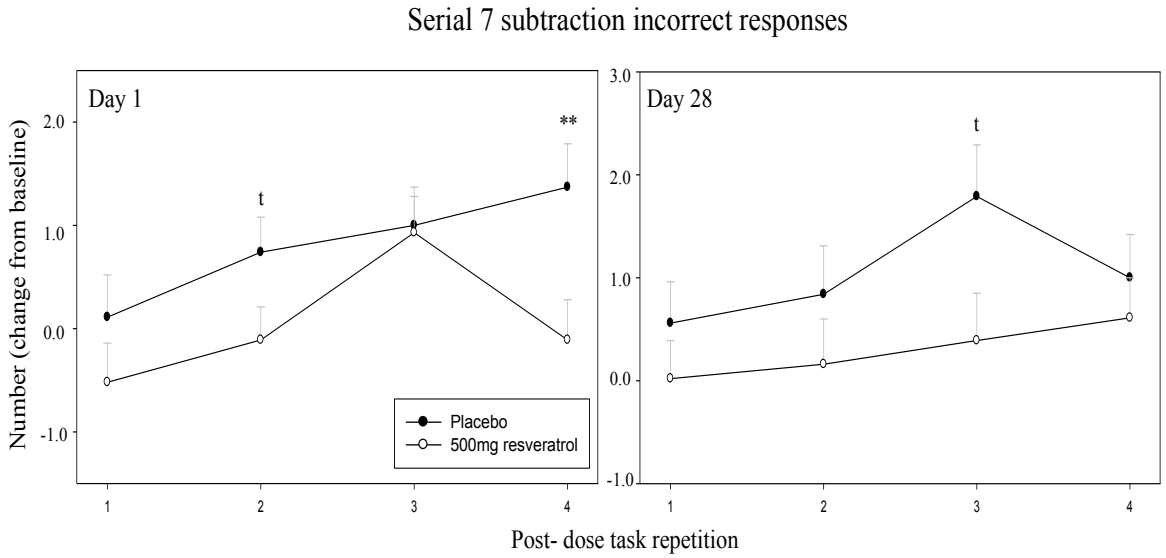


Figure 5.6. Effects of 500mg *trans*-resveratrol and placebo on number of serial 7 subtraction incorrect responses on day 1 and after 28 days supplementation. Graph displays mean (with SEM error bars), change from baseline, number of serial subtraction incorrect responses on day 1 (right panel) and day 28 (left panel) at all four post-dose task repetitions, after 500mg resveratrol and placebo. *<.05, **<.01 and t= trend.

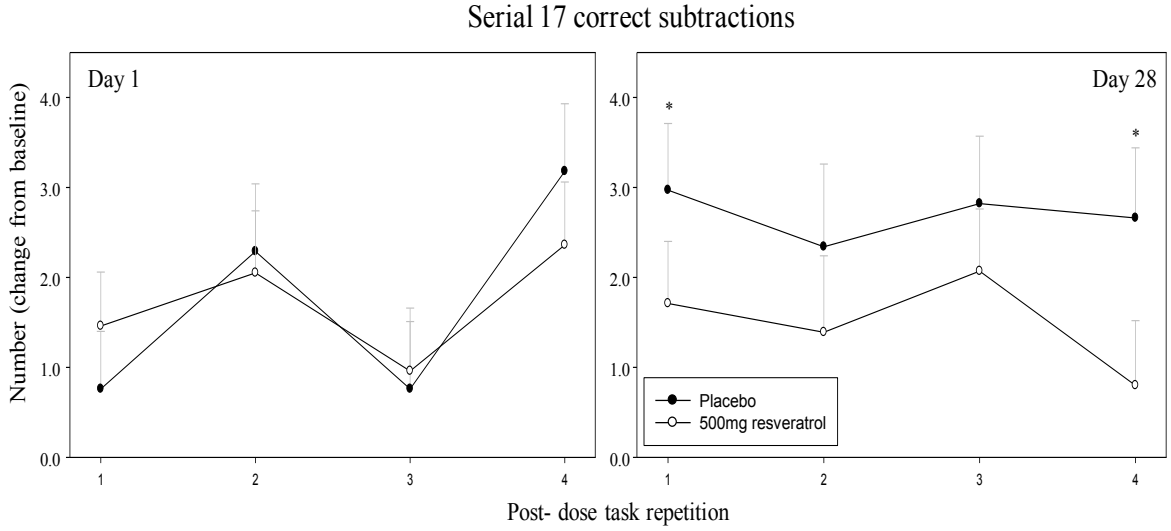


Figure 5.7. Effects of 500mg *trans*-resveratrol and placebo on number of serial 17 correct subtractions on day 1 and after 28 days supplementation. Graph displays mean (with SEM error bars), change from baseline, number of correctly completed serial subtractions on day 1 (right panel) and day 28 (left panel) at all four post-dose task repetitions, after 500mg resveratrol and placebo. *<.05, **<.01 and t= trend.

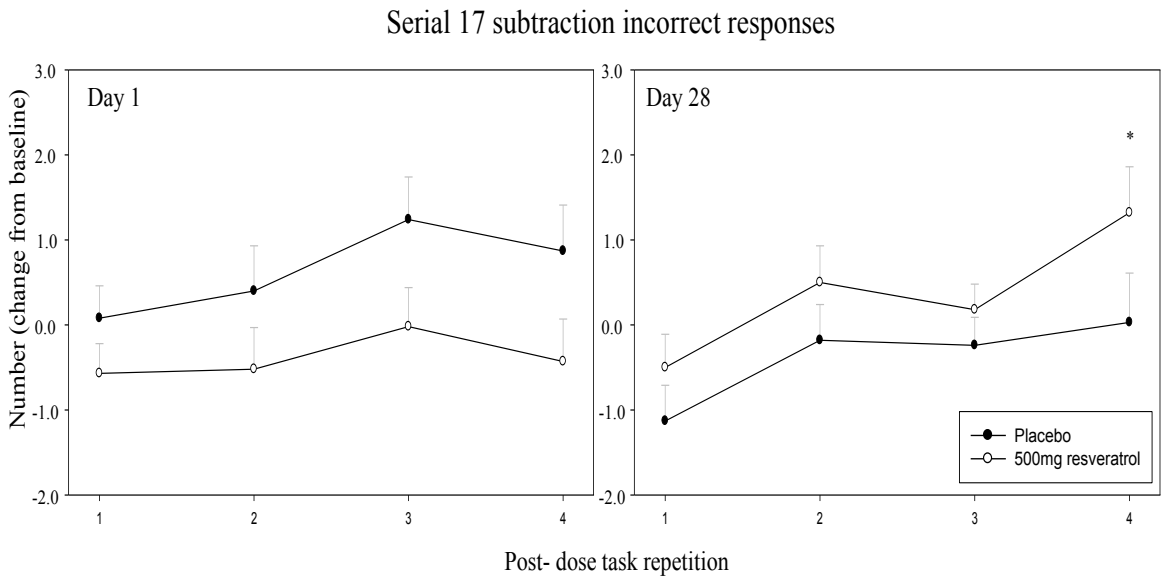


Figure 5.8. Effects of 500mg *trans*-resveratrol and placebo on number of serial 17 subtraction incorrect responses on day 1 and after 28 days supplementation. Graph displays mean (with SEM error bars), change from baseline, number of incorrect responses on serial 17 subtractions on day 1 (right panel) and day 28 (left panel) at all four post-dose task repetitions, after 500mg resveratrol and placebo. *<.05, **<.01 and t= trend.

5.3.5 General health

Results of the General Health Questionnaire (GHQ) ANOVAs showed no significant difference between treatments with regards any of the four variables (somatic symptoms, anxiety and insomnia, social dysfunction and severe depression) at any of the four post-dose weeks. (See table 5.6. for GHQ data table with ANOVA F and P values.)

Table 5.6. The acute and chronic effects of 500mg *trans*-resveratrol, compared to placebo, on subjective general health. Table displays raw baseline (day 1) mean and change from baseline means for day 7 (week 1), day 14 (week 2), day 21 (week 3) and day 28 (week 4) for the four sub-scales of the GHQ (A: Somatic symptoms; B: Anxiety and Insomnia; C: Social dysfunction; D: Severe depression) and the total comprised value for both the 500mg *trans*-resveratrol and placebo conditions. (SEM in italics, in brackets, underneath.) Table also displays ANOVA F and P values for main effects of treatment (T) and day (D) and an interaction between the two (T*D).

		Questionnaire time-point					ANOVA		
Measure	Treatment condition	Day 1 (Baseline)	Day 7 (Week 1)	Day 14 (Week 2)	Day 21 (Week 3)	Day 28 (Week 4)	Effect	F	P
A: Somatic Symptoms	500mg resveratrol	4.15 *(0.46)*	0.48 *(0.68)*	0.48 *(0.91)*	0.89 *(1.02)*	0.04 *(0.76)*	D	.762	.517
	Placebo	3.69 *(0.52)*	0.69 *(0.66)*	1.50 *(0.84)*	1.58 *(0.94)*	0.92 *(0.83)*	T	.532	.469
							T*D	.194	.871
B: Anxiety & Insomnia	500mg resveratrol	3.74 *(0.74)*	-0.33 *(0.74)*	-0.44 *(0.94)*	0.15 *(0.85)*	-0.33 *(0.86)*	D	.303	.823
	Placebo	4.00 *(0.65)*	-0.04 *(0.82)*	-0.38 *(0.60)*	-0.04 *(0.92)*	-0.27 *(0.62)*	T	.004	.951
							T*D	.073	.975
C: Social dysfunction	500mg resveratrol	6.78 *(0.40)*	-0.07 *(0.38)*	-0.04 *(0.50)*	0.56 *(0.72)*	-0.04 *(0.59)*	D	1.30	.278
	Placebo	6.81 *(0.33)*	-0.65 *(0.55)*	-1.15 *(0.45)*	-0.38 *(0.41)*	-0.35 *(0.57)*	T	1.43	.237
							T*D	.517	.621

D: Severe depression	500mg resveratrol	0.67 *(0.29)*	-0.22 *(0.32)*	0.26 *(0.36)*	0.67 *(0.62)*	-0.04 *(0.25)*	D	.441	.580
	Placebo	0.69 *(0.25)*	-0.04 *(0.30)*	-0.42 *(0.21)*	-0.38 *(0.22)*	-0.12 *(0.26)*	T	1.22	.275
							T*D	2.38	.115
Total	500mg resveratrol	14.74 *(1.17)*	0.44 *(1.33)*	0.85 *(1.85)*	2.85 *(2.45)*	0.22 *(1.52)*	D	.890	.435
	Placebo	15.12 *(1.19)*	0.04 *(1.63)*	-0.38 *(1.44)*	0.85 *(1.57)*	0.27 *(1.37)*	T	.230	.633
							T*D	.284	.805

5.3.6 Sleep

Results of the Pittsburgh Sleep Quality Index ANOVAs showed no significant treatment-related differences with regards any of the questionnaire subcomponents (subjective sleep quality; sleep latency; sleep duration; habitual sleep efficiency; sleep disturbances; use of sleep medication and daytime dysfunction) at any of the four post-dose weeks. The only main effects were with regards 'day' on the 'subjective sleep quality' [F(3,153)= 3.10; p= .029] and 'daytime dysfunction' [F(1,153)= 5.40; p= .001] measures. (See table 5.7 for PSQI data and ANOVA information.)

Table 5.7. The acute and chronic effects of 500mg *trans*-resveratrol, compared to placebo, on subjective sleep quality. Table displays raw baseline (day 1) mean and change from baseline means for day 7 (week 1), day 14 (week 2), day 21 (week 3) and day 28 (week 4) for the seven sub-scales of the PSQI (Subjective sleep quality; Sleep latency; Sleep duration; Habitual sleep efficiency; Sleep disturbances; Use of sleep medication; Daytime dysfunction) as well as a 'Global score', comprised of all seven, for both the 500mg *trans*-resveratrol and placebo conditions. (SEM in italics, in brackets.) Table also displays ANOVA F and P values for main effects of day (D) and treatment (T) and an interaction between the two (T*D). *<.05, **<.01 and t= trend.

		Questionnaire time-point					ANOVA		
Measure	Treatment condition	Day 1 (Baseline)	Day 7 (Week 1)	Day 14 (Week 2)	Day 21 (Week 3)	Day 28 (Week 4)	Effect	F	P
Subjective Sleep Quality	500mg resveratrol	0.81 *(0.09)*	0.00 *(0.13)*	-0.11 *(0.13)*	0.07 *(0.15)*	0.19 *(0.12)*	D T T*D	3.10 .050 .865	.029* .823 .461
	Placebo	1.00 *(0.12)*	-0.08 *(0.15)*	-0.04 *(0.18)*	-0.12 *(0.13)*	0.24 *(0.19)*			
Sleep Latency	500mg resveratrol	2.07 *(0.30)*	-0.52 *(0.25)*	-0.59 *(0.26)*	-0.26 *(0.30)*	-0.19 *(0.35)*	D T T*D	1.41 .221 1.36	.242 .640 .256
	Placebo	2.40 *(0.29)*	-0.16 *(0.36)*	-0.28 *(0.31)*	-0.64 *(0.29)*	0.04 *(0.40)*			

Sleep Duration	500mg resveratrol	0.11 *(0.06)*	-0.07 *(0.05)*	0.04 *(0.08)*	0.07 *(0.09)*	0.11 *(0.08)*	D T T*D	1.12 .031 .629	.337 .860 .561
	Placebo	0.08 *(0.08)*	0.08 *(0.06)*	-0.08 *(0.08)*	0.08 *(0.06)*	0.12 *(0.12)*			
Habitual Sleep Efficiency	500mg resveratrol	0.52 *(0.12)*	-0.11 *(0.11)*	0.11 *(0.12)*	0.37 *(0.13)*	0.07 *(0.15)*	D T T*D	2.81 .825 1.66	.051 .368 .186
	Placebo	0.56 *(0.16)*	-0.20 *(0.21)*	-0.04 *(0.15)*	-0.08 *(0.17)*	0.08 *(0.24)*			
Sleep Disturbances	500mg resveratrol	1.07 *(0.09)*	-0.11 *(0.08)*	-0.22 *(0.11)*	-0.11 *(0.11)*	-0.11 *(0.10)*	D T T*D	.915 .047 .602	.435 .829 .615
	Placebo	1.16 *(0.07)*	-0.04 *(0.09)*	-0.20 *(0.12)*	-0.20 *(0.12)*	-0.16 *(0.14)*			
Use of Sleep Medication	500mg resveratrol	0.00 *(0.00)*	0.00 *(0.00)*	0.00 *(0.00)*	0.00 *(0.00)*	0.00 *(0.00)*	D T T*D	N/A	
	Placebo	0.00 *(0.00)*	0.00 *(0.00)*	0.00 *(0.00)*	0.00 *(0.00)*	0.00 *(0.00)*			
Daytime Dysfunction	500mg resveratrol	0.78 *(0.11)*	-0.07 *(0.13)*	-0.37 *(0.16)*	-0.52 *(0.13)*	-0.44 *(0.15)*	D T T*D	5.40 2.91 .541	.001** .094 .655
	Placebo	0.56 *(0.13)*	0.20 *(0.14)*	0.04 *(0.17)*	-0.04 *(0.15)*	0.00 *(0.14)*			
Global Score	500mg resveratrol	5.37 *(0.47)*	-0.89 *(0.41)*	-1.15 *(0.55)*	-0.37 *(0.53)*	-0.37 *(0.64)*	D T T*D	1.42 .153 1.54	.238 .697 .206
	Placebo	5.76 *(0.60)*	-0.20 *(0.69)*	-0.44 *(0.67)*	-1.00 *(0.50)*	0.32 *(0.80)*			

5.3.7 Mood

The results of the Profile of Mood States (POMS) ANOVAs demonstrated a significant main effect of day for the 'vigour' measure [F(3,156)= 3.33; p= .030] and a trend for the 'tense' measure also [F(3, 156)= 2.60; p= .065]. No significant main effects of treatment were evinced but a significant treatment x day interaction was observed for the 'fatigue' measure. Further analysis with post-hoc students t tests demonstrated that subjective ratings of fatigue were significantly lower for resveratrol on day 7 (week 1) p<.05, day 21 (week 3) p<.05 and day 28 (week 4) p<.005[26]. Day 14 (week 2) p=.097.

(See table 5.8 for mood data and ANOVA information and figure 5.9 for 'fatigue' graph.)

Table 5.8. The acute and chronic effects of 500mg *trans*-resveratrol, compared to placebo, on mood. Table displays raw baseline (day 1) and change from baseline means (with SEM in brackets) for day 7 (week 1), day 14 (week 2), day 21 (week 3) and day 28 (week 4) for the 6 sub-scales of the POMS (Tense; Depression; Anger; Vigour; Fatigue; and Confusion) for both the 500mg *trans*-resveratrol and placebo conditions. Table also displays ANOVA F and P values for main effects of day (D) and treatment (T) and an interaction between the two (D*T). *<.05, **<.01 and t= trend.

		Questionnaire time-point					ANOVA		
Measure	Treatment condition	Day 1 (Baseline)	Day 7 (Week 1)	Day 14 (Week 2)	Day 21 (Week 3)	Day 28 (Week 4)	Effect	F	P
Tense	500mg resveratrol	7.07 *0.98)*	-0.72 *(0.91)*	-0.55 *(1.09)*	0.07 *(1.06)*	-1.93 *(0.74)*	D	2.60	.065 t
	Placebo	6.46 *(1.01)*	-0.04 *(1.08)*	-0.92 *(1.10)*	0.50 *(1.10)*	-0.38 *(0.92)*	T	.082	.776
							T*D	1.00	.384

[26] t's (52)= 2.11, 2.58 and 3.45 respectively.

Depression	500mg resveratrol	6.43 *(1.71)*	0.34 *(0.78)*	1.55 *(1.22)*	0.34 *(0.92)*	-1.38 *(0.71)*	D	1.37	.253
	Placebo	5.04 *(1.06)*	-0.96 *(1.15)*	-1.77 *(1.30)*	-0.31 *(0.93)*	-1.23 *(0.89)*	T	1.25	.270
							T*D	2.03	.113
Anger	500mg resveratrol	6.18 *(1.36)*	-1.24 *(0.60)*	-0.76 *(1.03)*	-0.48 *(1.03)*	-2.17 *(0.66)*	D	2.26	.093
	Placebo	6.50 *(1.08)*	-1.15 *(0.82)*	-2.69 *(1.31)*	-1.62 *(1.17)*	-2.54 *(1.09)*	T	.326	.571
							T*D	.718	.525
Vigour	500mg resveratrol	14.18 *(0.88)*	0.45 *(1.13)*	0.45 *(1.08)*	-0.28 *(1.21)*	-0.59 *(1.08)*	D	3.33	.030*
	Placebo	13.85 *(0.89)*	-0.54 *(0.95)*	-1.23 *(0.97)*	-2.19 *(1.13)*	-2.54 *(1.22)*	T	2.00	.163
							T*D	.082	.949
Fatigue	500mg resveratrol	8.04 *(0.88)*	-2.62 *(0.67)*	-2.21 *(0.95)*	-2.41 *(0.98)*	-3.34 *(0.81)*	D	.577	.591
	Placebo	5.54 *(0.90)*	-0.46 *(0.67)*	-0.27 *(0.76)*	0.65 *(0.88)*	0.04 *(0.67)*	T	9.37	.003**
							T*D	1.12	.337
Confusion	500mg resveratrol	7.68 *(0.73)*	-0.86 *(0.56)*	0.17 *(0.78)*	-0.55 *(0.77)*	-0.66 *(0.73)*	D	.345	.793
	Placebo	6.65 *(0.70)*	-0.58 *(0.58)*	-0.96 *(0.58)*	0.19 *(0.65)*	-0.31 *(0.63)*	T	.024	.878
							T*D	1.56	.203

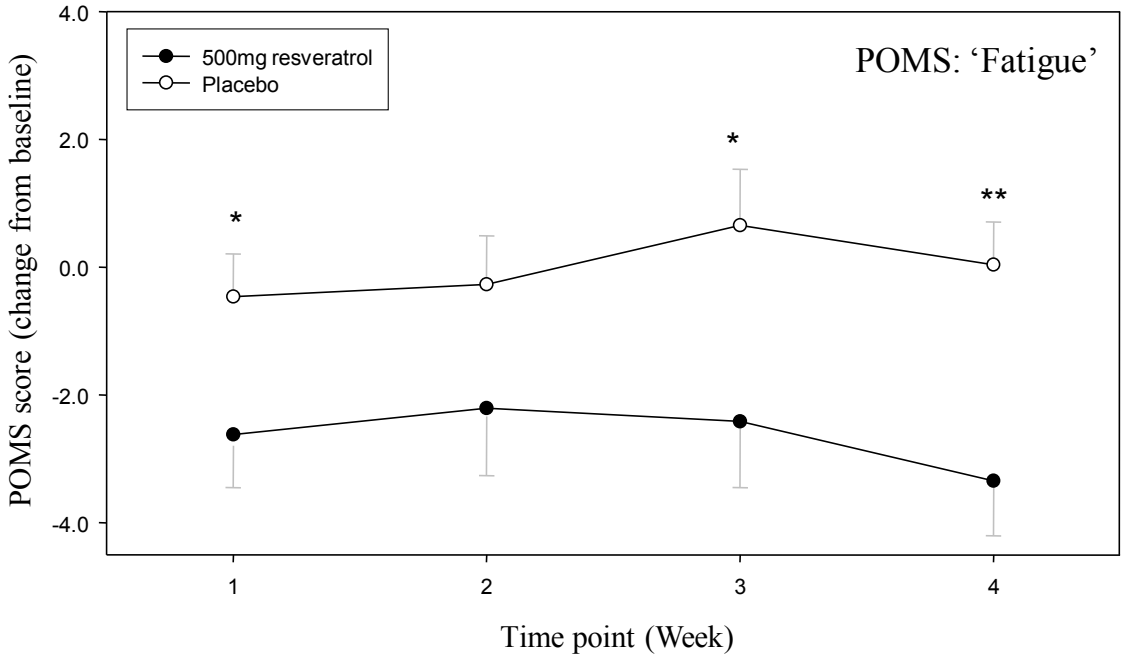


Figure 5.9. Subjective ratings of 'fatigue' after 500mg *trans*-resveratrol and placebo after 1, 2, 3 and 4 weeks supplementation. Graph displays mean (with SEM error bars), change from baseline ratings of fatigue at week 1 (day 7), week 2 (day 14), week 3 (day 21) and week 4 (day 28). *<.05 and **<.01.

5.3.8 Blood pressure (BP)

1. Pure chronic effects

The results of the ANOVA on day 28 pre-dose BP measurements (converted to change-from-day 1-baseline) comparing readings between 500mg resveratrol and placebo, demonstrated only a significant effect for diastolic BP [F(1, 28)= 5.86; p= .022] with levels higher in the resveratrol condition.

2. Acute, sub-chronic and superimposed effects

The results of the treatment x repetition x day ANOVA revealed a trend for a main effect of day for Systolic BP (p= .08) and Pulse Rate (p= .07). For Diastolic BP, a significant interaction between treatment x day was evinced [F(1, 22)= 6.61; p= .017] which revealed only 1 significant comparison, in the placebo condition, between day 1 and day 28 (<.05)[27], at the 40 minutes PD measurement. Here the mean was higher overall on day 28 compared to day 1.

(See table 5.9 for BP values and ANOVA F and P values.)

[27] t(14)= -2.19

Table 5.9. The acute effects of 500mg *trans*-resveratrol, compared to placebo, on blood pressure, and after a 28 day supplementation period. Table displays raw day 1 baseline scores and change-from-day 1-baseline values for 40 minutes and 85 minutes post-dose for day 1 and 28 after 500mg *trans*-resveratrol and placebo. (Standard error displayed in italics, in brackets, underneath.) Table also displays the F and P values for both ANOVAs. The number 1. 'Pure chronic' ANOVA displays the results of a main effect of treatment at day 28 pre-dose (where the values were changed from day 1 baseline). The number 2. 'Acute and superimposed chronic' ANOVA displays main effects of day (D), treatment (T), an interaction between the two (D*T), a main effect of repetition (R), an interaction between repetition and treatment (R*T), an interaction between day and repetition (D*R) and an interaction between day, repetition and treatment (D*R*T). *<.05, **<.01 and t=trend.

Measure	Treatment condition	Day 1			Day 28			ANOVAs				
		Baseline	40mins PD	85mins PD	Baseline	40mins PD	85mins PD	1. Pure chronic ANOVA		2. Acute and superimposed chronic ANOVA		
								F	P	Effect	F	P
Systolic Blood Pressure (mmHg)	500mg resveratrol	121.21 (*3.20)*	-3.44 *(9.60)*	1.22 *(3.32)*	-3.69 *(2.27)*	-0.56 *(5.15)*	0.00 *(4.96)*	1.21	.280	Tr	1.10	.305
										R	2.09	.162
										Tr*R	.013	.911
										D	3.38	.080 t
	Placebo	117.06 *(2.73)*	-0.20 *(3.16)*	3.47 *(3.87)*	0.07 *(2.56)*	6.73 *(3.03)*	7.53 *(3.17)*			Tr*D	1.84	.189
										R*D	.898	.354
										Tr*R*D	.029	.867
Diastolic Blood Pressure (mmHg)	500mg resveratrol	76.14 *(1.24)*	5.78 *(6.12)*	6.78 *(2.84)*	-3.31 *(1.12)*	2.56 *(2.03)*	4.44 *(2.19)*	5.86	.022*	Tr	.047	.830
										R	.120	.732
										Tr*R	1.67	.209
										D	.674	.421
	Placebo	77.44 *(2.51)*	2.67 *(3.09)*	4.27 *(2.71)*	0.71 *(1.24)*	6.53 *(1.90)*	3.27 *(2.37)*			Tr*D	6.61	.017*
										R*D	.909	.351
										Tr*R*D	1.90	.182

Pulse Rate (BPM)	500mg resveratrol	69.14 *(1.25)*	3.22 *(5.53)*	0.33 *(3.41)*	1.00 *(3.02)*	-3.44 *(5.97)*	-2.67 *(4.04)*	1.27 .269	Tr	.423 .522
									R	.067 .799
									Tr*R	.059 .811
	Placebo	71.69 *(3.18)*	-1.07 *(3.62)*	-3.67 *(4.06)*	5.36 *(2.27)*	-6.20 *(2.15)*	-3.67 *(4.23)*		D	3.63 .070 t
									Tr*D	.340 .566
									R*D	1.32 .263
									Tr*R*D	.037 .850

5.3.9 Bioavailability

No resveratrol (in any form) was found in baseline samples on day 1, indicating that all volunteers did not consume resveratrol containing products before the study. The results of the ANOVAs for each form of resveratrol are as follows:

Total metabolites: A significant effect of time was observed [F(1.3, 8.1)= 7.50; p= .020] for levels of total resveratrol metabolites (the sum of the below three metabolites) with pairwise comparisons showing that day 1 post-dose levels were higher than day 1 baseline (<.05), that day 28 pre-dose levels were higher than day 1 baseline (<.05) and that day 28 post-dose levels were higher than both day 1 baseline (<.005) and day-28 pre-dose levels (<.01).

Resveratrol 3-*O*-sulfate: A significant effect of time was observed [F(3, 18)= 7.53; p= .002] for levels of resveratrol 3-*O*-sulfate with pairwise comparisons showing that day 1 post-dose levels were higher than day 1 baseline (<.05), that day 28 pre-dose levels were higher than day 1 baseline (<.05) and that day 28 post-dose levels were higher than both day 1 baseline (<.005) and day 28 pre-dose levels (<.01).

Resveratrol 4-O-glucuronide: A significant effect of time was observed [F(1.2, 7.4)= 6.81; p= .029] for levels of resveratrol 4-*O*-glucuronide with pairwise comparisons showing that day 1 post-dose levels were higher than day 1 baseline (<.05), that day 28 pre-dose levels were higher than day 1 baseline (<.05) and that day 28 post-dose levels were higher than both day 1 baseline (<.01) and day 28 pre-dose levels (<.05).

Resveratrol 3-O-glucuronide: A significant effect of time was observed [F(1.2, 7.3)= 5.95; p= .039] for levels of resveratrol 3-*O*-glucuronide with pairwise comparisons showing that day 1 post-dose levels were higher than day 1 baseline (<.05), that day 28 pre-dose levels were higher than day 1 baseline (<.05) and that day 28 post-dose levels were higher than both day 1 baseline (<.005) and day 28 pre-dose levels (<.005).

No aglycone resveratrol was quantifiable in plasma at any time-point, on either day. Resveratrol 3-*O*-sulfate was the predominant metabolite in all volunteers, contributing 73-77% of total metabolites. The 4'- and 3-*O*-glucuronide forms evinced roughly equal contributions to the remaining metabolites in circulation.

Mean plasma concentration values (μM) for resveratrol metabolites on day 1 and day 28 shown in figure 5.10 with means and ANOVA F and P values shown in table 5.10.

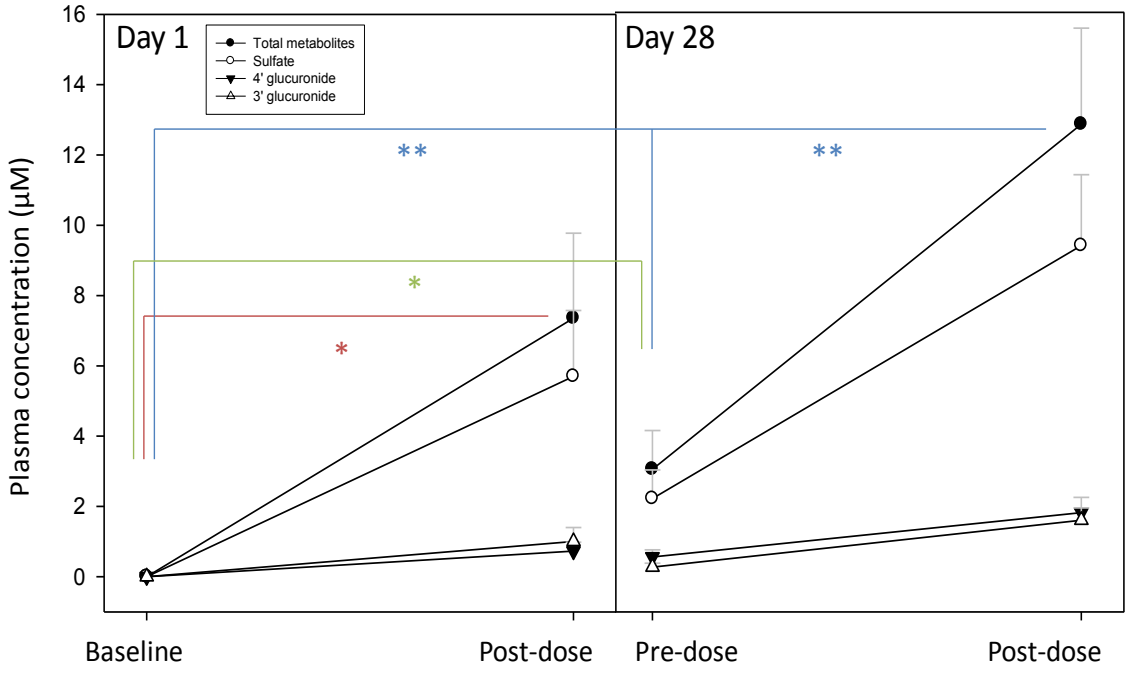


Figure 5.10. Bioavailability of resveratrol metabolites after 500mg *trans*-resveratrol acutely, and after a 28 day supplementation period. Graph displays mean plasma concentration (µM) values (with SEM error bars) of resveratrol metabolites in plasma at baseline and post-dose (110mins post administration) on day 1 and day 28, after 500mg *trans*-resveratrol, in 7 healthy, young adults. Significance on graph demonstrated for total metabolites, with *<.05 and **<.01, although all 3 metabolites demonstrate the same pattern.

Table 5.10. Mean plasma levels of resveratrol metabolites on day 1 and day 28 after 500mg *trans*-resveratrol. Table displays mean (with SEM in brackets) plasma levels of resveratrol metabolites (depicted graphically above) at baseline and post-dose on day 1 and at pre-dose and post-dose on day 28 after 500mg resveratrol. Table also displays ANOVA F and P values where *<.05 and **<.01.

Resveratrol metabolite	Day 1		Day 28		ANOVA	
	Baseline	Post-dose	Pre-dose	Post-dose	F	P
Total	0.00 *(0.00)*	7.36 *(2.42)*	3.04 *(1.10)*	12.83 *(2.72)*	7.50	.020*
3-*O*-sulfate	0.00 *(0.00)*	5.70 *(1.88)*	2.21 *(0.80)*	9.39 *(2.01)*	7.53	.002**
4-*O*-glucuronide	0.00 *(0.00)*	0.73 *(0.25)*	0.56 *(0.20)*	1.81 *(0.43)*	6.81	.029*
3-*O*-glucuronide	0.00 *(0.00)*	1.00 *(0.40)*	0.27 *(0.11)*	1.60 *(0.35)*	5.95	.039*

5.4 Discussion

The overall aim of this study was to investigate the effects of 500mg resveratrol on mood, subjective sleep, cognitive function and cerebral blood flow (CBF) in healthy, young humans over an extended period of time. Specifically, this chapter aimed to address the lack of cognitive effects of resveratrol observed in chapters 2, 3 and 4 which, it was argued, may have been due to acute, bolus administration of resveratrol being insufficient to elicit the hemodynamic effects necessary to improve cognitive performance. Hence, a 28 day supplementation period was utilized here; in a paradigm which also incorporated the measurement of subjective perceptions of health, mood and sleep. A quantitative measure of CBF (specifically cerebral blood flow volume (CBFV)) was provided by trans-cranial Doppler (TCD) sonography due to the lack of a quantitative measure of change in CBF across time from the NIRS.

To summarise the results of the current chapter briefly: the results support the acute augmentation of CBF by resveratrol, which has been reported in previous chapters, but did not observe any chronic changes in CBF (as assessed by NIRS), in either treatment condition, or CBFV (as assessed by TCD) acutely or chronically. Increased BP was seen chronically in resveratrol-treated participants only. No subjective effects of resveratrol on health or sleep quality were found but fatigue was significantly lower at three out of four of the weekly assessments in the resveratrol condition. Chronic dosing of resveratrol was observed to increase plasma levels of resveratrol metabolites and, in terms of cognitive effects, resveratrol was associated with significantly better performance on the NBack task chronically, as compared to placebo, and the performance in the placebo condition evinced both better and poorer performance on the subtraction tasks acutely and chronically. The following will now discuss each of these findings in more depth.

In terms of the acute CBF effects observed here, results demonstrate that 500mg resveratrol is able to augment the CBF response to cognitive task demands, relative to placebo, albeit to a weaker extent than those seen in Chapters 3 and 4. Nevertheless, the acute augmentation of CBF is broadly in line with the findings from chapter 3, following the same dose, and also following 250mg resveratrol with 20mg piperine in chapter 4 and manifested in small, significantly higher levels of total-Hb at the ends of the absorption and post-dose task periods and a consistent pattern of significantly higher levels of oxy-Hb across some of the absorption and post-dose task periods on day 1 (See the top panels of figures 5.2 and 5.3 for day-1 total- and oxy-Hb graphs respectively.)

The acute effects of resveratrol on deoxy-Hb levels however, were the opposite to those seen in the previous two studies. Chapters 3 and 4 reported that resveratrol, at 250- and 500mg and 250mg with 20mg piperine, respectively, evinced significantly higher levels of deoxy-Hb, i.e. enhanced oxygen extraction, as compared to placebo. In the current study however, planned comparisons showed that deoxy-Hb was significantly lower than placebo at several of the final epochs of the absorption period and most epochs across the post-dose task period. The reason for this discrepancy, i.e. why two chapters should evince a pattern of effects suggesting enhanced oxygen utilization in response to resveratrol (which would be anticipated considering the direct effects of resveratrol on mitochondrial function (Lagouge et al., 2006)) whilst this study demonstrates opposite effects, is unclear. However, it may be notable that, of these three comparable chapters, the current is the first to utilize a between-subjects design and this may introduce an unanticipated degree of variability in CBF.

Chronically, resveratrol was not found to alter CBF parameters; with no significant differences observed in levels of total- or oxy-Hb on day 28 between resveratrol and placebo. The acute effects of deoxy-Hb found on day 1, with placebo evincing higher levels, did not emerge on day 28 with both treatment groups demonstrating a gradual decline in levels across the testing session.

These results suggest that the acute CBF effects of 500mg resveratrol seen on day 1, and replicated previously (chapter 3), with significantly higher levels of total- and oxy-Hb compared to placebo, are not present after chronic consumption; with no significant difference in levels of either total- or oxy-Hb between resveratrol and placebo on day 28. The difficulty with this interpretation is akin to the issue faced in the pilot study (reported in chapter 2) and reflects the manner in which NIRS calculates haemoglobin levels. The continuous-wave (C-W) NIRS used here presents haemoglobin levels as a concentration (μmol/L) change rather than in terms of absolute levels. As such, if a gross increase in CBF levels had taken place in resveratrol-supplemented participants across the chronic supplementation period, meaning that this group began the day 28 NIRS recording with higher CBF than the placebo participants, NIRS would be unable to quantify this and would only provide concentration change data within day 28 itself. Naturally, if chronic consumption of resveratrol had resulted in increased CBF it may well be the case that the lack of effects seen on day 28 reflects a reduced need for additional activity dependent blood-flow during task performance. Alternatively, the lack of an effect may well reflect the participants approaching a ceiling in terms of CBF or indeed that participants had habituated to the effects of resveratrol. Taken together then, direct comparison between day 1 and day 28 NIRS readings might not provide a completely reliable picture of CBF changes.

In an attempt to clarify this issue however; if we compare the CBF results from the current chapter and chapter 2 (both hindered in interpretation by concentration change levels of haemoglobin only measured post-potential-treatment effect taking place), we see that both studies demonstrate a similar phenomenon, i.e. that of placebo evincing greater post-dose CBF effects than resveratrol (chapter 2) or no difference in levels between the resveratrol and placebo conditions (day 28 results here). This adds credence to the argument made in the discussion of chapter 2; i.e. that resveratrol-treated participants had already experienced a global increase in CBF and that this was unable to rise further when recording recommenced. This is further supported by the fact that the studies in this PhD which have measured the CBF response to resveratrol across the entire testing session (chapters 3 and 4) have both observed that resveratrol evinces a significantly higher CBF response during post-dose task completion, as compared to placebo.

As stated above, it was hypothesized that the above constraints with regards NIRS measurements could be mitigated by the use of TCD to provide a quantitative CBFV measurement that would give an indication of gross changes in CBF between day 1 and day 28. However, no significant differences between treatments were observed with TCD readings and so, unfortunately, this data cannot help to clarify the above chronic NIRS results. With regards potential explanations for this null effect of resveratrol on CBFV, both acutely and chronically, it could be argued that significant CBFV changes were lacking as, during the TCD recording periods (which were prior to and following cognitive tasks), participants were not in a state of cognitive demand and, therefore, did not require an increase in metabolic substrates to the prefrontal cortex. It might also be the case that the 5 minute recording periods utilized, which yield only two measurements per minute, is simply not sufficient to measure treatment-related CBFV effects and that longer assessments might be more sensitive. Ideally the TCD and NIRS would both have been used to record concomitantly throughout the absorption and cognitive task periods. Unfortunately, due to the constraints of the equipment utilized here, this was not possible.

The current study does, however, report vascular effects of resveratrol in the periphery on day 28; with an analysis of pure chronic effects (derived by comparing BP levels between resveratrol and placebo at pre-dose on day 28) demonstrating higher diastolic BP in resveratrol-supplemented participants. No baseline differences in BP readings, nor acute effects within day 1 or day 28 were observed. The rationale for investigating the potential for BP and heart rate effects of resveratrol here was an attempt to elucidate whether the higher levels of diastolic BP, seen in chapter 4, was as a result of piperine or merely a piperine-mediated enhancement of an ineffective dose of

resveratrol. That this effect has been replicated here, albeit after chronic supplementation of a higher dose of resveratrol, supports the role of resveratrol alone in increasing BP. This finding is intuitively unexpected as resveratrol has demonstrated efficacy as a vasodilator previously (Wong et al., 2011 and 2012); a phenomenon associated with lowered BP. This is seen, for example, after 18 weeks supplementation with 30mg daily cocoa polyphenols where systolic and diastolic BP were significantly reduced, alongside significantly higher levels of S-nitrosoglutathione (NO), in hypertensive humans. Conversely, here we find that diastolic BP is increased after 28 days supplementation. Without measuring NO levels here it is not possible to clarify whether resveratrol was indeed exerting vasodilatory effects, as per the hypothesis, on day 28 or not. Certainly the lack of CBF (NIRS) and CBFV (TCD) effects on day 28 could support the argument that it was not. This does somewhat muddy the argument however by introducing the notion that resveratrol may have been acting as a vasoconstrictor: hence reduced arterial size and the increased BP we observe here. Whether resveratrol can act as a vasoconstrictor is, at present, unknown but if we consider structurally similar polyphenols, e.g. the tea polyphenol epigallocatechin-3-gallate (EGCG), then we see that polyphenols are capable of acting both as vasodilators and vasoconstrictors depending on dose and the time of assessment (Alvarez, Campos, Justiniano, Lugnier, & Orallo, 2006). EGCG has also been investigated with regards its cognitive and CBF effects in humans where it was reported that 135mg, administered acutely, evinced significantly lower CBF as compared to placebo; which might indeed be suggestive of vasoconstriction.

With regards the results of the weekly questionnaires completed by participants across the supplementation period, no significant effects of resveratrol were observed for subjective perceptions of general health (as assessed by the GHQ) or sleep (as assessed by the PSQI). With regards subjective perceptions of mood, the only variable on the POMS questionnaire which evinced any significant difference was ‘fatigue’; where levels were significantly lower in the resveratrol-treated group at weeks 1, 3 and 4, as compared to placebo. Very little research exists regarding the effects of polyphenols on mood but this anti-fatigue effect may find an explanation in *in vitro* and animal work which report the ability of resveratrol to inhibit Monoamine Oxidase-A and B (MAO-A/B) activity. This inhibition was reported to lead to an increase in monoamine neurotransmitter concentrations, namely 5-hydroxytryptophan (5-HT), noradrenaline and dopamine, with a concomitant improvement in mood; similar to that seen with imipramine and fluoxetine, in mice (Xu, Li, et al., 2010). Another potential anti-fatigue mechanism is predicated on resveratrol’s structural similarity to the flavonoid quercetin, which has been reported to increase energy expenditure and endurance capacity in

mice (Davis, Murphy, Carmichael, & Davis, 2009; Stewart et al., 2008) and power output in elite male cyclists when taken as part of a cocktail of supplemented compounds (MacRae & Mefferd, 2006). Putative mechanisms for these effects include increased blood flow; due to vasorelaxation (Chen & PaceAsciak, 1996), and oxygenation; with Davis et al. also reporting SIRT-mediated increases in mitochondrial gene expression in brain and skeletal muscles. Both mechanisms are shared with resveratrol (Chen & PaceAsciak, 1996; Lagouge et al., 2006) and could explain the increased energy levels seen here. It is worth noting here that, whilst there was no statistically significant difference in baseline (pre-dose, day 1) levels of fatigue between resveratrol and placebo participants, the baseline values were nevertheless numerically higher in the former group (8.04 compared to 5.54 respectively) which might suggest that this effect represents a return to normal levels for the resveratrol group following an unusually high baseline.

One of the hypotheses made in the introduction was that repeated dosing, over 28 days, would lead to an increase in plasma levels of resveratrol. This was based on data from supplementation with 200µg/kg and 2mg/kg daily which evinced clinically efficacious chemopreventive effects (results reported in Gescher & Steward, 2003). The results here support this hypothesis; with plasma levels of resveratrol metabolites significantly higher at day 28 pre-dose compared to day 1 pre-dose baseline; demonstrating that a pure chronic increase (irrespective of treatment on day 28) had taken place. That the day 1 baseline mean levels were 0 does render this comparison, statistically, problematic. However, disregarding statistical significance, the fact that metabolites were present at all (considering that levels were 0 at baseline on day 1) is indicative that an increase in plasma levels of resveratrol had taken place.

However, the argument could be made here that the presence of resveratrol metabolites on day 28 pre-dose could represent residual levels from the day 27 dose and/or that it could be the result of non-compliance by participants; perhaps consuming treatment later than instructed on day 27 or even consuming treatment inadvertently on day 28 before attending the testing session, rather than accumulation that was the result of repeated, 28 day, dosing. With regards the first potential explanation, reference to the capsule logs completed daily by participants to note the time of treatment administration, and a capsule count of their returned treatment on day 28, shows that this group were between 96% (i.e. returning with 1 surplus capsule) to 100% compliant and that day 27 treatment was consumed between 8:13am-12:00pm. Whilst this time of 12:00pm is later than instructed, and could potentially account for a residue of plasma resveratrol in this participant, it could not account for a baseline

plasma level of 6.0μM which is also similar to the baseline plasma levels of participants who reported taking day 27 treatment at 7:40am (5.7μM) and 8:13am (5.8μM).

This argument is supported by previous studies which have charted the 24hr bioavailability profile of resveratrol in healthy humans. Broadly, these studies show that resveratrol peaks in human plasma, after oral administration, between ~45mins and 1hr post-dose and that a 2nd peak can emerge at ~6hrs post-dose due to enterohepatic recirculation (Walle et al., 2004). The plasma half-life of resveratrol has been reported at 9.2 ± 0.6hrs (Walle et al.) and can range between 3.2-11.5hrs for sulfate metabolites, 2.9-10.6hrs for glucuronides and 2.9-8.9hrs for the parent compound (Boocock et al., 2007). The latter study also reports that, after a 500mg oral resveratrol dose in healthy humans, levels of all measured resveratrol conjugates had returned to baseline between 20-24hrs post-dose. This was apart from the sulfate metabolite which only registered ~10ng/mL at 24hrs.

Taken together then, the results from previous pharmacokinetic studies on the plasma profile of resveratrol after acute, bolus, oral consumption support a maximum complete excretion of resveratrol at 24hrs post-dose. As such, the current study should not have anticipated baseline plasma levels of resveratrol metabolites on day 28 as a product of residual levels from day 27 consumption and, if this did occur at all (due to later consumption of day 27 treatment for example), concentrations would be expected in the ng range at most. With a paucity of research in the bioavailability of resveratrol generally, but specifically after repeated dosing, and without having taken intermittent 24hr blood samples here, it is not possible to categorically assert that the presence of baseline resveratrol on day 28 represents a cumulative 'topping-up' of plasma levels as a result of chronic consumption. However, as there is no evidence to counter this argument at present, this seems to be the fairest assumption based on the data presented here. In support of this, the significantly higher mean plasma metabolite levels at post-dose, compared to pre-dose, on day 28, may be suggestive of an enhancement of acute bioavailability due to chronic dosing.

The chronic 28 day dosing paradigm utilized in the current chapter was designed to address the potential ineffectiveness of resveratrol at eliciting cognitive performance effects after acute, bolus supplementation. The hypothesis being that chronic consumption of resveratrol might be more effective at augmenting CBF and, in turn, improving cognition. Analysis demonstrated that the only task measure to evince a pure chronic effect (derived by the comparison of changes in performance between resveratrol and placebo between day 1 and day 28 pre-dose) was N-Back % correct. After 28 days supplementation, participants in the 500mg resveratrol condition

completed significantly more correct 3-Back responses, as compared to placebo. No effects on this measure were observed following consumption of treatment on day 1 or day 28 nor were any effects observed on the other accuracy sub-measure assessed in this chapter. The results of acute, chronic and superimposed analysis revealed that, on day 28, participants in the placebo condition achieved more correct responses on the serial 17 subtractions. However, on day 1 and day 28, participants in the placebo condition also made significantly more incorrect responses on the serial 7 and serial 17 subtraction tasks respectively. Taken together, the performance of participants in the placebo condition appears to represent a speed-accuracy trade-off and the one significant finding of improved performance on the NBack task in the resveratrol condition, in the face of no other significant cognitive enhancement, is likely a type I error.

In conclusion, the current study found that chronic, 28 day supplementation of 500mg resveratrol daily results in an accumulation of plasma resveratrol metabolites in healthy, young humans. Acute (day 1) CBF effects of this dose were observed but no chronic (day 28) effects, as assessed by either NIRS or TCD, were found. No subjective effects of resveratrol on health or sleep quality were reported but fatigue was significantly lower at three out of four of the weekly assessments in the resveratrol condition. This could merely represent a return to baseline but it is tentatively suggested that this might be indicative of the effects of resveratrol on mitochondrial activity and energy expenditure. In terms of cognitive effects, only one sub-measure out of ten (from five tasks) was significantly affected by resveratrol and this is most likely the result of type I error. Performance in the placebo condition however, suggested a speed-accuracy tradeoff and so these results could represent a maintenance of consistent performance in the resveratrol condition. Finally, the analysis of BP measurements demonstrated, counter-intuitively, increased diastolic BP in resveratrol-treated participants after 28 days supplementation and this, coupled with the lack of day 28 CBF effects, might suggest that resveratrol was not acting as a vasodilator after chronic consumption.

Chapter 6.
The cognitive effects of 500mg *trans*-resveratrol in healthy, young humans.

6.1 Introduction

The hypothesis underlying this thesis is that the polyphenol resveratrol will be capable of evincing cognitive and cerebral blood flow (CBF) effects in healthy, young humans. The acute CBF effects of resveratrol in the prefrontal cortex of healthy, young humans has been confirmed in chapters 3, 4 and 5 after doses of 250- and 500mg of resveratrol and, 250mg resveratrol with 20mg piperine and so this aspect of the hypothesis has been consistently supported. The issue, however, resides with the secondary aspect of the hypothesis; i.e. the lack of cognitive effects of resveratrol despite the aforementioned CBF effects. This consistent lack of effect of resveratrol on cognitive parameters has persisted across a range of doses (i.e. 250mg, 250mg with 20mg piperine, 500mg and 1000mg), in acute (chapters 2, 3, 4 and day 1 of chapter 5) and chronic (day 28 of chapter 5) paradigms, and despite an augmentation of plasma metabolite levels (chapter 5). Chapters 3 and 4 failed to find any significant effects of resveratrol on cognitive function whatsoever and chapter 5 revealed only better performance on the NBack task, following 28 days supplementation or resveratrol, as compared to placebo. The argument made in the discussion of the previous chapter was that this pure chronic effect of resveratrol likely represented a type I error rather than a true resveratrol-induced improvement in performance. The basis of this argument was that this effect on the NBack task was not observed acutely, nor after treatment on day 28, and represents an effect on only 1/10 task sub-measures.

The aim of this final chapter is to address some potential limitations of the previous paradigms which may be responsible for masking any cognitive enhancing effect of resveratrol, if it exists. The first is the possibility that the cognitive tasks utilized throughout this PhD (namely the Serial subtraction, RVIP and 3-Back tasks) may be too narrow with regards the cognitive domains that they load upon and that potential effects of resveratrol on other aspects of cognitive performance have been missed. The second issue relates to the fact that the cohorts utilized throughout this thesis are all young, healthy and predominantly students, who could be conceived of as being at their cognitive peak (Rönnlund et al., 2005), who may find the cognitive tasks utilized relatively easy; thus making any change in task performance subtle and difficult to detect.

In order to address the first issue, the current chapter will employ a series of novel tasks, alongside those utilized previously. The tasks hitherto utilized in this programme of studies were chosen based both on their capacity to elicit cognitive demand and to activate the prefrontal cortex. Their selection beyond that was, due to a lack of any pre-existing research in the area of resveratrol and cognition in humans, somewhat exploratory. As such, there is a danger that by repeatedly using these same tasks, of predominantly attention/vigilance and working memory, that a potential effect of resveratrol on some other aspect of cognitive function could be missed. As such, the novel tasks will assess additional factors such as response inhibition (Stroop) and recall and recognition (word and picture immediate and delayed recall and recognition). The latter of these tasks have also previously proven sensitive to changes in neural fuel provision, i.e. oxygen (Moss et al., 1998).

With regards the second issue, previous intervention studies have also factored the potential for a ceiling effect on task performance, in this cohort, into their testing paradigms. For example, as well as utilizing cognitively demanding tasks in an attempt to impair performance (with the aim to reverse this with nutritional supplementation) Kennedy, Scholey and Wesnes (2001a) and Reay et al. (2010) also incorporated cognitively demanding testing protocols; namely repeated post-dose testing across the day. The current study will also adopt this approach; utilizing an extended period of post-dose testing, i.e. 40 minutes, 2.5hrs, 4hrs and 6hrs. These time-points mirror those utilized in the aforementioned investigations with the exception of the initial measurement being at 40 minutes post-dose rather than 1hr. The rationale for this was to be consistent with the commencement of post-dose cognitive testing in previous chapters. Importantly, this testing regimen also allows for the measurement of cognitive function at key time-points relating to the pharmacokinetic plasma profile of resveratrol: which has been observed to begin rising between 45-90 minutes (chapter 3); to peak between 0.8-1.5hrs and to re-peak at ~5-6hrs post oral consumption (Boocock et al., 2007).

To summarise, the current randomised, double-blind, placebo-controlled, cross-over study will investigate the effects of 500mg resveratrol on cognitive performance with a relatively larger cohort of healthy, young adults as they undertake a cognitively demanding testing protocol which incorporates novel tasks. Due to a change in paradigm, namely the omission of the neuroimaging component (which, in large part, dictated sample size), a potentially more adequately powered analysis of cognitive function is included here and is a key component of this design.

6.2 Method

6.2.1 Participants

This sample comprised 50 healthy adults (11 males, 39 females, mean age 19.5yrs, range 18-22yrs, SD 1.2yrs, 46 right handed, 4 left) who took part in both treatment conditions of the study. Due to data catchment errors, and/or tasks not being properly completed at one or more time-point, four data sets were excluded from the final word recall analysis, seven for N-Back, NWM, Stroop and CRT, and eleven for Bond-Lader, Serial subtractions and RVIP.

With regards inclusion criteria, participants were required to be in good health and free from illicit drugs, alcohol, prescription medication, herbal extracts/food supplements, and have no relevant food allergies, intolerances or digestive problems. In addition, participants who had suffered a head injury, neurological- or neuro-developmental disorder were excluded from participation, as were those who had uncorrected sight problems, were pregnant, or seeking to become so. All participants were non-smokers and did not consume excessive amounts of caffeine (>6 cups of coffee or equivalent/d).

6.2.2 Treatments and standardised lunch

During the two study visits participants received two single-dose treatments in a counterbalanced order dictated by random allocation. The treatments comprised two capsules which combined to give either:

i) Inert placebo or

ii) 500mg *trans*-resveratrol.

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. No member of the investigational team was aware of the contents of the capsules until a blind-data review was completed.

Standardised lunch:

As part of this study paradigm participants were required to consume the following standardised lunch on both visits to prevent hunger-induced effects on study outcomes:

1x portion of pasta (100g dry weight Sainsbury's own brand penne) and tomato sauce (150g Sainsbury's own brand tomato and herb pasta sauce) served warm, and 1x 150g pot of Ambrosia original Devon custard. Participants ate together in a purpose built kitchen environment and were informed to eat as much as they wished in as much time as they needed.

6.2.3 Cognitive tasks and mood

This study utilized a 25 minute selection of tasks which comprised, in order of completion: Bond-Lader visual analogue mood scales; Word presentation and immediate word recall; Picture presentation; 2 minutes each of Serial 3 and 7 subtractions (described in section 2.2.3); 2-minutes of RVIP (also described in section 2.2.3); 2-minutes of 3-Back (described in section 4.2.3); 2 minutes of the Numeric working memory task; 2 minutes of the Stroop task; 2 minutes of the Choice reaction time task; Delayed word recall; Delayed word recognition; Delayed picture recognition; and completion of visual analogue scales (VAS) rating 'mental fatigue' and 'Difficulty' (described in section 2.2.3). The novel tasks incorporated here are described below:

Choice reaction time (CRT)

The CRT task requires participants to indicate, by pressing the 'left' or 'right' response box button, the direction of the arrow presented on the computer screen. Fifty stimuli (arrows) are presented, with varying delays, taking ~2 minutes to complete, depending on participant reaction time. The task is scored for percentage correct responses and reaction time (msec).

Stroop

The computerised Stroop task involves the presentation of a series of names of colours, on screen, one at a time. Some of the words are congruent with the colour of ink they are written in (e.g. the word red written in red ink) and some incongruent (e.g. the word red written in blue ink); with these conditions presented in random order. Participants were required to indicate, by pressing the appropriate coloured button on the response box, as quickly as possible, the colour of ink that the presented word was written in. Sixty words are presented taking ~2 minutes to complete, depending on participant reaction time, with the task scored for percentage correct responses and

reaction time (msec) with both task outcomes broken down for congruent and incongruent performance.

Numeric working memory (NWM)

For this task, a set of five target numbers are presented on screen, separately, with an inter-stimulus time of 1000ms. A series of numbers are then presented to which participants must respond 'yes' or 'no' as to whether they were part of the originally presented set or not. This task is repeated three times consecutively (with different target numbers each time) and is scored for percentage correct detections and reaction time (msec).

Immediate and delayed word recall

For the immediate word recall task participants are presented with 15 target words at the beginning of the task battery, on screen, one at a time (with a display and inter-stimulus time of 1000ms), and instructed to try and remember as many as they can. The words utilized in this task are all proper nouns, e.g. 'area', 'physics', 'beach'. Immediately after word presentation participants are instructed to note down as many of these words within a 60 second time limit (a count-down timer is provided on-screen) and to turn over the piece of paper when completed.

The Delayed word recall task is completed at the end of the test battery. Participants are informed via a computerised instruction page that they have one minute to note down as many of the words from the list presented at the beginning of the task battery as they can remember. After 60 seconds participants are asked to turn over the piece of paper and continue with the next task. The only task outcome is the number of correctly recalled words.

Delayed word and picture recognition

At the end of the test battery, word and picture recognition tasks are completed which use the stimuli presented at the beginning of the test battery. Word and picture recognition are completed separately but both require participants to differentiate, by pressing 'yes' or 'no' on the response box, between the 15 target words and pictures presented at the beginning of the test battery and 15 decoy words and pictures. The tasks take ~2 minutes to complete and are scored for percentage of correctly recognised words and reaction time (msec).

Bond-Lader VAS (Bond & Lader, 1974)

These VAS scales require participants to, using the mouse, indicate how they currently feel “at this moment in time” by clicking at the appropriate point along a 100mm scale on screen. Sixteen scales are presented with antonyms at either end, e.g. ‘alert’ V ‘drowsy’, ‘lethargic’ V ‘energetic’ and ‘troubled’ V ‘tranquil’, with these 16 scores (% along the line towards the right-hand adjective) combining to create three overall measures of mood: ‘alert’, ‘content’ and ‘calm’.

6.2.4 Procedure

Upon arrival at the lab participants first sat for at least a 5 minute rest in a quiet room. After filling out a breakfast log, participants completed Bond-Lader mood VAS and one baseline repetition of the cognitive tasks. Participants were then administered their treatment for that day and rested, in the lab, for a 40 minute absorption period. (For all breaks participants remained within the lab: either in the testing room working quietly or in a comfortable waiting room and were instructed not to eat or drink (apart from water) or to exert themselves or fall asleep during this time.) Participants then completed the same mood VAS and cognitive tasks for the first post-dose repetition and rested for a further 1hr and 20 minute break. After this, participants completed the second post-dose repetition (at 2.5hrs post-dose) and during the next 1hr rest break consumed their standardised lunch. At 4hrs post-dose participants completed the third post-dose repetition of mood VAS and cognitive tasks followed by a 1hr 30 minute rest break. The last repetition of the mood VAS and tasks was completed at 6hrs post-dose. Participants were then free to leave the lab.

The timeline and running order of both testing sessions, which was identical, is shown in Figure 6.1.

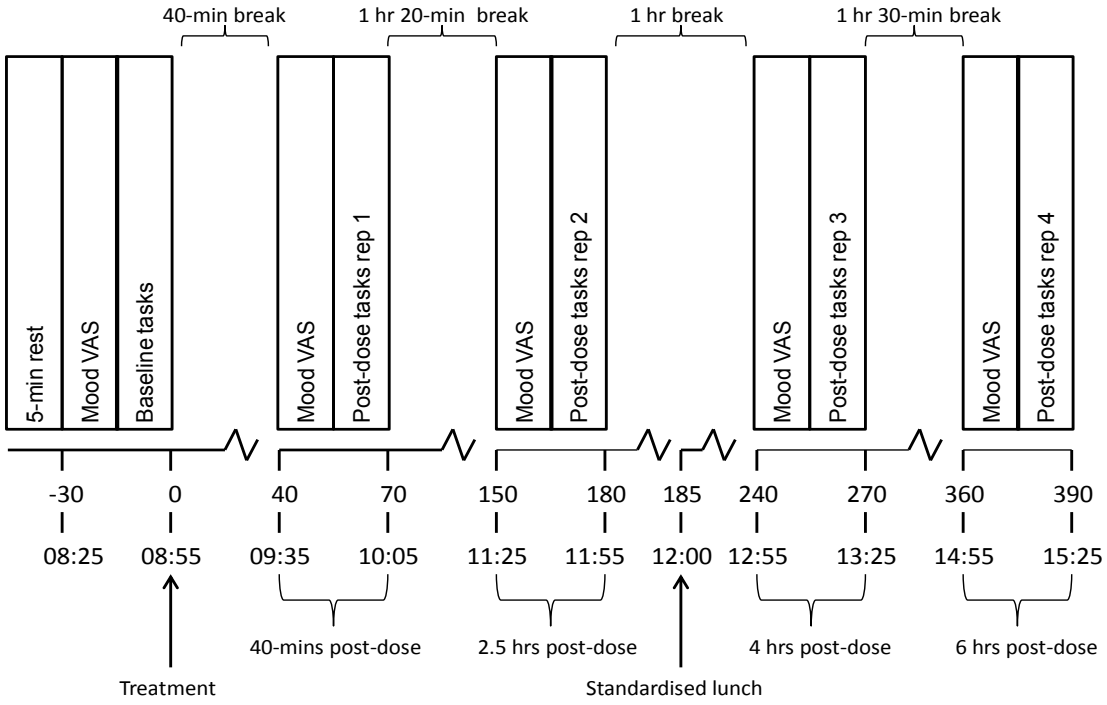


Figure 6.1. Chapter 6 study testing session timeline.

6.2.5 Statistics

Task performance and Bond-Lader mood data was analysed with SPSS version 16.0 for Windows (SPSS Inc, State College, PA) as change from pre-dose baseline, for each individual task outcome (Immediate word recall, Serial 3 and 7 subtractions, RVIP, 3-Back, Numeric working memory, Stroop, Choice reaction time, Delayed word recall, Delayed word recognition, Delayed picture recognition, 'mental fatigue', 'Difficulty', 'Alert', 'Calm' and 'Content') by within-subjects ANOVA (treatment x repetition) with Bonferroni corrected post-hoc comparisons conducted only if a significant main effect of treatment and/or treatment x repetition interaction was observed here. Prior to any analysis, baseline differences were investigated with regards these measures and any results only reported if significant.

6.3 Results

6.3.1 Cognitive task performance and mood

Bond-Lader VAS:

The omnibus ANOVA demonstrated no significant effects on the alert, calm and content Bond-Lader mood measures.

Cognitive tasks:

A significant interaction between treatment x repetition was seen on the 'delayed word recall' task [F(3, 135)= 3.35; p= .021]. There were no other treatment related effects on any measure. However, main effects of repetition were found on '3s correct' [F(3, 114)= 3.61; p= .016] and 'NBack' reaction time [F(3, 114)= 8.97; p< .001] with a trend for 'picture recognition' percentage correct also [F(3, 114)= 2.43; p= .069].

The pattern of effects over the four post-dose testing sessions for the 'delayed word recall' task measure were investigated further with post-hoc students t tests but revealed no significant differences between treatments at any of the four post-dose repetitions.

Figure 6.2 shows the data from the 'Delayed word recall' task at the four post-dose time points. The cognitive and mood data from all repetitions is presented in Table 6.1.

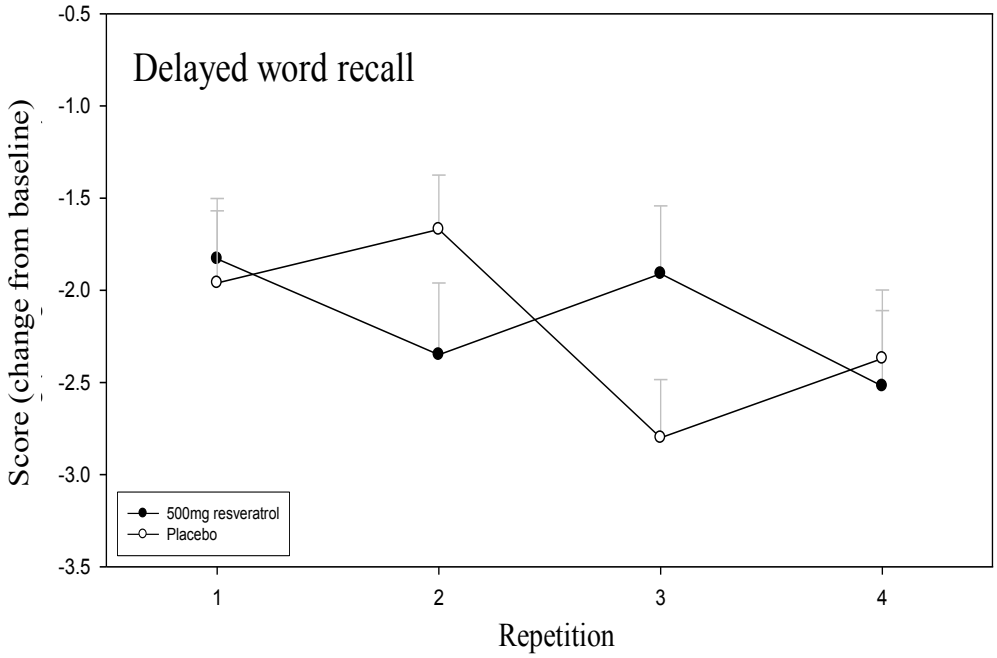


Figure 6.2. The effects of 500mg resveratrol and placebo on delayed word recall. Graph displays change from baseline means (with SEM error bars) for each of the four post-dose task repetitions.

Table 6.1. The effects of 500mg *trans*-resveratrol, compared to placebo, on mood and cognitive task performance across the day. Table displays raw baseline means (with SEM in italics, in brackets) and change from baseline values for all four post-dose time-points (i.e. 40mins, 2.5hrs, 4hrs, 6hrs) for both 500mg *trans*-resveratrol and placebo. Table also displays ANOVA F and P values for a main effect of treatment (T) and repetition (R) and an interaction between the two (T*R) with *<.05, **<.01 and t=trend.

Measure	Treatment condition	Pre-dose baseline score	Post-dose change from baseline score				ANOVA		
			40mins	2.5hrs	4hrs	6hrs	Effect	F	P
Bond-Lader 'Alert' (mm)	500mg resveratrol	52.07 *(1.89)*	3.73 *(1.17)*	3.75 *(2.32)*	6.02 *(2.17)*	3.01 *(2.16)*	T	.710	.004**
	Placebo	52.45 *(2.18)*	4.85 *(1.37)*	4.87 *(1.69)*	5.78 *(2.05)*	3.99 *(2.23)*	R	.256	.035*
							T*R	.883	.004**
Bond-Lader 'Calm' (mm)	500mg resveratrol	62.92 *(1.81)*	-2.40 *(1.50)*	-2.63 *(2.12)*	0.53 *(2.03)*	-2.14 *(2.40)*	T	.843	.001**
	Placebo	65.06 *(1.77)*	-2.01 *(1.29)*	-3.31 *(1.61)*	-0.92 *(1.64)*	-2.01 *(1.42)*	R	.077	.064 t
							T*R	.802	.009**
Bond-Lader 'Content' (mm)	500mg resveratrol	61.79 *(1.99)*	-1.33 *(1.28)*	-0.18 *(1.83)*	1.52 *(1.96)*	0.76 *(2.01)*	T	.893	<.001**
	Placebo	63.24 *(1.68)*	0.00 *(0.94)*	-0.49 *(0.93)*	0.08 *(1.17)*	0.27 *(1.20)*	R	.348	.028*
							T*R	.620	.015*
Immediate word recall (N^{o} correct)	500mg resveratrol	7.98 *(0.26)*	-1.15 *(0.25)*	-1.30 *(0.30)*	-0.35 *(0.27)*	-0.28 *(0.32)*	T	.624	.434
	Placebo	8.41 *(0.28)*	-1.13 *(0.32)*	-0.78 *(0.32)*	-1.04 *(0.32)*	-1.22 *(0.33)*	R	1.18	.148
							T*R	.004	.095
3s Correct (Number)	500mg resveratrol	36.38 *(1.62)*	2.87 *(0.95)*	2.62 *(1.15)*	5.23 *(1.00)*	5.64 *(1.16)*	T	.527	.472
	Placebo	38.46 *(1.86)*	2.95 *(0.93)*	2.72 *(0.97)*	4.05 *(1.08)*	3.54 *(1.06)*	R	3.61	.016*
							T*R	1.19	.316

3s Incorrect (Number)	500mg resveratrol	2.13 *(0.26)*	0.46 *(0.45)*	0.49 (0.37)	0.28 (0.38)	0.03 (0.35)	T R T*R	.001 .328 .491	.975 .805 .689
	Placebo	1.87 *(0.35)*	0.10 *(0.37)*	0.49 *(0.42)*	0.33 *(0.36)*	0.38 *(0.39)*			
7s Correct (Number)	500mg resveratrol	20.64 *(1.21)*	0.44 *(0.78)*	2.0 *(0.93)*	2.36 *(0.98)*	0.90 *(0.78)*	T R T*R	2.17 2.23 .599	.149 .096 .617
	Placebo	20.03 *(1.50)*	2.95 *(1.05)*	3.64 *(1.07)*	3.31 *(0.94)*	2.59 *(1.11)*			
7s Incorrect (Number)	500mg resveratrol	2.10 *(0.34)*	0.03 *(0.44)*	0.0 *(0.39)*	0.13 *(0.42)*	0.97 *(0.50)*	T R T*R	.785 1.98 .758	.381 .134 .520
	Placebo	3.05 *(1.01)*	-0.82 *(1.09)*	-0.92 *(1.05)*	-0.54 *(0.97)*	-0.54 *(0.99)*			
RVIP Correct (%)	500mg resveratrol	59.78 *(3.48)*	-2.56 *(2.42)*	-1.28 *(3.07)*	-0.16 *(2.56)*	0.96 *(2.71)*	T R T*R	.040 .263 .702	.843 .852 .553
	Placebo	63.78 *(3.62)*	0.0 *(2.90)*	1.44 *(3.05)*	-0.80 *(2.39)*	-0.96 *(2.70)*			
RVIP Reaction Time (ms)	500mg resveratrol	487.37 *(16.75)*	-13.09 *(8.72)*	-18.67 *(19.63)*	8.30 *(17.07)*	7.55 *(19.87)*	T R T*R	.084 .309 1.53	.773 .309 .217
	Placebo	486.41 *(8.63)*	-2.79 *(7.78)*	-10.91 *(10.25)*	-14.15 *(7.47)*	-5.10 *(10.20)*			
N-Back Correct (%)	500mg resveratrol	85.63 *(1.85)*	-0.10 *(1.03)*	0.67 *(1.02)*	-0.52 *(1.10)*	-1.03 *(1.59)*	T R T*R	.664 1.09 .208	.420 .349 .856
	Placebo	83.72 *(2.41)*	0.31 *(1.56)*	1.81 *(1.23)*	1.19 *(1.31)*	0.47 *(1.23)*			
N-Back Reaction Time (ms)	500mg resveratrol	806.32 *(37.28)*	-68.21 *(17.64)*	-100.04 *(23.80)*	116.40 *(25.65)*	-150.12 *(26.13)*	T R T*R	.684 8.97 .705	.413 <.001** .551
	Placebo	747.94 *(36.01)*	46.61 *(21.71)*	-85.73 *(19.60)*	-102.38 *(22.20)*	-106.42 *(24.72)*			
NWM Correct (%)	500mg resveratrol	95.87 *(0.44)*	-2.02 *(0.59)*	-1.45 *(0.62)*	-1.81 *(0.68)*	-1.86 *(0.61)*	T R T*R	.530 .238 .786	.471 .869 .504
	Placebo	94.78 *(0.66)*	-0.44 *(0.78)*	-1.37 *(0.94)*	-1.09 *(0.91)*	-1.37 *(1.00)*			

NWM Reaction Time (ms)	500mg resveratrol	744.43 *(21.68)*	-51.03 *(12.11)*	-48.51 *(14.73)*	-62.85 (15.25)	-44.43 (17.77)	T R T*R	.382 .822 .401	.540 .484 .752
	Placebo	736.95 *(22.80)*	-32.12 *(12.93)*	-42.38 *(14.33)*	-47.33 *(16.33)*	-42.17 *(15.84)*			
Stroop correct (%)	500mg resveratrol	96.55 *(0.43)*	-0.66 *(0.48)*	-0.35 *(0.60)*	-0.62 *(0.51)*	-0.70 *(0.55)*	T R T*R	.063 .034 .218	.803 .991 .884
	Placebo	96.40 *(0.46)*	-0.35 *(0.46)*	-0.54 *(0.50)*	-0.50 *(0.50)*	-0.34 *(0.48)*			
Stroop Reaction Time (ms)	500mg resveratrol	630.00 *(12.88)*	-10.25 *(9.32)*	5.69 *(33.33)*	-31.77 *(13.99)*	0.0 *(44.56)*	T R T*R	.088 .723 .705	.769 .463 .488
	Placebo	623.63 *(11.85)*	1.59 *(18.81)*	-22.70 *(9.10)*	-24.17 *(11.01)*	-23.87 *(22.70)*			
CRT Correct (%)	500mg resveratrol	97.19 *(0.52)*	-0.65 *(0.45)*	-0.84 *(0.43)*	-0.65 *(0.50)*	-1.26 *(0.43)*	T R T*R	.216 2.12 .395	.645 .102 .757
	Placebo	96.60 *(0.55)*	0.09 *(0.48)*	-0.65 *(0.61)*	-0.56 *(0.59)*	-1.21 *(0.67)*			
CRT Reaction Time (ms)	500mg resveratrol	406.28 *(9.47)*	10.69 *(6.44)*	50.59 *(51.58)*	1.43 *(13.90)*	30.50 *(43.53)*	T R T*R	.000 .483 1.01	.987 .614 .398
	Placebo	396.18 *(6.17)*	43.31 *(42.24)*	-0.89 *(6.65)*	9.57 *(8.56)*	38.91 *(43.00)*			
Delayed Word Recall (N^{o} correct)	500mg resveratrol	5.83 *(0.28)*	-1.83 *(0.33)*	-2.35 *(0.39)*	-1.91 *(0.37)*	-2.52 *(0.41)*	T R T*R	.012 1.89 3.35	.913 .134 .021*
	Placebo	6.02 *(0.31)*	-1.96 *(0.39)*	-1.67 *(0.30)*	-2.80 *(0.32)*	-2.37 *(0.37)*			
Delayed Word Recognition (% correct)	500mg resveratrol	82.33 *(1.24)*	-3.41 *(1.44)*	-3.81 *(1.49)*	-5.87 *(1.70)*	-4.92 *(1.69)*	T R T*R	.001 1.19 1.40	.982 .318 2.46
	Placebo	82.94 *(1.21)*	-6.27 *(1.64)*	-3.49 *(1.38)*	-5.40 *(1.67)*	-3.02 *(1.35)*			
Picture Recognition (% correct)	500mg resveratrol	93.49 *(1.09)*	-5.87 *(1.10)*	-6.43 *(1.48)*	-6.27 *(1.33)*	-6.59 *(1.47)*	T R T*R	.386 2.43 1.67	.538 .069 t .176
	Placebo	92.22 *(1.49)*	-3.25 *(1.34)*	-5.56 *(1.30)*	-7.94 *(1.77)*	-4.76 *(1.27)*			

Mental Fatigue (mm)	500mg resveratrol	45.19 *(2.60)*	6.91 *(2.90)*	4.52 *(2.82)*	3.43 *(2.79)*	6.07 *(3.55)*	T R T*R	.003 .993 .330	.953 .398 .753
	Placebo	45.98 *(2.48)*	5.48 *(1.97)*	3.69 *(3.39)*	5.24 *(2.98)*	7.36 *(3.54)*			
Difficulty (mm)	500mg resveratrol	42.70 *(2.35)*	5.10 *(2.44)*	3.88 *(2.29)*	3.36 *(2.56)*	3.00 *(2.90)*	T R T*R	.088 .195 1.54	.769 .870 .216
	Placebo	40.88 *(1.86)*	0.62 *(2.02)*	3.19 *(2.44)*	4.45 *(3.06)*	3.10 *(3.24)*			

6.4 Discussion

The overall aim of this study was to investigate the cognitive effects of 500mg resveratrol in healthy, young humans. The specific aim of this chapter was to broaden the cognitive domains assessed by the cognitive tasks used in previous chapters and to delineate the time course of any effects by testing at four time points over the six hours following consumption of the day's intervention. It was argued that these potential confounds were the most prevalent methodological issues that could explain the null/weak effects of resveratrol on cognitive performance despite its consistent CBF effects.

The results of the current study once again show that a single dose of 500mg of resveratrol is incapable of beneficially modulating cognitive function. The only significant treatment related effect was restricted to a single task outcome (delayed word recall) and was largely interpretable; most likely representing a chance finding. However, there was some evidence of modulation of transient mood, with a decrease in 'alertness', but increases in 'contentedness' and 'calmness' across the post-dose assessments associated with resveratrol consumption.

The tasks utilized in this chapter comprised those used throughout this PhD (serial subtractions, RVIP and N-Back) alongside the novel tasks; Stroop, NWM and word and picture immediate and delayed word recall and recognition. As no study to date has investigated the potential cognition enhancing effects of resveratrol in humans (thus providing no indication of the aspects of cognition which might be sensitive to the effects of resveratrol) the rationale for incorporating these tasks was to provide a broad assessment across the major cognitive domains. It therefore seemed logical to expand the aspects of cognition under assessment from attention, working memory and executive functioning (serial subtractions, RVIP, N-Back) to include response inhibition (Stroop) and aspects of long-term memory (word and picture immediate and delayed recall and recognition). The latter of these tasks have also previously proven sensitive to changes in neural fuel provision, i.e. oxygen (Moss et al., 1998).

In order to increase workload demands in this young, healthy sample (at their cognitive peak (Rönnlund et al., 2005)) a more intensive testing regimen was utilized to amplify the demand for cognitive resources inculcated by the demanding tasks alone. The rationale here was predicated on the assumption that the young, healthy participants (which comprise the samples throughout this programme of studies), might not be sufficiently cognitively compromised by the tasks alone and not to an extent which could be reversed by resveratrol-mediated increases in CBF and oxygen utilization. Thus the current study adopted a similar protocol to Kennedy et al. (2001) and Reay et

al. (2010) by repeating cognitive assessment at 40 minutes, 2.5hrs, 4hrs and 6hrs post-dose.

However, despite the assessment of novel cognitive domains and the extension of the assessment period throughout the time-frame corresponding to peak bioavailability for resveratrol's metabolites, the current study demonstrates that 500mg resveratrol is unable to affect cognitive performance. This finding supports the lack of cognitive effects observed in chapters 2, 3 and 4 and suggests that the weak effect of resveratrol on N-Back % correct, in chapter 5, is more likely a chance finding rather than a replicable effect of resveratrol on cognitive function.

In terms of mood, this chapter reports that participants in the placebo condition reported themselves to be significantly more alert, on average, across the day, as compared to placebo. Participants in the resveratrol condition however, reported themselves to be significantly more calm and content across the day as a whole. No baseline differences were observed with either of these three mood measures and no significant differences between treatments were observed at specific time-points during the day; suggesting that resveratrol was conferring anxiolytic effects and that this was a general improvement in mood across the day rather than restricted to specific times. Research has yet to investigate the potential mood effects of resveratrol in humans but a number of animal studies suggest that resveratrol may have beneficial anxiolytic effects and that this may be as a result of modulation of monoamine neurotransmitters. Xu et al. (2010), for example, observed reduced immobility in mice during despair tasks with 10-, 20-, 40- and 80mg/kg *trans*-resveratrol administered by intestinal gavage. These results were similar to those evinced by imipramine and fluoxetine and all treatments increased levels of monoamine neurotransmitters; namely serotonin (5-HT), noradrenaline and dopamine, in the frontal cortex. At doses of 40- and 80mg/kg resveratrol also increased 5-HT levels in the hippocampus and, at 80mg/kg it increased levels of noradrenaline. This modulation was attributed to the ability of resveratrol to inhibit MAO-A (and MAO-B in the case of 80mg/kg), an effect that was seen ~30-minutes after administration.

In conclusion, this chapter investigated the cognitive enhancing effects of 500mg resveratrol utilizing novel cognitive tasks and a more demanding testing paradigm, as compared to previous chapters. The lack of cognitive effects reported here is supported by chapters 2, 3 and 4; where no effects were seen, and chapter 5; where resveratrol affected performance on only one task sub-measure; likely a type I error. Calmness and contentedness were improved by resveratrol suggesting anxiolytic properties. These mood effects are supported by animal data but further investigation is required

in humans to clarify the strength and duration of this effect and whether it extends to other aspects of mood.

Chapter 7.

General discussion

The rationale for investigating the potential cognition enhancing effects of resveratrol was predicated on its putative ability to increase neural access to metabolic substrates, i.e. oxygen and glucose (supplementation of which has previously been shown to enhance aspects of cognitive performance (Kennedy & Scholey, 2000; Moss et al., 1998; Scholey et al., 1999)), via vasorelaxation-induced increases in cerebral blood flow (CBF) (Ritz, Curin, Mendelowitsch, & Andriantsitohaina, 2008). It may also augment utilization of this fuel by enhancing oxidative phosphorylation of mitochondria directly (Lagouge et al., 2006). This PhD aimed to test the hypothesis that resveratrol is capable of enhancing cognitive function, via CBF augmentation, in young, healthy human participants with five intervention studies.

7.1 Summary of empirical study findings

The first study was a double-blind, placebo-controlled, crossover pilot study (chapter 2) with 22 participants which demonstrated that resveratrol, at doses of 1000- and 500mg, was potentially able to modulate the haemodynamic CBF response to task performance and oxygenation, but in the opposite direction to that predicted. Cognitive performance was unaffected by either dose. The overall conclusion of chapter 2, however, was that the interpretation of the results from this study was muddied by the methodological limitations of the measurement of CBF with NIRS; i.e. commencing recording of the hemodynamic response after a treatment effect is likely to have taken place. The constraints of NIRS are discussed further in sections 7.2 and 7.6.1.

The second study (chapter 3), a placebo-controlled, double-blind, crossover study with 22 participants, addressed the aforementioned methodological issues in the first study by monitoring CBF from treatment administration through the absorption and post-dose task period. The acute hemodynamic effects of resveratrol now became much clearer and supported the hypothesis of resveratrol mediated enhancement of CBF. In this study it was demonstrated that 500mg resveratrol significantly augmented CBF (i.e. increased total-Hb and oxy-Hb) in the prefrontal cortex in response to cognitively demanding tasks that activate this brain region, beginning 45 minutes after the treatment administration. Increased utilization of this fuel, as evidenced by higher levels of deoxy-Hb, was also evinced following 250- and 500mg resveratrol; with this effect beginning 36 minutes post-dose. Consistent with chapter 2, no effects of either dose of resveratrol were observed on cognitive task performance. The potential explanation for

a lack of cognitive effects here, and taken forward to inform the rationale of the subsequent study, was that the bioavailability of resveratrol (as assessed in a separate sample of nine males) may have been too low (with a C_{max} of the aglycone at 5.65- and 14.4ng/mL after 250- and 500mg resveratrol respectively; of the glucuronide metabolite: 48.9- and 202.2ng/mL; and of the sulfate: 300.4- and 746.1ng/mL respectively) to induce effects on CBF parameters and mitochondrial function sufficient to influence cognitive function.

The third study (chapter 4), also a double-blind, placebo controlled, crossover study with 22 participants, therefore aimed to alter the natural metabolism of resveratrol (via co-supplementation with piperine) *in vivo* such that bioavailability may be increased and its efficacy, in terms of cognitive function, might be enhanced. Co-supplementation of 250mg resveratrol with 20mg piperine did indeed increase the efficacy of resveratrol in terms of enhancing CBF (as 250mg had proved to be less effective than 500mg in influencing CBF in chapter 3); as demonstrated by significantly higher levels of total-Hb, oxy-Hb and deoxy-Hb, compared to placebo (although a piperine-only condition was not incorporated into this design and would be required before fully committing to the statement that piperine was enhancing resveratrol rather than exerting these effects itself), and potentially BP; although this was opposite to the anticipated direction of effects and could have been the product of some unknown vascular effect of piperine. Interestingly, the results of a bioavailability study in a separate sample of six males demonstrated no significant differences in plasma levels of resveratrol metabolites (aglycone not measureable) between the resveratrol alone condition and when co-supplemented with piperine. This suggested that another piperine-mediated mechanism was enhancing the efficacy of resveratrol with regards CBF enhancement, perhaps as a result of thermogenesis in vascular and neural tissue. Once again there was no interpretable effect on cognitive task performance. The argument was posited that the persistence of no effects of resveratrol on cognitive performance might be as a result of the ineffectiveness of acute, bolus supplementation of resveratrol; i.e. that this treatment regimen was not sufficient to inculcate the necessary plasma levels required to influence CBF and to the extent that concomitant enhancement of cognitive function could be induced.

The fourth study (chapter 5), and the penultimate study in this programme, was therefore a placebo-controlled, double-blind, between subjects investigation assessing the acute and chronic (28 day) effects of 500mg resveratrol on CBF as assessed by NIRS and TCD (NIRS: N=46; TCD: N=46) and cognitive performance (N=41). This study also assessed subjective perceptions of health (N=53), mood (N=54) and sleep quality (N=53) across the 28 day period. The hypothesis here was that repeated dosing

may evince a cumulative increase in plasma resveratrol levels or might impact some unknown mechanism that requires longer term activation, and that this more ecologically valid method of supplementation might also influence overall health and wellbeing. Results of plasma samples taken from a sub-sample of seven participants showed that repeated dosing did indeed result in cumulative plasma levels of resveratrol, with metabolites present at pre-dose on day 28 and significantly higher at day 28 post-dose. The acute CBF effects of resveratrol, as assessed by NIRS, were consistent with the preceding investigations, albeit slightly weaker in effect; which may be due to the increased variability in this between-subjects design. No chronic NIRS effects of resveratrol were found however, and whilst this might represent a lack of efficacy of resveratrol, the argument was again made here that the constraints of NIRS measurement (discussed in sections 7.2 and 7.6.1) might be masking an effect. A further neuroimaging technique was applied alongside NIRS in this chapter to try and mitigate the constraints of NIRS and provide a quantitative measure of CBF change across the 28 days. However, as no acute or chronic CBFV effects were observed with TCD, this technique could not clarify the NIRS effects observed here. No subjective effects of resveratrol on health or sleep quality were reported but fatigue was significantly lower in the resveratrol condition at three out of four post-dose weekly self-assessments made by the participants. This could be tentatively interpreted as being indicative of the effects of resveratrol on mitochondrial activity and energy expenditure. This chapter was the first to report any cognitive effects of resveratrol but, as this was restricted to the gross-chronic improvement of only one sub-measure of one task, with no indication of efficacy in previous chapters, this finding was considered to represent a type I error rather than a true effect of resveratrol. Finally, the analysis of BP measurements demonstrated, counter-intuitively, increased diastolic BP in resveratrol-treated participants after 28 days supplementation and this, coupled with the lack of day 28 CBF effects, might suggest that resveratrol was not acting as a vasodilator after chronic consumption. Further investigation, beyond the scope of this PhD, would be needed to clarify this argument however.

The fifth and final study (chapter 6) in this programme; a placebo-controlled, double blind, crossover investigation, addressed the foremost remaining issues with the previous investigations; that the testing protocols might have been relatively un-demanding on the young, healthy cohorts utilized and that the tasks might be too narrow in their assessment of the cognitive effects of resveratrol. Chapter 6 also aimed to assess the time-course of resveratrol's effects across the period of its greatest bioavailability with assessments pre-dose and at 40 minutes, 2.5-, 4-, and 6hrs post-dose. The study also incorporated novel tasks alongside those previously used to

provide a more comprehensive assessment of cognitive function. However, despite the assessment of novel cognitive domains, and the extended time-course of assessment, this final study demonstrated that 500mg resveratrol was unable to significantly affect cognitive performance. This finding supports the lack of cognitive effects observed in chapters 2, 3 and 4 and adds further support to the argument that the weak effects of resveratrol on N-Back % correct, in chapter 5 was more likely to be a type I error rather than true and replicable effects of resveratrol on aspects of cognitive function.

7.2 Discussion of the cerebral blood flow and oxygenation effects of resveratrol

The hypothesis that resveratrol would be able to engender increased CBF, and enhanced oxygen utilization, has been confirmed in two out of the four studies which have utilized NIRS within this thesis (chapters 3 and 4 respectively) with confirmation of this effect from the acute measurement in chapter 5 (albeit to a weaker extent). All of these studies monitored CBF in the prefrontal cortex from before treatment administration and throughout the absorption and post-dose task periods. Chapter 3 demonstrated increased CBF (as evidenced by higher concentrations of total- and oxy-Hb) throughout the entire post-dose task period after 500mg resveratrol, and increased oxygen utilization (as evidenced by higher concentrations of deoxy-Hb) at this dose and 250mg, in comparison to placebo (see figure 3.3). Chapter 4 confirmed these findings with a 250mg dose of resveratrol, which proved less effective than the 500mg dose in chapter 3, but only when its efficacy was enhanced with 20mg piperine co-supplementation (see figure 4.3). Here NIRS again recorded increased CBF (i.e. significantly higher levels of total- and oxy-Hb) and oxygen utilization (i.e. higher concentrations of deoxy-Hb) across the entire post-dose task period.

That CBF enhancement was evinced by resveratrol predominantly during periods of increased cognitive workload (i.e. the post-dose task period) supports the hypothesis that resveratrol's mode of action here is by amplifying the natural nitric oxide (NO)-driven vasodilation that takes place in response to increased neural activity. The normal haemodynamic response to task performance can be seen in the observation of increased CBF (see figure 3.3) in the placebo condition in chapter 3 from the last epoch of the absorption period to the first epoch of the post-dose task period; demonstrating a natural task-induced demand for metabolic substrates. Both 500mg and 250mg (albeit to a lesser extent with the latter dose) resveratrol amplified this natural response and evinced a significantly greater CBF response than placebo.

The enhanced oxygen utilization (i.e. higher deoxy-Hb compared to placebo) reported in both chapter 3 and 4 supports findings which have hitherto only been investigated in

animal models, i.e. that resveratrol is capable of enhancing oxidative phosphorylation of mitochondria (Lagouge et al., 2006). However, in the face of the scant significant outcomes of resveratrol in terms of cognitive function throughout this programme of studies, it is not possible to assert whether or not this augmentation of oxygen utilization was of positive benefit.

That significant CBF effects of resveratrol were not observed in the pilot study (chapter 2), or in the chronic arm of chapter 5, can be seen as a product of the methodological flaws relating to NIRS measurement. In both studies the interpretation of CBF effects is constrained by the existence of a break in NIRS recording between treatment administration and the recording period (95 minutes post-dose in chapter 2 and 28-days after the commencement of daily 500mg resveratrol supplementation in chapter 5). The flaw here regards the manner in which NIRS calculates haemoglobin levels. The continuous-wave (C-W) NIRS used here presents haemoglobin levels as a concentration (μmol/L) change rather than in absolute levels. As such, if a gross increase in CBF levels had taken place in resveratrol-supplemented participants across the break in recording (meaning that this group began the resumed NIRS recording period with higher CBF than placebo participants) C-W NIRS would be unable to quantify this and would only provide concentration change data within the resumed period itself. This is further compounded by the possibility that, if resveratrol-supplemented participants had experienced a global increase in CBF across the break their requirement for additional local blood flow during neural demand may well have been reduced, or alternatively their resting blood flow may have been nearer to ceiling and unable to rise as much as in the placebo condition.

However, the fact that both studies demonstrate the same effect, i.e. that of placebo evincing greater CBF effects than resveratrol or, no difference in levels, in the resumed NIRS recording period, adds credence to the argument that the resveratrol-treated participants had already experienced a global increase in CBF prior to the commencement of recording. This is further supported by the fact that the two aforementioned studies which have measured the CBF response to resveratrol across the entire testing session, i.e. with no break (chapters 3 and 4), have both observed that resveratrol evinces a significantly higher CBF response during post-dose task completion, as compared to placebo.

The CBF response to resveratrol, as recorded by NIRS, has also raised some unanticipated areas for discussion. These relate to the relatively weaker acute effects of resveratrol evinced during day 1 in chapter 5 and the observation in chapters 3 and

4 of deoxy-Hb evincing significantly higher levels at the end of the absorption period (pre the post-dose task period) after resveratrol, compared to placebo.

To take the first issue, in chapters 3 and 4 the pattern of CBF effects evinced by 500mg resveratrol and 250mg resveratrol with 20mg piperine respectively, were largely consistent across the entire post-dose task period for all three NIRS chromophores. In the acute arm of chapter 5 however, this was not the case; with 500mg resveratrol failing to achieve a significant effect on the ANOVA and in terms of planned comparisons (undertaken without reference to the ANOVA) only evincing significantly higher concentrations of total-Hb, as compared to placebo, at half of the post-dose task epochs (10/20) and only 2 post-dose task epochs with regards oxy-Hb levels. Unexpectedly, and inexplicably, the levels of deoxy-Hb (acutely) were significantly higher after placebo, as compared to 500mg resveratrol, across the post-dose task period; which is contrary to those findings in chapters 3 and 4. The only possible explanation that can be provided for these weaker, and contrary, CBF results at present is that chapter 5 utilizes a between-subjects paradigm whereas chapters 3 and 4 are both crossover designs. This suggests that individual differences with regards the CBF response to treatment and tasks is more marked than might be anticipated and might be responsible for the weaker results in chapter 5. As support for this argument, studies utilizing NIRS to monitor the CBF response to cognitive tasks only, have reported large individual differences with regards neural activation (Meek et al., 1998; Shibuya-Tayoshi et al., 2007).

Secondly, chapters 3 and 4 both demonstrate significantly higher levels of deoxy-Hb (i.e. enhanced oxygen utilization) in response to resveratrol which begin during the last epochs of the absorption period. This represents a period when participants are in a non-aroused state and, therefore, if resveratrol's mechanism of action is to enhance demand-driven CBF, this effect should not exist. The only potential explanation here is that resveratrol may be facilitating a preparatory enhanced CBF response to impending increased workload.

Finally, this thesis also utilized a secondary neuroimaging methodology; trans-cranial Doppler (TCD) in chapter 5 in order to monitor cerebral blood flow velocity (CBFV) in the middle cerebral artery (MCA). This converging of operations with a measure of quantitative hemodynamics was intended to compliment the concentration change CBF data from NIRS. The two techniques have been used successfully in conjunction previously (Ide et al., 1999) and it was hoped that the constraints in NIRS measurement (discussed in sections 7.2 and 7.6.1) could be countered by TCD; which lends itself better to repeated testing over periods of time. Unfortunately, resveratrol

failed to elicit any effect on CBFV and so TCD was unable to offer any clarification of the NIRS results obtained in chapter 5. These results were somewhat unexpected; chiefly because resveratrol had evinced such a consistent pattern of effects on CBF, as assessed by NIRS, in chapters 3 and 4. Previous research with cocoa flavanols had also demonstrated the efficacy of vasodilatory polyphenols on CBFV, as measured by TCD (Sorond et al., 2008). The only explanation that could be provided for a lack of results from TCD, despite significant hemodynamic modulation data from NIRS, was that the two were recorded separately; the latter during cognitive task demands and the former prior to and following this cognitive task period. As such, metabolic substrate demands would have been less during the TCD recording periods and an increase in the hemodynamic response unnecessary.

To summarise the CBF effects of resveratrol: the ability of resveratrol to amplify the natural neural demand-driven, NO-mediated enhancement of CBF in the prefrontal cortex has been confirmed by this thesis. This manifested as significantly higher concentrations of total-, oxy-, and deoxy-Hb in response to 500mg resveratrol (chapter 3 and, to a lesser extent, chapter 5) and 250mg resveratrol alone (chapter 3) and with 20mg piperine (chapter 4). That placebo also evinced an increase in CBF in response to cognitively demanding tasks (chapter 3), although not significant, supports that resveratrol is enhancing a natural workload increase in metabolic substrate demand. Both findings support the hypothesis that resveratrol is exerting CBF effects by interacting with the vasodilatory mediator NO.

The hemodynamic effects of resveratrol reported in this thesis are, in many respects, broadly in line with previous intervention studies. The typical hemodynamic response to increased neural activity, whether assessed by NIRS (Herman, Ehlis & Falgatter, 2003) or FMRI (Tamura, Hoshi & Okado, 1997), is an up-regulation of total-Hb and oxy-Hb as the fuel demands of neurons increases. By the simple fact of there now being more oxygen present, we also see a reduction in the levels of deoxy-Hb. This natural neurovascular coupling between preceding neuronal electrical activity and the subsequent CBF response is mediated, in part, by the actions of the vasodilator NO. It is this mechanism that many of the interventions previously discussed in this thesis are argued to interact with; e.g. docosahexaenoic acid (Jackson et al., 2012a, 2012b), caffeine (Kennedy & Haskell, 2011) and the polyphenols epigallocatechin gallate (EGCG) (Wightman et al., 2012) and cocoa flavanols (Francis et al., 2006). If this argument holds then what we would expect to see is merely an amplification of this natural, NO-mediated, hemodynamic response following consumption of the above interventions. Indeed, broadly, this is what we do see. Both EGCG and caffeine are associated with reduced CBF and their actions are attributed to a down-regulation of

NO. Long-chain fatty acids and cocoa flavanols, however, are observed to enhance the hemodynamic response. With regards the former, a 12 week supplementation of 1g daily DHA-rich fish oil was reported to significantly increase concentrations of total- and oxy-Hb in the prefrontal cortex of 22 healthy adults (Jackson et al., 2012a); as assessed by NIRS. However, no effects were found with levels of deoxy-Hb. With regards cocoa flavanols, a significant amplification of the BOLD signal was seen following 5 days supplementation with 172mg flavanols daily, in 16 healthy, young (18-30yrs) females (Francis et al. 2006). Evidence of a link here between flavanol-augmented CBF and NO can be seen in an earlier study which demonstrated significant peripheral vasodilation in response to 4 weeks of 821mg daily cocoa; an effect which was abolished following the NO inhibitor LNAME (Fisher et al., 2003). That levels of total- and oxy-Hb are observed to be significantly higher following resveratrol consumption in this thesis, is arguably indicative that it too is amplifying the CBF response by interacting with NO.

Where the hemodynamic responses between resveratrol and the other above vasodilatory compounds differ however, is with regards levels of deoxy-Hb. As stated above, the anticipated response of deoxy-Hb would be to fall in the presence of increased levels of oxy-Hb; indeed the BOLD signal is predicated on this response. However, in all chapters of this thesis where hemodynamics were monitored throughout the testing session (i.e. chapters 3 and 4[28]), levels of deoxy-Hb are higher during cognitive demand following the consumption of resveratrol, as compared to placebo. In the above chapters, these higher levels of deoxy-Hb are concomitant with higher levels of oxy-Hb also; and so this anomalous finding is not due to some reversal of the ratio of oxy-Hb to deoxy-Hb. Rather, in line with the hypothesis, what this increase in deoxy-Hb levels likely represents is an increase in the extraction of oxygen from these increased levels of oxy-Hb. This assumption is based on the ability of resveratrol to influence cellular oxygenation by interacting with mitochondrial phosphorylation; a mechanism most likely facilitated indirectly by its influence on the SIRT pathway. SIRT-1 deacetylates PGC-1α (Rodgers et al., 2005), a gene which controls mitochondrial biogenesis and function, and the ability of resveratrol to interact with this mechanism most likely explains the increase in mitochondrial number (Baur et al., 2006) and function (Lagouge et al., 2006) observed in resveratrol-treated animals. With regards the latter study, 400mg/kg/day resveratrol, for a 15 week supplementation period, significantly enhanced mitochondrial structure size and activity in mice; an effect which resulted in increased oxygen consumption and physical performance. In humans, whilst this effect has not been investigated directly with resveratrol, indirect

[28] Why this wasn't also the case in the acute arm of chapter 5 is discussed previously in this section.

evidence from another red wine polyphenol; quercetin, also finds increased physical performance. Specifically, this performance was high-intensity cycling and was observed in 11 elite male athletes after consumption of quercetin, as part of an antioxidant drink, twice daily for 6 weeks (MacRae & Mefferd, 2006). Taken together, these studies suggest that resveratrol is potentially capable of augmenting oxygen extraction and that this can increase the performance of metabolically active tissue. If we extend this premise to active neural tissue, then we should anticipate higher levels of deoxy-Hb in the prefrontal cortex; and indeed that was the case. Of course it was also anticipated that the cognitive performance sub-served by this metabolically active brain region would also be enhanced but, the findings in this thesis suggest that this wasn't the case.

7.3 Discussion of the cognitive and mood effects of resveratrol

The hypothesis of this programme of studies was that resveratrol would enhance CBF in healthy, young humans and, in turn, that the increased neural access and utilization of metabolic substrates would enhance cognitive performance. Resveratrol-mediated enhancement of CBF has been consistently seen throughout this thesis but cognitive performance effects have not.

Chapters 2, 3, 4 and 6 observed no effects of 1000- and 500mg resveratrol on any task measure. To put this lack of effects into perspective: chapters 2 and 3 comprised three tasks; equalling six and seven sub-measures respectively, chapter 4 utilized five tasks with 10 sub-measures and chapter six comprised 10 potential tasks with 18 potential sub-measures. That none of these 41 sub-measures were affected by resveratrol really does quite clearly demonstrate its lack of efficacy in terms of modulating cognitive performance. Chapter 5 was the only chapter to report any cognitive effects of resveratrol: a pure chronic improvement in accuracy on the N-Back task. This represented improved performance on 1/10 sub-measures, from five tasks, but wasn't found acutely or following treatment administration on day 28. When considering this finding in light of the lack of cognitive effects of resveratrol in all other chapters as well, it is most likely that this represents a type I error rather than a true modulation of performance by resveratrol.

It would be interesting at this point to compare the cognitive effects, or lack thereof, of resveratrol here to previous investigations. As stated in the aims for conducting this thesis however, there is no existing research assessing the cognitive effects of resveratrol in humans. The hypothesis for anticipating cognition enhancing effects of resveratrol here was based on the potential for increased access to metabolic

substrates facilitated by augmented CBF. Again, however, this latter mechanism hadn't even been confirmed directly in animal or humans models. It is, however, possible to draw some comparisons with studies assessing the effects of structurally similar polyphenols- principally cocoa flavanols.

In healthy, young adults improvements have been observed on spatial memory performance, the detection of stimuli movement and sensitivity to visual contrast following acute supplementation of 720mg cocoa flavanols (Field, Williams & Butler, 2011). In a cohort of older adults suffering from mild cognitive impairment, 8 weeks supplementation with 990- and 520mg cocoa flavanols was associated with improved verbal fluency and performance on the trail maker task (Desideri et al., 2012). If we look now to research which incorporates some of the cognitive tasks utilized in this thesis, we see that 994- and 520mg cocoa flavanols can improve performance on the RVIP task in healthy, young adults (Scholey et al., 2010). However, a significant increase in errors was reported on the serial 7s subtractions following the higher dose. Further, a later study by the same lead author failed to find any cognitive enhancing effects following 30 days consumption with 500- or 250mg cocoa polyphenols in healthy, young adults although mood was improved with the higher dose (Scholey et al., 2013).

The cognitive enhancement observed in the above polyphenol research may be predicated on the augmented CBF delivery of metabolic substrates to active regions of the brain. As such, the hypothesis of potential cognitive enhancement of resveratrol; as a result of enhancing this fuel provision via vasodilation and CBF augmentation, was based on research which has investigated the cognitive effects of this metabolic substrate supplementation alone. As an example, inspiration of pure oxygen for 1- and 3 minutes has been observed to significantly improve immediate and delayed word recall and tests of attention following 30 seconds of inspired O_2 (Moss et al., 1998; Scholey et al., 1999) in a group of young (mean age 24.5yrs) adult volunteers. In another sample of young (mean age 20.4yrs) undergraduate students, the administration of 25g glucose improved performance on the serial 7s subtractions; increasing the number of subtractions completed (Kennedy & Scholey, 2000). Whilst the oxygen supplementation literature is quite small the cognitive effects of glucose, particularly the facilitation of memory (Smith et al., 2011), is larger and more robust and, when taken together, suggests that compounds which can up-regulate access to these metabolic fuels should also be capable of improving cognitive function.

This thesis, however, failed to support this hypothesis finding only improved accuracy on the NBack task, in one chapter, following resveratrol. It is striking that this

represents cognitive effects in only 1/5 of the experimental chapters in this thesis and, as discussed previously, is more likely indicative of type I error rather than an actual resveratrol-mediated improvement in performance. However, this thesis (chapter 5) did observe attenuated ratings of fatigue following chronic supplementation of 500mg resveratrol; specifically at three out of the four post-dose weekly measurements, as compared to placebo.

Very little research exists regarding the effects of polyphenols on mood and research has yet to investigate the effects of resveratrol on mood, in humans, specifically. However, increases in ratings of calm and contentedness have been seen following cocoa supplementation (Pase et al., 2013) and this effect may find an explanation in *in vitro* and animal work which report the ability of resveratrol to inhibit Monoamine Oxidase-A and B (MAO-A/B) activity. This inhibition was reported to lead to an increase in monoamine neurotransmitter concentrations, namely 5-hydroxytryptophan (5-HT), noradrenaline and dopamine, with a concomitant improvement in mood; similar to that seen with imipramine and fluoxetine, in mice (Xu, Li, et al., 2010). Another potential anti-fatigue mechanism, specifically, is predicated on resveratrol's structural similarity to the flavonoid quercetin, which has been reported to increase energy expenditure and endurance capacity in mice (Davis, Murphy, Carmichael, & Davis, 2009; Stewart et al., 2008) and power output in elite male cyclists when taken as part of a cocktail of supplemented compounds (MacRae & Mefferd, 2006). Putative mechanisms for these effects include increased blood flow; due to vasorelaxation (Chen & Pace-Asciak, 1996), and oxygenation; with Davis et al. also reporting SIRT-mediated increases in mitochondrial gene expression in brain and skeletal muscles. Both mechanisms are shared with resveratrol (Chen & Pace-Asciak, 1996; Lagouge et al., 2006) and could explain the increased energy levels seen here.

The above provides some potential explanations for the mood effects of resveratrol reported in chapter 5. In the face of no directly comparable research however, these arguments are hypothetical and, of course, one has to accept that resveratrol could also be interacting with any number of unseen mechanisms in order to facilitate these effects. It could also be argued that the numerical difference (although not statistically different) in baseline fatigue ratings could represent a return to 'normal' levels for those in the resveratrol condition after an unusually high baseline. These findings do, however, provide an interesting avenue for future research where the strength and duration of these mood effects can be investigated further.

7.4 Discussion of the bioavailability of resveratrol

Within this thesis, three studies included an investigation into the bioavailability of resveratrol in plasma. Chapter 3 measured concentrations (ng/mL) of resveratrol aglycone and two metabolites (a sulfate and glucuronide) after 250- and 500mg resveratrol at baseline, 45-, 90- and 120 minutes post dose in a separate (from the cognitive/NIRS assessment) group of nine young (mean age 24.8yrs) males. The rationale for investigating plasma levels here was to ascertain if resveratrol was bioavailable during key time-points in the aforementioned cognitive/NIRS assessment and, therefore, had the potential to exert physiological effects during this time. Chapter 4 was able to measure concentrations (μM) of resveratrol metabolites only (one sulfate and two glucuronides) after 250mg resveratrol alone and when co-supplemented with 20mg piperine at the same time-points as previously, i.e. baseline, 45-, 90-, and 120 minutes post-dose. Again this was in a separate sample of participants to the cognitive/NIRS aspect of this investigation due to practicalities of not being able to take intermittent blood samples during NIRS testing. This sample comprised six healthy, young (mean age 25.8yrs) males also. The rationale for assessing bioavailability here was to observe whether the purported bio-enhancer piperine was capable of increasing plasma levels of resveratrol and, in turn, influence its efficacy. Finally, chapter 5 measured concentrations (μM) of the same metabolites as above at baseline and post-dose (110 minutes post treatment administration) on day 1 and day 28 of a chronic dosing regimen of 500mg resveratrol daily. The participants here were a sub-sample of the larger cognitive/NIRS aspect of this study and comprised six females and one male with a mean age of 19.43yrs. The rationale for assessing plasma bioavailability of resveratrol here mirrored the above in many respects in that the aim of the study was to ascertain if the treatment regimen, in this case chronic dosing, could inculcate an enhancement in bioavailability and, in turn, efficacy.

The findings of these three studies demonstrate several key discussion points: firstly, that, in line with all previous literature regarding the bioavailability of resveratrol, the plasma levels of the aglycone were extremely low/un-measurable; secondly, that resveratrol, at least in metabolite form, was bioavailable during key time-points relating to cognitive/NIRS assessment; and, thirdly, that methods to alter the bioavailability of resveratrol *in vivo* provided mixed results.

With regards the first point, the analysis of samples taken in chapter 3 demonstrated that resveratrol was predominantly available in metabolite form (and with higher sulfate than glucuronide metabolites) with the parent/aglycone form negligible or trace at all three sample time-points (e.g. 5.65ng/mL and 14.4ng/mL for 250- and 500mg

resveratrol respectively at 90 minutes post-dose (the t_{max})). Importantly, the concentrations observed here are broadly in line with previous data. Boocock et al (2007) observed an aglycone C_{max} of 72.6ng/mL compared to 14.4ng/mL in this thesis; of the glucuronide metabolites, 369.5- 404.6ng/mL versus 202.2ng/mL here; and of the sulfate metabolite, 1,135ng/mL compared to 746.1ng/mL here after a 500mg oral dose. Whilst these concentrations are not grossly dissimilar to those reported by Boocock et al., nevertheless they are lower, most likely accounted for by the large individual differences that exist with the metabolism and absorption of resveratrol (Wenzel & Somoza, 2005), and still represent levels consistent with those found previously.

With regards plasma levels of resveratrol in chapters 4 and 5, levels of the aglycone were completely undetectable, in both studies, at all time-points. The results from attempts to alter the natural metabolism in both of these studies (i.e. co-supplementation with piperine and chronic dosing) will be discussed below but if we take merely the acute, resveratrol metabolite concentrations after pure resveratrol doses, again we see very low levels. In chapter 4, following oral intervention with 250mg of resveratrol, plasma concentrations of total resveratrol metabolites evinced a mean of 5.18μM at the 45 minute post-dose sample time-point; 9.98μM At the 90 minute time-point; and 6.69μM at 120 minutes. Resveratrol-3-*O*-sulfate was the predominant metabolite in all volunteers, contributing 59-81% of total metabolites and the 4'- and 3-*O*-glucuronide forms made roughly equal contributions to the remaining metabolites in circulation. In study 4, following acute oral intervention with 500mg resveratrol (110mins post-dose on day-1), plasma concentrations of total resveratrol metabolites averaged 7.4μM and, again, resveratrol 3-*O*-sulfate was the predominant metabolite in all volunteers, contributing 73-77% of total metabolites with the 4'- and 3-*O*-glucuronide forms evincing roughly equal contributions to the remaining metabolites in circulation.

With regards reasons for the poor bioavailability observed here, the primary factor limiting the bioavailability of resveratrol, after oral administration, appears to be the high rate of first pass glucuronidation and the fact that the intestinal efflux pumps (specifically the Multi-drug resistance protein-3 (MRP3) pump) preferentially displace these glucuronidated metabolites into the blood stream whilst the Breast Cancer Resistance Protein (BCRP) pump effluxes the purported bioactive form of resveratrol, the aglycone, into the intestinal lumen (van de Wetering et al., 2008). The broader reason for the poor bioavailability of resveratrol, facilitated by the above mechanisms, is likely that the body identifies this compound as a xenobiotic with potentially toxic effects; hence rapid excretion to protect the host.

As mentioned previously, this poor bioavailability of resveratrol represents somewhat of a paradox however, and one which has hitherto failed to be addressed by the resveratrol literature: if these concentrations are considered 'low' then how do we explain the plethora of effects attributed to resveratrol? This is predicated on the assumption that concentrations must reach at least 5μmol/L in order to be capable of exerting physiological effects (Alarcón de la Lastra & Villegas, 2005) and also that the aglycone is the only active form of resveratrol. Both assumptions, however, fail to take into account that resveratrol *is* associated with physiological effects at lower doses and that resveratrol metabolites are always (again to the best of current knowledge) observed at higher concentrations than the aglycone after oral consumption; indeed sometimes representing the only form present in plasma. This suggests then, that this 'low' bioavailability might not be 'low' in terms of the subsequent efficacy of resveratrol, and that metabolites may exert activity also.

To take the second of the above key discussion points, in order to provide objective support (or not) for the attribution of physiological effects in the cognitive/NIRS assessments to the actions of resveratrol, blood plasma concentrations were measured at key time-points. Chapters 3 and 4 (utilizing doses of 500- and 250mg resveratrol) share similar testing session time-points and the profile of total resveratrol metabolite concentrations are similar in both studies also. Both demonstrate that resveratrol's metabolites were present at the 45 minute post-dose time-point (which represents the time at which participants began the first of four post-dose task battery repetitions in the cognitive/NIRS aspect of both studies) and that concentrations peaked at the 90 minute sample time-point: thus demonstrating that resveratrol was bioavailable, indeed rising in concentration, during this post-dose task period (45 to 85 minutes post-dose in both studies). At the 120 minute post-dose sample time-point total resveratrol metabolite levels began to be decline in both studies. In chapter 5, as participants in the bioavailability assessment were a sub-sample of the larger cognitive/NIRS study, blood samples could only be taken at the beginning and end of the testing session, rather than intermittent samples as above. However, baseline samples in the acute (day 1) arm of this study evinced a total metabolite concentration mean of 0μM, which rose to 7.4μM at 110 minutes post-dose following oral intervention with 500mg resveratrol. Whilst this does not indicate the concentrations of plasma levels during the 45-85 minute post-dose task period specifically, nevertheless it does support that resveratrol was bioavailable during the testing session as a whole.

And finally, to take the third of the above key discussion points, the aim of two (chapters 4 and 5) of the empirical research studies within this programme was to attempt to alter plasma levels of resveratrol and, in turn, improve the efficacy of

resveratrol with regards cognitive performance and CBF. As mentioned above, these methods provided mixed results. The first (chapter 4) attempted to inhibit the rate of aglycone glucuronidation, the primary factor limiting the bioavailability of resveratrol due to the preferential efflux of this metabolite into the blood stream and the aglycone back to the intestine, by co-supplementation of 250mg resveratrol with the purported bioenhancer piperine. The second (chapter 5) attempted to evince cumulative plasma levels of resveratrol via repeated dosing of 500mg daily for 28 days.

With regards the former, no significant difference was observed in the plasma levels of total metabolites between treatment conditions. However, contrary to the hypothesis of piperine-induced bioenhancement, the pattern of effects actually suggest inhibition rather than enhancement of plasma levels, e.g. the C_{max} of total metabolites after just resveratrol was 9.98μM compared to 4.82μM in the co-supplemented condition. Piperine also appeared to be inhibiting the transit of resveratrol; evidenced by the t_{max} of metabolites in the resveratrol alone condition occurring at the 90 minute sample time-point compared to the 120 minute time-point in the co-supplemented condition. This study did observe enhanced efficacy of resveratrol, with regards CBF, in the co-supplemented condition however, but in the face of no significant enhancement of bioavailability it is argued that these findings suggest an alternative mechanism of piperine-mediated enhanced resveratrol efficacy. The very small amount of literature in this area fails to provide any evidenced-based argument for this effect of piperine; with the only theoretical explanation concerning the thermogenic (i.e. heat-giving) effects it may confer (Badmaev et al., 1999). The possibility here is that piperine may be able to enhance the activity of resveratrol, the neuronal vasculature, and/or some other factor relevant to CBF via thermogenic properties. As evidence of piperines' heat-proffering properties, specifically in neural tissue, Reanmongkol et al. (1988) report on the ability of piperine to stimulate activity of ATPase (but inhibition of oxidative phosphorylation) which produces heat as a by-product (Clapham & Arch, 2006). Thermogenic increases in tissue activity have previously been proposed as an explanation for piperine-mediated increases in plasma beta-carotene levels in humans (Badmaev et al.) via increasing the absorption rate of the intestinal epithelium and, as a mechanism, could exist without piperine evincing an overall increase in resveratrol bioavailability: a phenomenon observed previously (Badmaev et al., 1999; Lambert et al., 2004; Shoba et al., 1998) but not replicated here.

Chapter 5, investigated the potential to cumulatively increase plasma levels of resveratrol by repeated dosing (28 days) of 500mg. The rationale for this was predicated on the results from three preclinical chemopreventive efficacy papers which reported that extremely low daily doses of resveratrol (between 200μg/kg and 2mg/kg)

were sufficient to produce peak plasma concentrations of aglycone resveratrol in the range of ~20nM- 2μM and in turn exert beneficial chemopreventive effects (results reported in Gescher & Steward, 2003). The bioavailability results from chapter 5 support this hypothesis finding that resveratrol metabolites were significantly higher at day 28 pre-dose compared to day 1 baseline; demonstrating that a pure chronic increase (irrespective of treatment on day 28) had taken place.

The logical following argument here would be that the presence of metabolites on day 28 might merely represent a residual carryover from day 27 dosing or that this could be the result of non-compliance, e.g. participants consuming treatment late on day 27 or even before they arrive for testing on day 28. With regards the first potential explanation, reference to the capsule logs completed daily by participants to note the time of treatment administration, and a capsule count of their returned treatment on day 28, shows that this group were between 96% (i.e. returning with one surplus capsule) to 100% compliant and that day 27 treatment was consumed between 8:13am-12:00pm. Whilst this time of 12:00pm is later than instructed, and could potentially account for a residue of plasma resveratrol in this participant, it is unlikely to account for a baseline plasma level of 6.0μM which is also similar to the baseline plasma levels of participants who reported taking day 27 treatment at 7:40am (5.7μM) and 8:13am (5.8μM). In terms of pre-dose day-28 levels representing a carryover from the previous days treatment, the pharmacokinetic profile of resveratrol, whilst only a small literature, is a pretty robust finding. Here the plasma half-life of resveratrol is reported at 9.2 ± 0.6hrs (Walle et al.) although this can range between 3.2-11.5hrs for sulfate metabolites, 2.9-10.6hrs for glucuronides and 2.9-8.9hrs for the parent compound (Boocock et al., 2007). The latter study also reports that, after a 500mg oral resveratrol dose in healthy humans, levels of all measured resveratrol conjugates had returned to baseline between 20-24hrs post-dose. This was apart from the sulfate metabolite which only registered ~10ng/mL at 24hrs. Taken together, residual levels should not necessarily have been anticipated at day 28 pre-dose and, if this did occur at all (due to later consumption of day 27 treatment for example), concentrations would be expected in the ng range at most.

To summarise, the results of all three bioavailability studies within this thesis support previous research that shows that resveratrol achieves very poor plasma levels after oral supplementation, especially of the parent/aglycone form. They also support the larger studies they inhabit by evidencing that resveratrol is bioavailable during key time-points. And, thirdly, the results from two of these bioavailability studies demonstrate that altering the plasma levels of resveratrol is possible to some extent but

that enhancing plasma levels does not necessarily enhance efficacy with regards cognitive function and CBF.

7.5 Discussion of statistical methods

The method of analysis chosen for NIRS data in this thesis was planned comparisons. The rationale for utilizing this statistical method is predicated on the clear hypotheses underpinning this programme of studies which evinced specific, focused questions of the data. To reiterate, the hypothesis was that resveratrol would enhance CBF and, as such, the specific *a priori*-derived questions concerned only how concentrations in resveratrol supplemented conditions differed to placebo at each time point, not how treatment conditions differed to each other, or how the effects differed at differing time points.

The main criticism levied towards planned comparisons regards the higher probability (compared to pairwise comparisons for example) of finding a significant effect/s and, specifically, of such effects representing type I errors. This is due to the fact that the alpha levels associated with each comparison does not necessarily need to be adjusted with planned comparisons, thus optimizing their power. To mitigate this, oftentimes protective mechanisms are employed. For example, reporting of significant planned comparisons might only occur if a significant main and/or interaction effect had been observed on a prior F-test. The issue here is the over-conservative nature of this purported 'protective' method, that it is unnecessary to the interpretation of planned comparisons and, finally and relatedly, that it can often be detrimental to the interpretation of planned comparisons; where the F-test can be non-significant despite a consistent pattern of significant planned comparisons (Rosenthal & Rosnow, 1985). A second potential protective mechanism involves correcting the alpha levels of comparisons after they have been made, e.g. by Bonferroni correction, in an attempt to control the family-wise error rate. The issue here concerns the fact that *a priori* planned comparisons are conducted in order to optimize the power of un-adjusted analysis and, as such, conducting post-hoc corrections on these comparisons is counter to the theoretical rationale of utilizing planned comparisons as the chosen statistical method. The use of either, or both, of these mechanisms to protect against type I error rates when performing planned comparisons can, therefore, be deemed as unnecessarily conservative and counter to the interpretation of such comparisons. This overly-conservative approach also has the potential to impede the research process, especially in a hitherto unexplored area of investigation, utilizing novel techniques,

where new and subtle effects might be missed due to unnecessarily over-conservative statistical methods (Keppel, 1991).

With this in mind, in order to drive forward the novel area of research investigated within this thesis, the single protective mechanism utilized here was to interpret significant planned comparisons only if they evinced a consistent pattern of effects. The rationale for this approach is two-fold; firstly, if resveratrol exerts a true effect on the outcomes measured then, for this to be meaningful, the effects should be consistent and, secondly, this approach is validated by the exponentially reducing probability of 'chance' significant differences occurring for one treatment at two or more consecutive time-points by chance. In order to incorporate a more meaningful indicator of error from the data, the planned comparison t tests utilized the mean squared error value from the prior conducted omnibus ANOVA and the results of these ANOVAs were reported for completeness throughout.

The findings evinced by planned comparisons throughout this PhD further validate its use as the statistical analysis of NIRS data here. All relevant chapters (i.e. 2-5) demonstrate consistent patterns of significant effects in response to resveratrol which could not be merely the product of chance (see figures 2.3, 3.3, 4.3, 5.2, 5.3 and 5.4). These studies also report dose-response effects of resveratrol treatment (e.g. 500mg versus 250mg in chapter 3, figure 3.3) which would not be apparent if chance were dictating the pattern of significance. Further, the CBF effects of resveratrol are consistent throughout the programme of studies (discounting the results from chapter 2 and the chronic aspect of chapter 5 which are arguably the result of methodological issues); demonstrating increased total-, oxy- and deoxy-Hb. Further, these findings are completely consistent with the underlying hypothesis of this thesis, based on the mechanisms of action of resveratrol. Undoubtedly, if the outcomes of planned comparison analysis were skewed by type I errors, then the consistency of NIRS results within studies (e.g. all eight post-dose time-points being significantly higher for total-Hb after 500mg resveratrol in chapter 3; and 17/20 post-dose time-points being significantly higher for total-Hb after 250mg resveratrol with 20mg piperine in chapter 4), and across the programme of studies (i.e. the direction of haemoglobin concentrations increasing in response to resveratrol in all studies); thus highlighting consistent patterns as well as themes, would not be possible.

As a final point about planned comparisons, it could be argued that this method doesn't represent the most appropriate method of analysis to utilize in studies where comparisons were not k-1 (i.e. chapters 5 and 6), i.e. where only two possible comparisons existed anyway. However, again in the interests of driving novel research

areas forward and in order to be consistent with the three other investigations in this thesis, planned comparisons were deemed to be the most appropriate statistical method here.

Planned comparisons however, were not deemed appropriate for the statistical analysis for cognitive performance data and other data with few continuous comparisons. This was because a significant pattern of effects, representing a 'true' effect, is much clearer to observe where multiple continuous comparisons exist, i.e. with NIRS data. With fewer comparisons, e.g. with four post-dose task comparison time-points, this pattern is less clear. To give an example, with planned comparisons, if 50% of 20 NIRS epoch comparisons were significant, this would be considered a true effect and reported as such. If 50% of four task time-point comparisons were significant however, this would be less easy to defend; especially if these were the first and fourth time-points for example; would this represent a significant 'pattern'? As such, to ease interpretation of any data which was not NIRS, and to protect against the example of potential type I error described above, all other data was analysed via corrected pairwise comparison.

7.6 Discussion of methodologies

7.6.1 Near-Infrared Spectroscopy (NIRS)

Near-Infrared Spectroscopy (NIRS) is a neuroimaging technique which has hitherto predominantly been utilized to monitor CBF changes in response to stimuli and/or cognitive tasks. The typical CBF response pattern here manifests as an increase in the total concentration of haemoglobin (total-Hb), a mirrored rise in concentrations of oxygenated haemoglobin (oxy-Hb) and a comparative decrease in deoxygenated haemoglobin (deoxy-Hb) (e.g. Schroeter, Zysset, Kupka, Kruggel, & von Cramon, 2002; Steinbrink et al., 2005). Certain cognitive tasks however, have been observed to elicit a relatively atypical CBF response (e.g. the Wisconsin card sort task (Fallgatter & Strik, 1998)) and hemispheric differences (Tsujimoto, Yamamoto, Kawaguchi, Koizumi, & Sawaguchi, 2004) and individual differences (Shibuya-Tayoshi et al., 2007) are reported to effect CBF, as detected by NIRS.

More recently, NIRS has been utilized to investigate the CBF response to pharmacological interventions, in response to cognitive tasks, where the pattern of effects is less consistent. The over-the-counter nausea and motion sickness treatment Dimenydrinate, containing both sedative and CNS-stimulatory ingredients, evinced no significant effects on CBF (Kanamaru et al., 2008). The vasoactive alkaloid Vinpocetine, a purported CBF and memory enhancing compound, also failed to evince

any significant modulation of CBF in a cohort of ischemic stroke patients although trends were observed for increased total- and oxy-Hb (Bönöczk et al., 2002). With regards nutritional interventions, since the start of the programme of research described in this thesis, NIRS has demonstrated sensitivity to the effects of polyunsaturated fatty acids (PUFAs); where a 12 week supplementation of 1g daily DHA-rich fish oil was reported to significantly increase concentrations of total- and oxy-Hb in the prefrontal cortex of 22 healthy adults during cognitive task performance. No effects were found with levels of deoxy-Hb (Jackson et al., 2012a). Conversely, caffeine (75mg) and the green tea polyphenol epigallocatechin gallate (EGCG) (135mg) are observed to acutely decrease CBF; with NIRS demonstrating decreased concentrations of total-Hb in the prefrontal cortex (although with regards caffeine this was only the case in non-consumers) of young, healthy participants (Kennedy & Haskell, 2011; Wightman et al., 2012).

Thus NIRS can be considered an appropriate technique to investigate the CBF effects of nutritional interventions; demonstrating sensitivity to both vasorelaxatory (PUFAs) and vasoconstricting (caffeine and EGCG) compounds. Indeed, findings throughout this programme of studies confirm this sensitivity and, in turn, the appropriateness of the NIRS technique here.

Firstly, as discussed in full in section 7.2, NIRS was able to detect the resveratrol-amplified cognitive workload-mediated increase in CBF in chapters 3 and 4 (and to a lesser extent the acute aspect of chapter 5) which was the rationale for utilizing NIRS within this programme of studies. This sensitivity is most apparent in chapter 3 where the post-dose cognitive task period evinced a rise in total-Hb in response to placebo alone; demonstrating the ability of NIRS to detect the natural CBF response to increased cognitive workload (as described above). As the aforementioned DHA fish oil study also reports, NIRS was able to detect the amplification of this natural response; as evidenced by higher concentrations of total-Hb in the 500mg (and to a lesser extent 250mg) resveratrol condition and, in turn, was able to provide insight into the mechanisms of action of resveratrol, i.e. that it exploits the nitric oxide (NO)-mediated vasorelaxatory response to preceding neural activation, as part of the neurovascular coupling, to enhance access to metabolic substrates via increased CBF.

Resveratrol also evinced a dissimilar pattern of CBF effects to those evinced in the aforementioned task and nutritional intervention studies, which had not been demonstrated before. The cognitive-NIRS studies outlined above report that the typical CBF response to tasks manifests as an increase in total- and oxy-Hb and a concomitant reduction in deoxy-Hb concentrations. The only other nutritional

intervention study with a vasorelaxatory compound (DHA fish oil) observed increased total- and oxy-Hb but no effect of deoxy-Hb levels. The current programme of studies (specifically chapters 3 and 4) saw a rise in all three NIRS chromophores (total-, oxy- and deoxy-Hb) which is indicative of the more complex mechanisms of resveratrol, i.e. vasorelaxatory effects alongside potential enhancement of oxidative phosphorylation which would be anticipated to evince increased total-, oxy- and deoxy-Hb, and also further supports the sensitivity of NIRS in detecting this hitherto un-investigated effect of NIRS on CBF.

Secondly, NIRS was able to detect the very subtle task-related, resveratrol-amplified changes in CBF; as seen in chapter 3. Here concentration levels of total- and deoxy-Hb (i.e. oxygen demand and utilization respectively) were observed numerically to rise in response to the serial subtraction tasks and fall during the Rapid Visual Information Processing (RVIP) task; with this oscillating pattern evident across the entire post-dose task period in the 500mg (and to a lesser extent 250mg) resveratrol condition, as compared to placebo (see figure 3.3). The subtraction task (3 and 7) can be considered more cognitively demanding, relative to RVIP, in terms of the level of cognitive workload it requires; i.e. to simultaneously hold and manipulate numerical information as well as to utilize mathematical skills and motor skills to type the response into the computer. The RVIP task, on the other hand, in less taxing with regards workload demands in that it merely requires participants to monitor the screen and to adopt a vigilant state whilst cognitively updating the on-screen information. Previous research utilizing NIRS demonstrates that more cognitively demanding tasks evince a greater CBF response than easier tasks/conditions; e.g. the incongruent versus congruent condition of the Stroop task (Schroeter et al., 2002), part B versus part A of the trail-maker task (Shibuya-Tayoshi et al., 2007) and increasing memory load in an item recognition task (Tsujimoto et al., 2004). Thus, the finding in chapter 3, of resveratrol amplifying the enhanced CBF response to increased workload demands in the serial subtraction task, versus the RVIP task, would be entirely expected based on previous research with NIRS utilizing other cognitively demanding tasks. It's important to highlight here, that in the subjective perceptions of task difficulty and mental demand study (appendix I), participants rated RVIP as more difficult and mentally demanding than the subtraction tasks. However, it is argued that when rating tasks with reference to 'mental demand', participants may have regarded this as another facet of 'difficulty' rather than relating to the level of mental/cognitive workload demanded of the task.

7.6.2 Trans-Cranial Doppler (TCD)

Trans-cranial Doppler (TCD) sonography has a smaller literature than NIRS and includes use as a tool to investigate the cerebral blood flow velocity (CBFV) response to cognitive tasks (Harders et al., 1989) and pharmacological interventions such as caffeine (Jones et al., 2000) and cocoa polyphenols. With regards the latter, a daily 900mg cocoa flavanol drink significantly increased mean blood flow velocity (MBFV) at 1- and 2 weeks in healthy older adults with these effects more pronounced than in a lower flavanol (36mg) condition (Sorond et al, 2008). The rationale for its use in this thesis (in chapter 5 only) was to compliment the concentration change CBF data provided by NIRS by recording quantitative CBFV from the right middle cerebral artery (MCA) pre- and post-testing on day 1 and day 28. The hemodynamic response in the MCA is strongly correlated to CBF changes in the prefrontal cortex (Rollnik et al. 2002; Ide et al., 1999) and so, based on the CBF effects of resveratrol reported in chapters 3 and 4, it was hypothesized that hemodynamic effects would also be seen in the MCA in chapter 5.

Unfortunately, no CBFV effects of resveratrol were observed here and so the aim of clarifying the results of the NIRS data from this chapter weren't realised. It could be that the converging of NIRS and TCD here simply wasn't effective, although Ide et al. observed this to be a successful partnership. Or that the TCD simply wasn't sufficiently sensitive to detect the region-specific, i.e. prefrontal cortex, modulation of haemoglobin as detected by NIRS. However, the most likely explanation for the lack of TCD effects, explained further in section 5.4, was that recording took place during periods of rest before and after cognitive demand. As such, a hemodynamic response might not have been anticipated during this time. In order to clarify whether or not this was the case, future research would be required to monitor CBF during periods of cognitive workload although this wasn't practically possible with the equipment utilized here.

7.6.3 Cognitive tasks

The rationale for utilizing the cognitive tasks used throughout this thesis are three-fold: firstly, and most importantly, to assess cognitive function and potential resveratrol-induced changes; secondly, to activate the prefrontal cortex and inculcate CBF changes which could be detected by NIRS (which measures from the forehead); and thirdly, to induce cognitive demand. This latter aspect was particularly important due to the young (18-35yrs), healthy (see exclusion criteria throughout) participants utilized in all of the studies who are presumably at their cognitive peak (Rönnlund et al., 2005)

and, therefore, likely to perform well on moderate cognitive tasks. As such, any potential enhancement evinced by resveratrol might be small and difficult to detect. Therefore, in order to encumber cognitive function in this high-performing group, such that a performance-gap could be created and potentially reversed by resveratrol (with this reversal large enough to detect statistically), a range of cognitively demanding tasks were utilized.

Chapters 2, 3 and 4 all utilized the cognitive demand battery (CDB); which comprises 2 minutes each of serial 3 and 7 subtractions and 5 minutes of rapid visual information processing (RVIP). Chapter 4 also utilized the 3-Back version of the N-Back task. Chapter 5 extended the range of cognitive tasks used based on the findings of a supplementary investigation (see appendix I) into the subjective perceptions of task difficulty and cognitive demand. These tasks comprised 2 minutes each of serial 7s, 13s and serial 17s subtractions, RVIP and 3-Back. Chapter 6 adopted a more mentally fatiguing testing regimen (akin to Kennedy, Scholey, & Wesnes, 2001; Reay et al., 2010) by assessing cognitive function at 40 minutes, 2.5-, 4-, and 6hrs post-dose. The tasks utilized here comprised serial 3s and 7s subtractions, RVIP, 3-Back, numeric working memory (NWM), Stroop, choice reaction time (CRT) and immediate and delayed word and picture recall and recognition. The aim of utilizing novel tasks in this final study was to ensure that other cognitive processes (i.e. response inhibition (Stroop) and recall and recognition (word and picture immediate and delayed recall and recognition)) other than working memory and executive functioning (serial subtractions, RVIP, N-Back), which resveratrol may also be able to effect, were not missed.

In terms of the appropriateness of these tasks to assess cognitive function, a wealth of previous literature has demonstrated their sensitivity to the effects of numerous nutritional interventions. The CDB, for example, has detected the cognitive enhancing effects of ginkgo biloba and ginseng (Scholey & Kennedy, 2002), ginseng and glucose (Reay, Kennedy & Scholey, 2006), and glucose and caffeine (Kennedy & Scholey, 2004). The effects of consumption of PUFA's (Narendran et al, 2012), ginseng (Reay, Scholey & Kennedy, 2010) and changes in fuel provision (Handa et al., 2009) have been detected by the N-Back task. The CRT task has proven sensitive to the effects of ginseng (Ziemba et al., 1999) and oxygen inspiration (Moss, Scholey & Wesnes, 1998). The Stroop task has detected changes in cognition evinced by ginkgo (Lovera et al., 2007) and caffeine (Kenemans et al., 1999). The effects of ginkgo (Kennedy, Scholey & Wesnes, 2000), ginseng (Kennedy, Scholey & Wesnes, 2001) and a ginkgo/ginseng combination (Kennedy, Scholey & Wesnes, 2001) have been evidenced by the NWM task. And, finally, immediate and delayed word recall has proven sensitive to the effects of a multitude of interventions, including oxygen inspiration (Moss, Scholey &

Wesnes, 1998; Scholey et al., 1999) and delayed word and picture recognition to the effects of protein and glucose (Jones, Sünram-Lea & Wesnes, 2012) and caffeine (Kuchinke & Lux, 2012). Taken together, the above tasks, all utilized throughout this programme of studies, have a wealth of literature to evidence their sensitivity to the effects of other nutritional interventions; many of which are associated with changes in blood flow (e.g. caffeine and ginseng evincing vasoconstriction and vasodilation respectively) and access to metabolic substrates (Kennedy & Scholey, 2000; Moss et al., 1998). In terms of the ability of these tasks to demonstrate sensitivity to the effects of resveratrol here, only chapter 5 observed any significant task performance modulation. In light of the proven sensitivity of these tasks previously, the weak effect of resveratrol on task performance here is likely more indicative of the ineffectiveness of resveratrol, rather than the inadequacy of the tasks themselves.

To take the second aforementioned rationale for the use of these cognitive tasks within this thesis: i.e. 'to activate the prefrontal cortex and inculcate CBF changes which could be detected by NIRS' again, a wealth of literature evidences the appropriateness of the tasks in this regard. With regards the subtraction tasks, bilateral prefrontal as well as parietal activation has been observed (Kazui, Kitagaki & Mori, 2000). Fronto-parietal activation has also been reported in response to the RVIP and N-Back tasks (Coull et al., 1996; Jansma et al., 2000 respectively). The CRT task is observed to activate the ventromedial prefrontal cortex (Heekeren et al., 2003), the Stroop task the left-lateral prefrontal cortex (Adleman et al., 2002) and the NWM task, i.e. working memory for digits, is observed to be sub-served by parietal, temporal and right-frontal regions (Gullick, Sprute & Temple, 2011). Finally, immediate and delayed word recall, i.e. retrieval, and recognition is reported to activate right prefrontal regions (Cabeza et al., 1997; Nobre, Allison & McCarthy, 1994 respectively). Taken together then, all tasks utilized within this programme of studies have previously been reported to activate, among other regions, the prefrontal cortex and could therefore be expected to inculcate CBF changes here which could be detected by NIRS.

In evidence of this, chapters 3 and 4 and the acute aspect of chapter 5 all, to a greater or lesser extent, show an increase in the CBF response in the prefrontal cortex upon the commencement of post-dose tasks in the placebo condition (see figures 3.3, 4.3, 5.2, 5.3 and 5.4 for NIRS graphs of these studies respectively). Importantly, as discussed in section 7.2, NIRS also detected the amplification of this prefrontal cortex activation in response to resveratrol. With regards hemispheric differences however, although several tasks have previously been reported to demonstrate some degree of lateralisation (e.g. Stroop, NWM and word retrieval) and despite investigating this statistically prior to further NIRS analysis in each chapter, none of the four studies

utilizing NIRS within this thesis observed any treatment related hemispheric differences between the right and left NIRS channels. Hence the data from both channels was averaged for analysis throughout. Potential explanations for a lack of hemispheric differences observed in this thesis include, firstly, that simply none existed in response to these tasks here; perhaps due to differences in format and, secondly, that NIRS may not have been sensitive enough to detect such differences; perhaps due to the placement of the NIRS channels on the head and the region of cortical measurement evinced by the resultant photon path.

Finally, the third rationale for the choice of tasks used in this thesis regards their use as a tool to elicit cognitive demand. To reiterate, the aim in using these tasks was to compensate for the existing high cognitive function of the young, health participants utilized by impeding cognitive resources and creating a gap in cognitive performance which could hypothetically be reversed by resveratrol supplementation. This programme of studies provides both subjective and objective evidence of the capacity of the above tasks to elicit such cognitive demand. With regards subjective perceptions of cognitive demand, results of the subjective perceptions of task difficulty and mental demand study (see appendix I) evidence that five of the above tasks were rated as both the most 'mentally fatiguing' and 'difficult'; those being RVIP (5 minute version), serial 17s subtractions, serial 13s subtractions, RVIP (2 minute version) and the 3-Back version of the N-Back task. In terms of subjective ratings of mental fatigue and difficulty on the VAS scales utilized in some of the experimental chapters however, no significant effects were evinced. Objectively, task-induced cognitive demand can arguably be seen in the enhanced CBF response to tasks in the placebo conditions of chapters 3 and 4 and the acute aspect of chapter 5; a finding observed in response to increased workload/demand in previous investigations also (Hasegawa, Carpenter & Just, 2002; Schroeter et al., 2002; Shibuya-Tayoshi et al., 2007; Tsujimoto et al., 2004).

7.6.4 Blood pressure

The rationale for monitoring blood pressure (BP) within this programme of studies was predicated on the incredibly small existing literature; with just a handful of papers in animal and human models to date. This literature suggested that vasodilatory polyphenols, like cocoa flavanols, which can influence vascular function, might also affect BP. These effects manifested as significant reductions in systolic and diastolic BP and mean arterial pressure (Shrime et al., 2011; Hooper et al., 2012) and are likely the result of the vascular dilatory effects of cocoa. As noted previously (section 1.6.4.3)

resveratrol shares these vasodilatory mechanisms and might, therefore, also be anticipated to affect the peripheral vasculature and BP as a result. Research into the BP effects of resveratrol in animals and humans however, has hitherto only investigated in overweight models. These cohorts potentially have compromised vasculature which might not relate to the healthy cohorts tested within this thesis. As an example, a reduction in BP has been observe in obese rats (but not lean rats) following 8-weeks supplementation with 10mg/kg resveratrol daily (Rivera et al., 2009). Similarly, in overweight/obese humans, significantly lowered systolic BP and mean arterial pressure was observed in 11 otherwise healthy males after a 30-day supplementation period of 150mg resveratrol daily (Timmers et al., 2011).

BP was assessed in chapters 4 and 5 only; with both chapters observing significantly higher levels of diastolic BP in response to resveratrol, although only when co-supplemented with piperine in chapter 4. This finding suggested that either piperine was exerting this effect or that it was merely enhancing the efficacy of an ineffective dose of resveratrol. The results of chapter 5 added clarification to this issue; observing increased diastolic BP after chronic supplementation of resveratrol, as compared to placebo. Why the opposite BP effects to those anticipated from the above research in cocoa flavanols, and in obese rats and humans with resveratrol, should be observed here is unclear. If there was a complete lack of an effect then an argument could be posited for resveratrol having no effect on the peripheral vasculature of healthy, young humans. But, that there appears to be a resveratrol-induced increase in BP, suggests that resveratrol might not be evincing vasodilatory effects in the periphery and may in fact be acting as a vasoconstrictor. Having not measured NO levels in these chapters it is not possible to clarify this argument here but, indirectly, the lack of CBF (NIRS) and CBFV (TCD) effects on day 28 would support the argument. Whether resveratrol can act as a vasoconstrictor is, at present, unknown but if we consider structurally similar polyphenols, e.g. the tea polyphenol epigallocatechin-3-gallate (EGCG), then we see that polyphenols are capable of acting both as vasodilators and vasoconstrictors depending on dose and the time of assessment (Alvarez et al. 2004). EGCG has also been investigated with regards its cognitive and cerebral blood flow effects in humans where it was reported that 135mg, administered acutely, evinced significantly lower CBF as compared to placebo; which might be suggestive of vasoconstriction (Wightman et al., 2012).

To summarise, resveratrol was found to increase blood pressure following supplementation of 500mg daily for 28 days, in healthy, young participants. Whether this was due to vasoconstrictive effects is unknown but certainly requires further investigation. The indirect measurement of NO, which has been measured previously

following cocoa supplementation (Taubert, Roesen, & Schomig, 2007), might also shed some light on this unexpected effect.

7.7 Future directions

In 2011 Smoliga, Baur and Hausenblas published a review which stated that, of the 4000 articles published between 1990 and 2010, only a handful were conducted in humans, the rest consisting almost entirely of *in vitro* work with a small number of animal model studies. A PubMed search using the search term "Resveratrol" within the title/abstract of "randomised controlled trials" with the MeSH term "humans" identifies 29 articles published between 2005 and 2012. The majority of these relate to health: one in the area of obesity, three in diabetes, six in cardiovascular/cardioprotection, two in cancer and seven relating to blood flow and endothelial function. Three articles investigate the antioxidant/anti-inflammatory effects of resveratrol and a further three can be regarded as miscellaneous. The final paper is the only one to be published investigating the cognitive and cerebral blood flow (CBF) effects of resveratrol. This is despite the efficacy of structurally similar cocoa flavanols on CBF and cognitive performance in humans and the potential for resveratrol to mirror these effects due to evidence of similar mechanisms of action; namely pertaining to vasodilation. Research also shows that resveratrol is capable of enhanced and preserved cognitive function in animal models (Sharma & Gupta, 2002; Tsai et al., 2007; Oomen et al., 2009) and suggests that the mechanisms might be related to blood flow (Oomen et al.).

This single paper to have investigated the CBF and cognitive effects of resveratrol in humans is the second empirical research study of this thesis, published in 2010[29]. The aim of this paper was to instigate research into the effects of polyphenols, specifically resveratrol, in humans and to break away from animal work which has sufficiently demonstrated its efficacy and safety. The specific aim of this thesis was to investigate the CBF and cognitive enhancing effects of resveratrol in healthy, young humans and, the results of the five intervention studies reported here, do indeed demonstrate the potent CBF enhancing properties of various doses of resveratrol but not cognitive enhancement.

In taking this research area forward, if continuing with young, healthy samples, future studies may want to bear in mind the difficulty in observing cognitive enhancement in this type of sample, following resveratrol-mediated CBF enhancement. These are a group who are already likely at their cognitive peak (Ronnlund et al., 2005) and

[29] With a further paper (the study from chapter 4 of this thesis) currently In press.

therefore any treatment-related improvement would undoubtedly be small. These are also a group who would not be expected to have any decrements in CBF and/or fuel provision/utilization and, as such, an acute enhancement may not be of benefit unless resources were significantly compromised.

One example here would be to investigate young, healthy samples in a state of reduced CBF and/or oxygenation. An attenuation of the neurovascular coupling response could be achieved via trans-cranial magnetic stimulation, for example, i.e. an area of the cortex could be depolarised with the hypothesis that those supplemented with resveratrol would experience a quicker return of CBF to this area when placed under cognitive demand; due to the ability of resveratrol to amplify the natural NO-mediated demand-driven vasorelaxation and increase in blood flow. This is seen in the periphery of obese humans when undergoing flow mediated dilatation, for example (Wong et al., 2011). Reduced neural oxygenation in young, healthy participants could also be achieved during maximal exercise performance, where oxygen utilization might preferentially be enhanced in muscle tissue rather than the brain, and in environments which mimic the reduced oxygen levels at altitude, i.e. an environmental chamber.

Alternatively, future research may wish to investigate groups who experience natural decrements in CBF and oxygenation, e.g. those with anaemia, those working at altitude, cohorts experiencing natural age-associated reductions in CBF and/or cognitive function and clinical populations who experience this also; i.e. those with neurodegenerative diseases and stroke patients. In terms of benefit to quality of life, it is certainly the latter groups here who would benefit from the CBF and cognitive effects of natural and safe compounds like resveratrol. Whether such benefits could be achieved after decrements have been experienced, or chronic, prophylactic consumption is necessary to stave off these deleterious effects of aging is also worthy of investigation.

As an overall comment on the future of resveratrol research, the hope hereafter is that the literature on resveratrol and human intervention begins to flourish as researchers begin to recognise the efficacy of resveratrol in humans. In terms of key areas, certainly research needs to clarify the pharmacokinetic profile of resveratrol in humans after oral supplementation with well controlled, multiple time-point, investigations and to elucidate the active components. Secondly, a greater range of doses requires investigation and a move towards investigating lower doses, i.e. those achievable by dietary consumption, should be encouraged. Thirdly, longer-term supplementation studies would be of great interest, especially those which follow participants over a period of years, and even decades, to observe whether chronic protection can be

provided by resveratrol, and dietary polyphenols more generally, against age-associated disorders.

7.8 General summary

The rationale for investigating the potentially cognitive enhancing effects of *trans*-resveratrol was predicated on its ability to both increase neural access to metabolic substrates and to augment utilization of this fuel by enhancing oxidative phosphorylation of the mitochondria. This PhD aimed to test the hypothesis that resveratrol is capable of eliciting cognitive enhancement via CBF augmentation in young, healthy human participants. The five intervention studies here demonstrate the ability of resveratrol to enhance CBF in the prefrontal cortex. They also show the appropriateness of utilizing a novel neuroimaging technique (NIRS) to monitor CBF following nutritional interventions. The resveratrol-mediated CBF enhancement manifested as significantly higher concentrations of total-Hb, oxy-Hb, and deoxy-Hb in response to 500mg resveratrol (chapter 3, 4 and, to a lesser extent, chapter 5) and 250mg resveratrol (chapter 3) with 20mg piperine (chapter 4). Only chapter 5 reported any modulation of cognitive performance but the lack of any other findings, in any chapter, supports the argument that this was the product of type I error rather than a true effect of resveratrol. However, this chapter did find a significant attenuation of fatigue across the chronic supplementation period in the resveratrol condition and, coupled with the higher calm and content ratings, as compared to placebo, in chapter 6 suggests that resveratrol may have promise as an anxiolytic and/or energy enhancing compound; certainly this requires further investigation.

Taken together, this thesis demonstrates the CBF enhancing effects of the polyphenol resveratrol in healthy, young human participants but argues that this increased access and utilization of metabolic substrates is not sufficient to inculcate increases in cognitive performance in this sample.

References

Abd El-Mohsen, M., Bayele, H., Kuhnle, G., Gibson, G., Debnam, E., Kaila Srai, S., Rice-Evans, C., & Spencer, J. (2006). Distribution of trans-resveratrol in rat tissues following oral administration. *The British journal of nutrition, 96*(1), 62-70.

Adams, R., McKie, V., Carl, E., Nichols, F., Perry, R., Brock, K., McKie, K., Figueroa, R., Litaker, M., & Weiner, S. (1997). Long term stroke risk in children with sickle cell disease screened with transcranial doppler. *Annals of Neurology, 42*(5), 699-704.

Adrian, M., Jeandet, P., Bessis, R., & Joubert, J. M. (1996). Induction of phytoalexin (resveratrol) synthesis in grapevine leaves treated with aluminum chloride (AlCl3). *Journal of Agricultural and Food Chemistry, 44*(8), 1979-1981.

Adrian, M., Jeandet, P., Douillet-Breuil, A. C., Tesson, L., & Bessis, R. (2000). Stilbene content of mature Vitis vinifera berries in response to UV-C elicitation. *Journal of Agricultural and Food Chemistry, 48*(12), 6103-6105.

Agarwal, B., & Baur, J. A. (2011). Resveratrol and life extension. *Annals of the New York Academy of Sciences, 1215*(1), 138-143.

Alarcón de la Lastra, C., & Villegas, I. (2005). Resveratrol as an anti-inflammatory and anti-aging agent: Mechanisms and clinical implications. *Molecular Nutrition & Food Research, 49*(5), 405-430.

Albers, A. R., Varghese, S., Vitseva, O., Vita, J. A., & Freedman, J. E. (2004). The antiinflammatory effects of purple grape juice consumption in subjects with stable coronary artery disease. *Arteriosclerosis, thrombosis, and vascular biology, 24*(11), e179-e180.

Almeida, L., Vaz-da-Silva, M., Falcao, A., Soares, E., Costa, R., Loureiro, A. I., Fernandes-Lopes, C., Rocha, J. F., Nunes, T., & Wright, L. (2009). Pharmacokinetic and safety profile of trans-resveratrol in a rising multiple-dose study in healthy volunteers. *Molecular Nutrition & Food Research, 53*.

Alvarez, E., Campos, M., Justiniano, H., Lugnier, C., & Orallo, F. (2006). Study of the mechanisms involved in the vasorelaxation induced by (-)-epigallocatechin-3-gallate in rat aorta. *British journal of pharmacology., 147*(3), 269-280.

Andriambeloson, E., Stoclet, J. C., & Andriantsitohaina, R. (1999). Mechanism of endothelial nitric oxide-dependent vasorelaxation induced by wine polyphenols in rat thoracic aorta. *Journal of cardiovascular pharmacology, 33*(2), 248.

Arab, L., Liu, W., & Elashoff, D. (2009). Green and black tea consumption and risk of stroke: a meta-analysis. *Stroke, 40*(5), 1786.

Ayata, C., Ma, J., Meng, W., Huang, P., & Moskowitz, M. A. (1996). L-NA-sensitive rCBF augmentation during vibrissal stimulation in type III nitric oxide synthase mutant mice. *Journal of Cerebral Blood Flow & Metabolism, 16*(4), 539-541.

Badmaev, V., Majeed, M., & Norkus, E. P. (1999). Piperine, an alkaloid derived from black pepper increases serum response of beta-carotene during 14-days of oral beta-carotene supplementation. *Nutr Res, 19*(3), 381-388.

Bais, H. P., Vepachedu, R., Gilroy, S., Callaway, R. M., & Vivanco, J. M. (2003). Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science, 301*(5638), 1377.

Barberger-Gateau, P., Raffaitin, C., Letenneur, L., Berr, C., Tzourio, C., Dartigues, J.-F., & Alpérovitch, A. (2007). Dietary patterns and risk of dementia The Three-City cohort study. *Neurology, 69*(20), 1921-1930.

Bate-Smith, E. C. (1973). Haemanalysis of tannins: the concept of relative astringency. *Phytochemistry, 12*(4), 907-912.

Bauer, S. R., Ding, E. L., & Smit, L. A. (2011). Cocoa consumption, cocoa flavonoids, and effects on cardiovascular risk factors: an evidence-based review. *Current Cardiovascular Risk Reports, 5*(2), 120-127.

Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J. S., Lopez-Lluch, G., Lewis, K., Pistell, P. J., Poosala, S., Becker, K. G., Boss, O., Gwinn, D.,

Wang, M., Ramaswamy, S., Fishbein, K. W., Spencer, R. G., Lakatta, E. G., Le Couteur, D., Shaw, R. J., Navas, P., Puigserver, P., Ingram, D. K., de Cabo, R., & Sinclair, D. A. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature, 444*(7117), 337-342.

Benton, D., & Parker, P. Y. (1998). Breakfast, blood glucose, and cognition. *The American journal of clinical nutrition, 67*(4), 772S-778S.

Bhardwaj, A., Sethi, G., Vadhan-Raj, S., Bueso-Ramos, C., Takada, Y., Gaur, U., Nair, A. S., Shishodia, S., & Aggarwal, B. B. (2007). Resveratrol inhibits proliferation, induces apoptosis, and overcomes chemoresistance through down-regulation of STAT3 and nuclear factor-κB–regulated antiapoptotic and cell survival gene products in human multiple myeloma cells. *Blood, 109*(6), 2293-2302.

Black, H. S., & Rhodes, L. E. (2001). Systemic photoprotection Dietary intervention and therapy. *Comprehensive series in photosciences, 3*, 573-591.

Bond, A., & Lader, M. (1974). Use of analog scales in rating subjective feelings. [Article]. *British Journal of Medical Psychology, 47*(SEP), 211-218.

Bönöczk, P., Panczel, G., & Nagy, Z. (2002). Vinpocetine increases cerebral blood flow and oxygenation in stroke patients: a near infrared spectroscopy and transcranial Doppler study. *European Journal of Ultrasound, 15*(1-2), 85-91.

Boocock, D. J., Faust, G. E. S., Patel, K. R., Schinas, A. M., Brown, V. A., Ducharme, M. P., Booth, T. D., Crowell, J. A., Perloff, M., Gescher, A. J., Steward, W. P., & Brenner, D. E. (2007). Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. *Cancer Epidemiology Biomarkers & Prevention, 16*(6), 1246-1252. doi: 10.1158/1055-9965.epi-07-0022

Boots, A., Haenen, G., & Bast, A. (2008). Health effects of quercetin: from antioxidant to nutraceutical. *European journal of pharmacology, 585*(2-3), 325-337.

Borgwardt, S., Hammann, F., Scheffler, K., Kreuter, M., Drewe, J., & Beglinger, C. (2012). Neural effects of green tea extract on dorsolateral prefrontal cortex. *European Journal of Clinical Nutrition, 66*(11), 1187-1192.

Brito, P. M., Devillard, R., Nègre-Salvayre, A., Almeida, L. M., Dinis, T. C., Salvayre, R., & Augé, N. (2009). Resveratrol inhibits the mTOR mitogenic signaling evoked by oxidized LDL in smooth muscle cells. *Atherosclerosis, 205*(1), 126-134.

Brownlee, H. E., McEuen, A. R., Hedger, J., & Scott, I. M. (1990). Anti-fungal effects of cocoa tannin on the witches' broom pathogen Crinipellis perniciosa. *Physiological and Molecular Plant Pathology, 36*(1), 39-48.

Bryant, J. P., Chapin III, F. S., & Klein, D. R. (1983). Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, 357-368.

Buxton, R. B., Uluda, K., Dubowitz, D. J., & Liu, T. T. (2004). Modeling the hemodynamic response to brain activation. *Neuroimage, 23*, 220-233.

Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. . *Psychiatry Research, 28*, 193-213.

Cameron, I., & Caronna, J. (1976). The effect of local changes in potassium and bicarbonate concentration on hypothalamic blood flow in the rabbit. *The Journal of Physiology, 262*(2), 415-430.

Camfield, D., Scholey, A., Pipingas, A., Silberstein, R., Kras, M., Nolidin, K., Wesnes, K., Pase, M., & Stough, C. (2012). Steady state visually evoked potential (SSVEP) topography changes associated with cocoa flavanol consumption. *Physiology & behavior, 105*(4), 948-957.

Celsis, P., Agniel, A., Cardebat, D., Demonet, J., Ousset, P., & Puel, M. (1997). Age related cognitive decline: a clinical entity? A longitudinal study of cerebral blood flow and memory performance. *Journal of Neurology, Neurosurgery & Psychiatry, 62*(6), 601-608.

Chang, T. K., Lee, W. B., & Ko, H. H. (2000). Trans-resveratrol modulates the catalytic activity and mRNA expression of the procarcinogen-activating human cytochrome P450 1B1. *Canadian journal of physiology and pharmacology, 78*(11), 874-881.

Chaves, A. A., Joshi, M. S., Coyle, C. M., Brady, J. E., Dech, S. J., Schanbacher, B. L., Baliga, R., Basuray, A., & Bauer, J. A. (2009). Vasoprotective endothelial effects of a standardized grape product in humans. *Vascular pharmacology, 50*(1-2), 20-26.

Chen, C. K., & PaceAsciak, C. R. (1996). Vasorelaxing activity of resveratrol and quercetin in isolated rat aorta. *General Pharmacology, 27*(2), 363-366.

Cholet, N., Seylaz, J., Lacombe, P., & Bonvento, G. (1997). Local uncoupling of the cerebrovascular and metabolic responses to somatosensory stimulation after neuronal nitric oxide synthase inhibition. *Journal of Cerebral Blood Flow & Metabolism, 17*(11), 1191-1201.

Chong, J., Poutaraud, A., & Hugueney, P. (2009). Metabolism and roles of stilbenes in plants. *Plant Science, 177*(3), 143-155.

Chul, J., Tak, S., Jang, K. E., Jung, J., & Jang, J. (2009). NIRS-SPM: Statistical parametric mapping for near-infrared spectroscopy. *Neuroimage, 44*(2), 428-447.

Clapham, J., & Arch, J. (2006). Thermogenic and metabolic antiobesity drugs: rationale and opportunities. *Diabetes Obes and Metab, 9*(3), 259-275.

Clément, M.-V., Hirpara, J. L., Chawdhury, S.-H., & Pervaiz, S. (1998). Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signaling-dependent apoptosis in human tumor cells. *Blood, 92*(3), 996-1002.

Coley, P. D., Bryant, J. P., & Chapin, F. S. (1985). Resource availability and plant antiherbivore defense. *Science, 230*(4728), 895.

Commenges, D., Scotet, V., Renaud, S., Jacqmin-Gadda, H., Barberger-Gateau, P., & Dartigues, J. F. (2000). Intake of flavonoids and risk of dementia. *European journal of epidemiology, 16*(4), 357-363.

Crews, W., Harrison, D., & Wright, J. (2008). A double-blind, placebo-controlled, randomized trial of the effects of dark chocolate and cocoa on variables associated with neuropsychological functioning and cardiovascular health: clinical findings from a sample of healthy, cognitively intact older adults. *The American journal of clinical nutrition, 87*(4), 872-880.

Criqui, M. H., & Ringel, B. L. (1994). Does diet or alcohol explain the French paradox? *The Lancet, 344*(8939), 1719-1723.

Critchfield, J. W., Welsh, C. J., Phang, J. M., & Chao Yeh, G. (1994). Modulation of adriamycin® accumulation and efflux by flavonoids in HCT-15 colon cells:: Activation of P-glycoprotein as a putative mechanism. *Biochemical Pharmacology, 48*(7), 1437-1445.

Dal-Pan, A., Pifferi, F., Marchal, J., Picq, J. L., & Aujard, F. (2011). Cognitive Performances Are Selectively Enhanced during Chronic Caloric Restriction or Resveratrol Supplementation in a Primate. *PLoS One, 6*(1), e16581.

Daroch, F., Hoeneisen, M., Gonzalez, C. L., Kawaguchi, F., Salgado, F., Solar, H., & Garcia, A. (2001). In vitro antibacterial activity of Chilean red wines against Helicobacter pylori. *Microbios, 104*(408), 79-85.

Das, S., & Das, D. K. (2007). Anti-inflammatory responses of resveratrol. *Inflammation & allergy drug targets, 6*(3), 168-173.

Davis, J. M., Murphy, E. A., Carmichael, M. D., & Davis, B. (2009). Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance. *American Journal of Physiology- Regulatory, Integrative and Comparative Physiology, 296*(4), R1071.

Dawson, T. M., & Dawson, V. L. (1995). Nitric Oxide: Actions and Pathological Roles. *The neuroscientist, 1*(1), 7-18.

Deka, A., & Vita, J. A. (2011). Tea and cardiovascular disease. *Pharmacological research, 64*(2), 136-145.

Desideri, G., Kwik-Uribe, C., Grassi, D., Necozione, S., Ghiadoni, L., Mastroiacovo, D., Raffaele, A., Ferri, L., Bocale, R., & Lechiara, M. C. (2012). Benefits in Cognitive Function, Blood

Pressure, and Insulin Resistance Through Cocoa Flavanol Consumption in Elderly Subjects With Mild Cognitive ImpairmentNovelty and Significance The Cocoa, Cognition, and Aging (CoCoA) Study. *Hypertension, 60*(3), 794-801.

Devore, E. E., Kang, J. H., Breteler, M., & Grodstein, F. (2012). Dietary intakes of berries and flavonoids in relation to cognitive decline. *Annals of Neurology, 72*(1), 135-143.

Dewick, P. M. (1994). The biosynthesis of shikimate metabolites. *Natural Product Reports, 11*, 173-203.

Djousse, L. (2012). Observational studies find association between chocolate consumption and reduced risk of cardiovascular disease and diabetes. *Evidence Based Medicine, 17*(4), 128-129.

Docherty, J. J., Fu, M. M. H., Stiffler, B. S., Limperos, R. J., Pokabla, C. M., & DeLucia, A. L. (1999). Resveratrol inhibition of herpes simplex virus replication. *Antiviral research, 43*(3), 145-155.

Docherty, J. J., Sweet, T. J., Bailey, E., Faith, S. A., & Booth, T. (2006). Resveratrol inhibition of varicella-zoster virus replication in vitro. *Antiviral research, 72*(3), 171-177.

Donnelly, L. E., Newton, R., Kennedy, G. E., Fenwick, P. S., Leung, R. H. F., Ito, K., Russell, R. E. K., & Barnes, P. J. (2004). Anti-inflammatory effects of resveratrol in lung epithelial cells: molecular mechanisms. *American journal of physiology. Lung cellular and molecular physiology, 287*(4), 774-783.

Fallgatter, A. J., & Strik, W. K. (1998). Frontal brain activation during the Wisconsin Card Sorting Test assessed with two-channel near-infrared spectroscopy. *European archives of psychiatry and clinical neuroscience, 248*(5), 245-249.

Fan, E. G., Zhang, L. J., Jiang, S., & Bai, Y. H. (2008). Beneficial Effects of Resveratrol on Atherosclerosis. [Review]. *Journal of Medicinal Food, 11*(4), 610-614.

Field, D. T., Williams, C. M., & Butler, L. T. (2011). Consumption of cocoa flavanols results in an acute improvement in visual and cognitive functions. *Physiology & behavior, 103*(3), 255-260.

Fieschi, C., Agnoli, A., Battistini, N., & Bozzao, L. (1966). Regional Cerebral Blood Flow in Patients With Brain Infarcts: A Study With the 85Kr Clearance Technique. *Archives of Neurology, 15*(6), 653.

File, S. E., Hartley, D. E., Elsabagh, S., Duffy, R., & Wiseman, H. (2005). Cognitive improvement after 6 weeks of soy supplements in postmenopausal women is limited to frontal lobe function. *Menopause, 12*(2), 193-201.

Fisher, N. D., Hughes, M., Gerhard-Herman, M., & Hollenberg, N. K. (2003). Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. *Journal of Hypertension, 21*(12), 2281-2286.

Francis, S. T., Head, K., Morris, P. G., & Macdonald, I. A. (2006). The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. *Journal of cardiovascular pharmacology, 47*, 215-220.

Friedman, M. (2007). Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. *Molecular Nutrition & Food Research, 51*(1), 116-134.

Fukui, Y., Ajichi, Y., & Okada, E. (2003). Monte Carlo prediction of near-infrared light propagation in realistic adult and neonatal head models. *Applied optics, 42*(16), 2881-2887.

Gehm, B. D., McAndrews, J. M., Chien, P. Y., & Jameson, J. L. (1997). Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. *Proceedings of the National Academy of Sciences of the United States of America, 94*(25), 14138-14143.

Gescher, A. J., & Steward, W. P. (2003). Relationship between mechanisms, bioavailibility, and preclinical chemopreventive efficacy of resveratrol: a conundrum. *Cancer Epidemiology Biomarkers & Prevention, 12*(10), 953.

Ghesquiere, S. (2005). Anatomy overview of a human artery. Wikipedia.

Ghosh, H. S., McBurney, M., & Robbins, P. D. (2010). SIRT1 negatively regulates the mammalian target of rapamycin. *PLoS One, 5*(2), e9199.

Gojkovic-Bukarica, L., Novakovic, A., Kanjuh, V., Bumbasirevic, M., Lesic, A., & Heinle, H. (2008). A role of ion channels in the endothelium-independent relaxation of rat mesenteric artery induced by resveratrol. *Journal of pharmacological sciences, 108*(1), 124-130.

Goldberg, D. (1978). *Manual of the General Health Questionnaire*. Windsor, England.: NFER publishing.

Goldberg, D., Yan, J., & Soleas, G. (2003). Absorption of three wine-related polyphenols in three different matrices by healthy subjects. *Clinical biochemistry, 36*(1), 79-87.

Gori, D. F. (1989). Floral color change in Lupinus argenteus (Fabaceae): why should plants advertise the location of unrewarding flowers to pollinators? *Evolution*, 870-881.

Gupta, M., & Shaw, B. P. (2009). Uses of medicinal plants in Panchakarma Ayurvedic therapy. *Indian Journal of traditional knowledge, 8*(3), 372-378.

Han, Y. S., Bastianetto, S., Dumont, Y., & Quirion, R. (2006). Specific plasma membrane binding sites for polyphenols, including resveratrol, in the rat brain. *Journal of Pharmacology and Experimental Therapeutics, 318*(1), 238.

Haque, H. A., Musha, T., & Nakajima, M. (1998). *Numerical estimation of skull and cortex boundaries from scalp geometry.* Paper presented at the Engineering in Medicine and Biology Society, 1998. Proceedings of the 20th Annual International Conference of the IEEE.

Harborne, J. R. (1993). *Introduction to Ecological Biochemistry* (Fourth Edition ed.). London: Elsevier.

Harders, A., Laborde, G., Droste, D., & Rastogi, E. (1989). Brain activity and blood flow velocity changes: a transcranial Doppler study. *International Journal of Neuroscience, 47*(1-2), 91-102.

Harrison, D. E., Strong, R., Sharp, Z. D., Nelson, J. F., Astle, C. M., Flurkey, K., Nadon, N. L., Wilkinson, J. E., Frenkel, K., & Carter, C. S. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature, 460*(7253), 392-395.

Hasegawa, M., Carpenter, P. A., & Just, M. A. (2002). An fMRI study of bilingual sentence comprehension and workload. *Neuroimage, 15*(3), 647-660.

Haslam, E. (1998). *Practical polyphenolics: from structure to molecular recognition and physiological action*: Cambridge Univ Pr.

Haynes, M. P., Sinha, D., Russell, K. S., Collinge, M., Fulton, D., Morales-Ruiz, M., Sessa, W. C., & Bender, J. R. (2000). Membrane estrogen receptor engagement activates endothelial nitric oxide synthase via the PI3-kinase–Akt pathway in human endothelial cells. *Circulation research, 87*(8), 677-682.

Hedden, T., & Gabrieli, J. D. (2004). Insights into the ageing mind: a view from cognitive neuroscience. *Nature Reviews Neuroscience, 5*(2), 87-96.

Heredia, A., Davis, C., & Redfield, R. (2000). Synergistic inhibition of HIV-1 in activated and resting peripheral blood mononuclear cells, monocyte-derived macrophages, and selected drug-resistant isolates with nucleoside analogues combined with a natural product, resveratrol. *JAIDS Journal of Acquired Immune Deficiency Syndromes, 25*(3), 246-255.

Herning, R. I., King, D. E., Better, W. E., & Cadet, J. L. (1999). Neurovascular deficits in cocaine abusers. *Neuropsychopharmacology, 21*(1), 110-118.

Herrmann, M., Ehlis, A.-C., & Fallgatter, A. (2003). Frontal activation during a verbal-fluency task as measured by near-infrared spectroscopy. *Brain Research Bulletin, 61*(1), 51-56.

Hollman, P., Geelen, A., & Kromhout, D. (2010). Dietary flavonol intake may lower stroke risk in men and women. *The Journal of nutrition, 140*(3), 600-604.

Hollman, P., Van Trijp, J., Buysman, M., Vd Gaag, M., Mengelers, M., De Vries, J., & Katan, M. (1997). Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS letters, 418*(1-2), 152-156.

Holthoff, J. H., Woodling, K. A., Doerge, D. R., Burns, S. T., Hinson, J. A., & Mayeux, P. R. (2010). Resveratrol, a dietary polyphenolic phytoalexin, is a functional scavenger of peroxynitrite. *Biochemical Pharmacology, 80*(8), 1260-1265.

Hooper, L., Kay, C., Abdelhamid, A., Kroon, P. A., Cohn, J. S., Rimm, E. B., & Cassidy, A. (2012). Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. *The American journal of clinical nutrition, 95*(3), 740-751.

Hoshi, Y. (2007). Functional near-infrared spectroscopy: current status and future prospects. *Journal of Biomedical Optics, 12*, 062106.

Howitz, K. T., Bitterman, K. J., Cohen, H. Y., Lamming, D. W., Lavu, S., Wood, J. G., Zipkin, R. E., Chung, P., Kisielewski, A., & Zhang, L. L. (2003). Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. *Nature, 425*(6954), 191-196.

Hu, Y., Guo, T., Zhang, X. p., & Tan, H. (2006). Resveratrol as a novel agent for treatment of multiple myeloma with matrix metalloproteinase inhibitory activity. *Acta Pharmacologica Sinica, 27*(11), 1447-1452.

Huppert, T. J., Hoge, R. D., Diamond, S. G., Franceschini, M. A., & Boas, D. A. (2006). A temporal comparison of BOLD, ASL, and NIRS hemodynamic responses to motor stimuli in adult humans. *Neuroimage, 29*(2), 368-382.

Ide, K., Horn, A., & Secher, N. H. (1999). Cerebral metabolic response to submaximal exercise. *Journal of Applied Physiology, 87*(5), 1604-1608.

Izzetoglu, K., Bunce, S., Onaral, B., Pourrezaei, K., & Chance, B. (2004). Functional optical brain imaging using near-infrared during cognitive tasks. *International Journal of Human-Computer Interaction, 17*(2), 211-231.

Jackson, P. A., Reay, J. L., Scholey, A. B., & Kennedy, D. O. (2012a). DHA-rich oil modulates the cerebral haemodynamic response to cognitive tasks in healthy young adults: a near IR spectroscopy pilot study. *The British journal of nutrition, 107*(8), 1093.

Jackson, P. A., Reay, J. L., Scholey, A. B., & Kennedy, D. O. (2012b). Docosahexaenoic acid-rich fish oil modulates the cerebral hemodynamic response to cognitive tasks in healthy young adults. *Biological Psychology, 89*(1), 183-190.

Jia, Z., Zhu, H., Misra, B. R., Mahaney, J. E., Li, Y., & Misra, H. P. (2008). EPR studies on the superoxide-scavenging capacity of the nutraceutical resveratrol. *Molecular and Cellular Biochemistry, 313*(1), 187-194.

Jodoin, J., Demeule, M., & Béliveau, R. (2002). Inhibition of the multidrug resistance P-glycoprotein activity by green tea polyphenols. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1542*(1-3), 149-159.

Johnson, J. J., Nihal, M., Siddiqui, I. A., Scarlett, C. O., Bailey, H. H., Mukhtar, H., & Ahmad, N. (2011). Enhancing the bioavailability of resveratrol by combining it with piperine. *Molecular Nutrition & Food Research, 55*, 1169-1176.

Jones, H. E., Herning, R. I., Cadet, J. L., & Griffiths, R. R. (2000). Caffeine withdrawal increases cerebral blood flow velocity and alters quantitative electroencephalography (EEG) activity. *Psychopharmacology, 147*(4), 371-377.

Juan, M. E., Maijó, M., & Planas, J. M. (2010). Quantification of trans-resveratrol and its metabolites in rat plasma and tissues by HPLC. *Journal of Pharmaceutical and Biomedical Analysis, 51*(2), 391-398.

Juan, S.-H., Cheng, T.-H., Lin, H.-C., Chu, Y.-L., & Lee, W.-S. (2005). Mechanism of concentration-dependent induction of heme oxygenase-1 by resveratrol in human aortic smooth muscle cells. *Biochemical Pharmacology, 69*(1), 41-48.

Jung, H. J., Hwang, I. A., Sung, W. S., Kang, H., Kang, B. S., Seu, Y. B., & Lee, D. G. (2005). Fungicidal effect of resveratrol on human infectious fungi. *Archives of pharmacal research, 28*(5), 557-560.

Jung, H. J., Seu, Y., & Lee, D. (2007). Candicidal action of resveratrol isolated from grapes on human pathogenic yeast C. albicans. *Journal of microbiology and biotechnology, 17*(8), 1324.

Kaeberlein, M., Powers, R. W., Steffen, K. K., Westman, E. A., Hu, D., Dang, N., Kerr, E. O., Kirkland, K. T., Fields, S., & Kennedy, B. K. (2005). Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. *Science, 310*(5751), 1193-1196.

Kanamaru, Y., Kikukawa, A., Miyamoto, Y., & Hirafuji, M. (2008). Dimenhydrinate effect on cerebral oxygen status and salivary chromogranin-A during cognitive tasks. *Progress in Neuropsychopharmacology & Biological Psychiatry, 32*(1), 107-115.

Kanaze, F. I., Bounartzi, M. I., Georgarakis, M., & Niopas, I. (2006). Pharmacokinetics of the citrus flavanone aglycones hesperetin and naringenin after single oral administration in human subjects. *European Journal of Clinical Nutrition, 61*(4), 472-477.

Katiyar, S. K., Afaq, F., Perez, A., & Mukhtar, H. (2001). Green tea polyphenol (–)-epigallocatechin-3-gallate treatment of human skin inhibits ultraviolet radiation-induced oxidative stress. *carcinogenesis, 22*(2), 287.

Kennedy, D. O., & Haskell, C. F. (2011). Cerebral blood flow and behavioural effects of caffeine in habitual and non-habitual consumers of caffeine: A Near Infrared Spectroscopy study. *Biological Psychology, 86*(3), 298-306.

Kennedy, D. O., & Scholey, A. B. (2000). Glucose administration, heart rate and cognitive performance: effects of increasing mental effort. *Psychopharmacology, 149*(1), 63-71.

Kennedy, D. O., & Scholey, A. B. (2004). A glucose-caffeine'energy drink'ameliorates subjective and performance deficits during prolonged cognitive demand. *Appetite, 42*(3), 331.

Kennedy, D. O., Scholey, A. B., & Wesnes, K. A. (2001). Dose dependent changes in cognitive performance and mood following acute administration of Ginseng to healthy young volunteers. *Nutritional Neuroscience, 4*(4), 295-310.

Keppel, G. (1991). *Design and analysis*. New Jersey: Prentice Hall.

Kesse-Guyot, E., Fezeu, L., Andreeva, V. A., Touvier, M., Scalbert, A., Hercberg, S., & Galan, P. (2012). Total and specific polyphenol intakes in midlife are associated with cognitive function measured 13 years later. *The Journal of nutrition, 142*(1), 76-83.

Kitaura, H., Uozumi, N., Tohmi, M., Yamazaki, M., Sakimura, K., Kudoh, M., Shimizu, T., & Shibuki, K. (2007). Roles of nitric oxide as a vasodilator in neurovascular coupling of mouse somatosensory cortex. *Neuroscience research, 59*(2), 160-171.

Kopp, P. (1998). Resveratrol, a phytoestrogen found in red wine. A possible explanation for the conundrum of the'French paradox'? *European journal of endocrinology, 138*(6), 619.

Kritz-Silverstein, D., Von Mühlen, D., Barrett-Connor, E., & Bressel, M. A. (2003). Isoflavones and cognitive function in older women: the SOy and Postmenopausal Health In Aging (SOPHIA) Study. *Menopause, 10*(3), 196-202.

Kuhnle, G., Spencer, J. P., Chowrimootoo, G., Schroeter, H., Debnam, E. S., Srai, S. K., Rice Evans, C., & Hahn, U. (2000). Resveratrol is absorbed in the small intestine as resveratrol glucuronide. *Biochem Biophys Res Commun, 272*(1), 212-217.

Kumar, A., Naidu, P. S., Seghal, N., & Padi, S. S. V. (2007). Neuroprotective effects of resveratrol against intracerebroventricular colchicine-induced cognitive impairment and oxidative stress in rats. [Article]. *Pharmacology, 79*(1), 17-26.

Kundu, J. K., & Surh, Y.-J. (2008). Cancer chemopreventive and therapeutic potential of resveratrol: mechanistic perspectives. *Cancer Letters, 269*(2), 243-261.

Kuo, P.-L., Chiang, L.-C., & Lin, C.-C. (2002). Resveratrol-induced apoptosis is mediated by p53-dependent pathway in Hep G2 cells. *Life sciences, 72*(1), 23-34.

Kuriyama, S., Hozawa, A., Ohmori, K., Shimazu, T., Matsui, T., Ebihara, S., Awata, S., Nagatomi, R., Arai, H., & Tsuji, I. (2006). Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya Project 1. *The American journal of clinical nutrition, 83*(2), 355.

Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P., Geny, B., Laakso, M., Puigserver, P., & Auwerx, J. (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 alpha. *Cell, 127*(6), 1109-1122.

Lambert, J. D., Hong, J., Kim, D. H., Mishin, V. M., & Yang, C. S. (2004). Piperine enhances the bioavailability of the tea polyphenol (–)-epigallocatechin-3-gallate in mice. *J Nutr, 134*(8), 1948.

Lamming, D. W., Ye, L., Katajisto, P., Goncalves, M. D., Saitoh, M., Stevens, D. M., Davis, J. G., Salmon, A. B., Richardson, A., & Ahima, R. S. (2012). Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. *Science, 335*(6076), 1638-1643.

Leikert, J. F., Räthel, T. R., Wohlfart, P., Cheynier, V., Vollmar, A. M., & Dirsch, V. M. (2002). Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells. *Circulation, 106*(13), 1614-1617.

Letenneur, L., Proust-Lima, C., Le Gouge, A., Dartigues, J.-F., & Barberger-Gateau, P. (2007). Flavonoid intake and cognitive decline over a 10-year period. *American journal of epidemiology, 165*(12), 1364-1371.

Lippi, G., Franchini, M., Favaloro, E. J., & Targher, G. (2010). Moderate red wine consumption and cardiovascular disease risk: beyond the "French paradox". *Semin Thromb Hemost, 36*(1), 59-70.

Liu, C., Li, S., Liu, T., Borjigin, J., & Lin, J. D. (2007). Transcriptional coactivator PGC-1&agr; integrates the mammalian clock and energy metabolism. *Nature, 447*(7143), 477-481.

Liu, G.-S., Zhang, Z.-S., Yang, B., & He, W. (2012). Resveratrol attenuates oxidative damage and ameliorates cognitive impairment in the brain of senescence-accelerated mice. *Life sciences*.

Liu, H., Qiu, N., Ding, H., & Yao, R. (2008). Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. *Food research international, 41*(4), 363-370.

Lovett, J. V. (1990). Alternatives to the chemical control of weeds. *Rotorua, New Zealand*, 57-65.

Lovett, J. V., Ryuntyu, M. Y., & Liu, D. L. (1989). Allelopathy, chemical communication, and plant defense. *Journal of Chemical Ecology, 15*(4), 1193-1202.

Ludwig, T. M. (1981). Before Rikyū. Religious and Aesthetic Influences in the Early History of the Tea Ceremony. *Monumenta Nipponica, 36*(4), 367-390.

Macheix, J. J., Fleuriet, A., & Billot, J. (1990). *Fruit phenolics*. USA: CRC Press: Boca Raton.

MacRae, H. S., & Mefferd, K. M. (2006). Dietary antioxidant supplementation combined with quercetin improves cycling time trial performance. *International journal of sport nutrition and exercise metabolism, 16*(4), 405.

Magyar, K., Halmosi, R., Palfi, A., Feher, G., Czopf, L., Fulop, A., Battyany, I., Sumegi, B., Toth, K., & Szabados, E. (2012). Cardioprotection by resveratrol: a human clinical trial in patients with stable coronary artery disease. *Clinical hemorheology and microcirculation, 50*(3), 179-187.

Mahn, K., Borrás, C., Knock, G. A., Taylor, P., Khan, I. Y., Sugden, D., Poston, L., Ward, J. P., Sharpe, R. M., & Viña, J. (2005). Dietary soy isoflavone induced increases in antioxidant and eNOS gene expression lead to improved endothelial function and reduced blood pressure in vivo. *The FASEB Journal, 19*(12), 1755-1757.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability1, 2. *Am J Clin Nutr, 79*, 727-747.

Marchal, G., Rioux, P., Petit-Taboué, M.-C., Sette, G., Travère, J.-M., Le Poec, C., Courtheoux, P., Derlon, J.-M., & Baron, J.-C. (1992). Regional cerebral oxygen consumption, blood flow, and blood volume in healthy human aging. *Archives of Neurology, 49*(10), 1013.

Marcus, D. L., Thomas, C., Rodriguez, C., Simberkoff, K., Tsai, J. S., Strafaci, J. A., & Freedman, M. L. (1998). Increased peroxidation and reduced antioxidant enzyme activity in Alzheimer's disease. *Experimental Neurology, 150*(1), 40-44.

Marier, J. F., Vachon, P., Gritsas, A., Zhang, J., Moreau, J. P., & Ducharme, M. P. (2002). Metabolism and disposition of resveratrol in rats: extent of absorption,

glucuronidation, and enterohepatic recirculation evidenced by a linked-rat model. *Journal of Pharmacology and Experimental Therapeutics, 302*(1), 369-373.

Markus, H. S. (2000). Transcranial Doppler ultrasound. *British medical bulletin, 56*(2), 378-388.

Martin, S., Andriambeloson, E., Takeda, K., & Andriantsitohaina, R. (2002). Red wine polyphenols increase calcium in bovine aortic endothelial cells: a basis to elucidate signalling pathways leading to nitric oxide production. *British journal of pharmacology, 135*(6), 1579-1587.

Mazza, G., & Miniati, E. (1993). *Anthocyanins in fruits, vegetables, and grains*: CRC Press.

McCullough, M. L., Peterson, J. J., Patel, R., Jacques, P. F., Shah, R., & Dwyer, J. T. (2012). Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults. *The American journal of clinical nutrition, 95*(2), 454-464.

McNair, D. M., Lorr, M., & Droppleman, L. F. (1971). *Profile of mood states*. San Diego: Educational and Industrial testing service.

Meek, J. H., Firbank, M., Elwell, C. E., Atkinson, J., Braddick, O., & Wyatt, J. S. (1998). Regional Hemodynamic Responses to Visual Stimulation in Awake Infants. *Pediatric Research, 43*(6), 840.

Meléndez-Ackerman, E., Campbell, D. R., & Waser, N. M. (1997). Hummingbird behavior and mechanisms of selection on flower color in Ipomopsis. *Ecology, 78*(8), 2532-2541.

Messier, C. (2004). Glucose improvement of memory: a review. *European journal of pharmacology, 490*(1), 33-57.

Mitsunaga, Y., Takanaga, H., Matsuo, H., Naito, M., Tsuruo, T., Ohtani, H., & Sawada, Y. (2000). Effect of bioflavonoids on vincristine transport across blood-brain barrier1. *European journal of pharmacology, 395*(3), 193-201.

Mokni, M., Elkahoui, S., Limam, F., Amri, M., & Aouani, E. (2007). Effect of resveratrol on antioxidant enzyme activities in the brain of healthy rat. *Neurochemical Research, 32*(6), 981-987.

Moss, M. C., Scholey, A. B., & Wesnes, K. (1998). Oxygen administration selectively enhances cognitive performance in healthy young adults: a placebo-controlled double-blind crossover study. *Psychopharmacology (Berl), 138*(1), 27-33.

Nabekura, T., Kamiyama, S., & Kitagawa, S. (2005). Effects of dietary chemopreventive phytochemicals on P-glycoprotein function. *Biochemical and Biophysical Research Communications, 327*(3), 866-870.

Nathan, C. (1997). Inducible nitric oxide synthase: what difference does it make? *J Clin Invest, 100*(10), 2417.

Ng, T., Feng, L., Niti, M., Kua, E., & Yap, K. (2008a). Tea consumption and cognitive impairment and decline in older Chinese adults. [Article]. *American Journal of Clinical Nutrition, 88*(1), 224-231.

Ng, T., Feng, L., Niti, M., Kua, E., & Yap, K. (2008b). Tea consumption and cognitive impairment and decline in older Chinese adults. *The American journal of clinical nutrition, 88*(1), 224.

Nicoletto, H. A., & Burkman, M. H. (2009). Transcranial Doppler series part II: performing a transcranial Doppler. *American journal of electroneurodiagnostic technology, 49*(1), 14.

Novakovic, A., Bukarica, L. G., Kanjuh, V., & Heinle, H. (2006). Potassium channels-mediated vasorelaxation of rat aorta induced by resveratrol. *Basic & Clinical Pharmacology & Toxicology, 99*(5), 360-364.

Novakovic, A., Gojkovic-Bukarica, L., Peric, M., Nezic, D., Djukanovic, B., Markovic-Lipkovski, J., & Heinle, H. (2006). The mechanism of endothelium-independent relaxation induced by the wine polyphenol resveratrol in human internal mammary artery. *Journal of Pharmacological Sciences, 101*(1), 85-90.

Nurk, E., Refsum, H., Drevon, C. A., Tell, G. S., Nygaard, H. A., Engedal, K., & Smith, A. D. (2009). Intake of flavonoid-rich wine, tea, and chocolate by elderly men and women is associated with better cognitive test performance. *The Journal of nutrition, 139*(1), 120-127.

O'Brien, J. T., Erkinjuntti, T., Reisberg, B., Roman, G., Sawada, T., Pantoni, L., Bowler, J. V., Ballard, C., DeCarli, C., & Gorelick, P. B. (2003). Vascular cognitive impairment. *The Lancet Neurology, 2*(2), 89-98.

O'Byrne, D. J., Devaraj, S., Grundy, S. M., & Jialal, I. (2002). Comparison of the antioxidant effects of Concord grape juice flavonoids α-tocopherol on markers of oxidative stress in healthy adults. *The American journal of clinical nutrition, 76*(6), 1367-1374.

Obrig, H., & Villringer, A. (2003). Beyond the visible—imaging the human brain with light. *J Cereb Blood Flow Metab, 23*(1), 1-18.

Oike, H., & Kobori, M. (2008). Resveratrol regulates circadian clock genes in Rat-1 fibroblast cells. *Bioscience, biotechnology, and biochemistry, 72*(11), 3038-3040.

Okada, F. (1978). Antiviral effects of tea catechins and black tea theaflavins on plant viruses. *Jpn Agric Res Q, 12*, 27–32.

Oomen, C. A., Farkas, E., Roman, V., Van Der Beek, E. M., Luiten, P. G. M., & Meerlo, P. (2009). Resveratrol preserves cerebrovascular density and cognitive function in aging mice. *Frontiers in Aging Neuroscience, 1*.

Pace-Asciak, C. R., Hahn, S., Diamandis, E. P., Soleas, G., & Goldberg, D. M. (1995). The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. *Clinica Chimica Acta, 235*(2), 207-219.

Park, S.-K., Jung, I.-C., Lee, W. K., Lee, Y. S., Park, H. K., Go, H. J., Kim, K., Lim, N. K., Hong, J. T., & Ly, S. Y. (2011). A combination of green tea extract and l-theanine improves memory and attention in subjects with mild cognitive impairment: a double-blind placebo-controlled study. *Journal of Medicinal Food, 14*(4), 334-343.

Pase, M., Scholey, A., Pipingas, A., Kras, M., Nolidin, K., Gibbs, A., Wesnes, K., & Stough, C. (2013). Cocoa polyphenols enhance positive mood states but not cognitive performance: a randomized, placebo-controlled trial. *Journal of Psychopharmacology, 27*(5), 451-458.

Reanmongkol, W., Janthasoot, W., Wattanatorn, W., Dhumma-Upakorn, P., & Chudapongse, P. (1988). Effects of piperine on bioenergetic functions of isolated rat liver mitochondria. *Biochem Pharmacol, 37*(4), 753-757.

Reay, J. L., Kennedy, D. O., & Scholey, A. B. (2006). Effects of Panax ginseng, consumed with and without glucose, on blood glucose levels and cognitive performance during sustained'mentally demanding'tasks. *Journal of Psychopharmacology, 20*(6), 771.

Reay, J. L., Scholey, A. B., & Kennedy, D. O. (2010). Panax ginseng (G115) improves aspects of working memory performance and subjective ratings of calmness in healthy young adults. [Article]. *Human Psychopharmacology-Clinical and Experimental, 25*(6), 462-471.

Reen, R. K., Jamwal, D. S., Taneja, S. C., Koul, J. L., Dubey, R. K., Wiebel, F. J., & Singh, J. (1993). Impairment of UDP-glucose dehydrogenase and glucuronidation activities in liver and small intestine of rat and guinea pig in vitro by piperine. *Biochem Pharmacol, 46*(2), 229.

Ritz, M. F., Curin, Y., Mendelowitsch, A., & Andriantsitohaina, R. (2008). Acute treatment with red wine polyphenols protects from ischemia-induced excitotoxicity, energy failure and oxidative stress in rats. *Brain Research, 1239*, 226-234.

Ritz, M. F., Ratajczak, P., Curin, Y., Cam, E., Mendelowitsch, A., Pinet, F., & Andriantsitohaina, R. (2008). Chronic treatment with red wine polyphenol compounds mediates neuroprotection in a rat model of ischemic cerebral stroke. *J Nutr, 138*(3), 519.

Rivera, L., Morón, R., Zarzuelo, A., & Galisteo, M. (2009). Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. *Biochem Pharmacol, 77*(6), 1053-1063.

Rodgers, J. T., Lerin, C., Haas, W., Gygi, S. P., Spiegelman, B. M., & Puigserver, P. (2005). Nutrient control of glucose homeostasis through a complex of PGC-1 and SIRT1. *Nature, 434*(7029), 113-118.

Rollnik, J. D., Düsterhöft, A., Däuper, J., Kossev, A., Weissenborn, K., & Dengler, R. (2002). Decrease of middle cerebral artery blood flow velocity after low-frequency repetitive transcranial magnetic stimulation of the dorsolateral prefrontal cortex. *Clinical Neurophysiology, 113*(6), 951-955.

Rönnlund, M., Nyberg, L., Bäckman, L., & Nilsson, L.-G. (2005). Stability, growth, and decline in adult life span development of declarative memory: Cross-sectional and longitudinal data from a population-based study. *Psychology and aging, 20*(1), 3-18.

Rosenthal, R., & Rosnow, R. L. (1985). *Contrast analysis: focused comparison in the analysis of variance*: Cambridge University Press.

Rush, J. W. E., Quadrilatero, J., Levy, A. S., & Ford, R. J. (2007). Chronic resveratrol enhances endothelium-dependent relaxation but does not alter eNOS levels in aorta of spontaneously hypertensive rats. *Ex Biol Med (Maywood), 232*(6), 814-822.

Santizo, R., Baughman, V. L., & Pelligrino, D. A. (2000). Relative contributions from neuronal and endothelial nitric oxide synthases to regional cerebral blood flow changes during forebrain ischemia in rats. *Neuroreport, 11*(7), 1549.

Schaefer, H. M., McGraw, K., & Catoni, C. (2008). Birds use fruit colour as honest signal of dietary antioxidant rewards. *Functional Ecology, 22*(2), 303-310.

Schaefer, H. M., & Rolshausen, G. (2006). Plants on red alert: do insects pay attention? *BioEssays, 28*(1), 65-71.

Schecklmann, M., Ehlis, A. C., Plichta, M. M., Boutter, H. K., Metzger, F. G., & Fallgatter, A. J. (2007). Altered frontal brain oxygenation in detoxified alcohol dependent patients with unaffected verbal fluency performance. *Psychiatry Research: Neuroimaging, 156*(2), 129-138.

Schecklmann, M., Ehlis, A. C., Plichta, M. M., & Fallgatter, A. J. (2008). Functional near-infrared spectroscopy: A long-term reliable tool for measuring brain activity during verbal fluency. *Neuroimage, 43*(1), 147-155.

Scholey, A., Downey, L., Ciorciari, J., Pipingas, A., Nolidin, K., Finn, M., Wines, M., Catchlove, S., Terrens, A., & Barlow, E. (2012). Acute neurocognitive effects of epigallocatechin gallate (EGCG). *Appetite, 58*(2), 767-770.

Scholey, A., French, S., Morris, P., Kennedy, D., Milne, A., & Haskell, C. (2010). Consumption of cocoa flavanols results in acute improvements in mood and cognitive performance during sustained mental effort. *Journal of Psychopharmacology, 24*(10), 1505-1514.

Scholey, A., & Kennedy, D. (2002). Acute, dose-dependent cognitive effects of Ginkgo biloba, Panax ginseng and their combination in healthy young volunteers: differential interactions with cognitive demand. *Human Psychopharmacology-Clinical and Experimental, 17*(1), 35-44.

Scholey, A., Moss, M., Neave, N., & Wesnes, K. (1999). Cognitive performance, hyperoxia, and heart rate following oxygen administration in healthy young adults. *Physiology & behavior, 67*(5), 783-789.

Schroeter, M. L., Zysset, S., Kupka, T., Kruggel, F., & von Cramon, D. Y. (2002). Near-infrared spectroscopy can detect brain activity during a color-word matching Stroop task in an event-related design. *Human brain mapping, 17*(1), 61-71.

Schubert, R., Fischer, R., Hain, R., Schreier, P. H., Bahnweg, G., Ernst, D., & Sandermann Jr, H. (1997). An ozone-responsive region of the grapevine resveratrol synthase promoter differs from the basal pathogen-responsive sequence. *Plant molecular biology, 34*(3), 417-426.

Setchell, K. D. R. (1998). Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. *American Journal of Clinical Nutrition, 68*(6), 1333S.

Sevin, A., Özta, P., Senen, D., Han, Ü., Karaman, C., Tarimci, N., Kartal, M., & Erdo an, B. (2007). Effects of polyphenols on skin damage due to ultraviolet A rays: an experimental study on rats. *Journal of the European Academy of Dermatology and Venereology, 21*(5), 650-656.

Shan, B., Cai, Y.-Z., Brooks, J. D., & Corke, H. (2008). Antibacterial properties of Polygonum cuspidatum roots and their major bioactive constituents. *Food Chemistry, 109*(3), 530-537.

Shanmuganayagam, D., Warner, T. F., Krueger, C. G., Reed, J. D., & Folts, J. D. (2007). Concord grape juice attenuates platelet aggregation, serum cholesterol and development of atheroma in hypercholesterolemic rabbits. *Atherosclerosis, 190*(1), 135-142.

Sharma, M., & Gupta, Y. K. (2002). Chronic treatment with trans rosveratrol prevents intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats. *Life Sciences, 71*(21), 2489-2498.

Shibuya-Tayoshi, S., Sumitani, S., Kikuchi, K., Tanaka, T., Tayoshi, S. Y., Ueno, S. I., & Ohmori, T. (2007). Activation of the prefrontal cortex during the Trail-Making Test detected with multichannel near-infrared spectroscopy. *Psychiatry & Clinical Neurosciences, 61*(6), 616.

Shoba, G., Joy, D., Joseph, T., Majeed, M., Rajendran, R., & Srinivas, P. (1998). Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta medica, 64*, 353-356.

Shrime, M. G., Bauer, S. R., McDonald, A. C., Chowdhury, N. H., Coltart, C. E., & Ding, E. L. (2011). Flavonoid-rich cocoa consumption affects multiple cardiovascular risk factors in a meta-analysis of short-term studies. *The Journal of nutrition, 141*(11), 1982-1988.

Sies, H. (2010). Polyphenols and health: update and perspectives. *Archives of Biochemistry and Biophysics, 501*(1), 2-5.

Singh, J., Dubey, R. K., & Atal, C. K. (1986). Piperine-mediated inhibition of glucuronidation activity in isolated epithelial cells of the guinea-pig small intestine: evidence that piperine lowers the endogeneous UDP-glucuronic acid content. *J Pharmacol Exp Ther, 236*(2), 488-493.

Smith, M., Riby, L., Eekelen, J., & Foster, J. (2011). Glucose enhancement of human memory: a comprehensive research review of the glucose memory facilitation effect. *Neuroscience & Biobehavioral Reviews, 35*(3), 770-783.

Smoliga, J., Baur, J., & Hausenblas, H. (2011). Resveratrol and health–a comprehensive review of human clinical trials. *Molecular Nutrition & Food Research, 55*(8), 1129-1141.

Sofi, F., Abbate, R., Gensini, G. F., & Casini, A. (2010). Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. *The American journal of clinical nutrition, 92*(5), 1189-1196.

Son, I. Y., Guhe, M., Gray, W., Yazici, B., & Schoelles, M. J. (2005). *Human performance assessment using fNIR.* Paper presented at the Proceedings of SPIE: Biomonitoring for physiological and cognitive performance during military operations.

Song, J. M., Lee, K. H., & Seong, B. L. (2005). Antiviral effect of catechins in green tea on influenza virus. *Antiviral research, 68*(2), 66-74.

Sorond, F. A., Lipsitz, L. A., Hollenberg, N. K., & Fisher, N. D. L. (2008). Cerebral blood flow response to flavanol-rich cocoa in healthy elderly humans. *Neuropsychiatric Disease and Treatment, 4*(2), 433.

Steffens, D., Norton, M., Plassman, B., Tschanz, J. T., Wyse, B., Welsh-Bohmer, K., Anthony, J., & Breitner, J. (1999). Enhanced cognitive performance with estrogen use in non-demented community-dwelling older women. *Journal of the American Geriatric Society, 47*(10), 1171-1175.

Stein, J. H., Keevil, J. G., Wiebe, D. A., Aeschlimann, S., & Folts, J. D. (1999). Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation, 100*(10), 1050-1055.

Steinbrink, J., Villringer, A., Kempf, F., Haux, D., Boden, S., & Obrig, H. (2005, May 23-29). *Illuminating the BOLD signal: combined fMRI-fNIRS studies.* Paper presented at the International School on Magnetic Resonance and Brain Function, Erice, ITALY.

Stewart, L. K., Soileau, J. L., Ribnicky, D., Wang, Z. Q., Raskin, I., Poulev, A., Majewski, M., Cefalu, W. T., & Gettys, T. W. (2008). Quercetin transiently increases energy

expenditure but persistently decreases circulating markers of inflammation in C57BL/6J mice fed a high-fat diet. *Metabolism: clinical and experimental, 57*(7 Suppl 1), S39.

Steyn, W. J., Wand, S. J. E., Holcroft, D. M., & Jacobs, G. (2002). Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. *New Phytologist, 155*(3), 349-361.

Sudano, I., Flammer, A. J., Noll, G., & Corti, R. (2012). Vascular and platelet effects of cocoa *Chocolate and Health* (pp. 103-113): Springer.

Suganuma, M., Okabe, S., Oniyama, M., Tada, Y., Ito, H., & Fujiki, H. (1998). Wide distribution of [3H](-)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis, 19*(10), 1771.

Syková, E., Kříž, N., & Preis, P. (1983). Elevated extracellular potassium concentration in unstimulated spinal dorsal horns of frogs. *Neuroscience letters, 43*(2), 293-298.

Tamura, M., Hoshi, Y., & Okada, F. (1997). Localized near–infrared spectroscopy and functional optical imaging of brain activity. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 352*(1354), 737-742.

Tao, P. (1992). The inhibitory effects of catechin derivatives on the activities of human immunodeficiency virus reverse transcriptase and DNA polymerases. *Zhongguo yi xue ke xue yuan xue bao. Acta Academiae Medicinae Sinicae, 14*(5), 334.

Taubert, D., Roesen, R., & Schomig, E. (2007). Effect of cocoa and tea intake on blood pressure: a meta-analysis. *Archives of internal medicine, 167*(7), 626.

Teede, H. J., McGrath, B. P., DeSilva, L., Cehun, M., Fassoulakis, A., & Nestel, P. J. (2003). Isoflavones reduce arterial stiffness A placebo-controlled study in men and postmenopausal women. *Arteriosclerosis, thrombosis, and vascular biology, 23*(6), 1066-1071.

Timmers, S., Konings, E., Bilet, L., Houtkooper, R. H., van de Weijer, T., Goossens, G. H., Hoeks, J., van der Krieken, S., Ryu, D., & Kersten, S. (2011). Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell metabolism, 14*(5), 612-622.

Toda, N., & Okamura, T. (2003). The pharmacology of nitric oxide in the peripheral nervous system of blood vessels. *Pharmacological reviews, 55*(2), 271.

Tsai, S. K., Hung, L. M., Fu, Y. T., Cheng, H., Nien, M. W., Liu, H. Y., Zhang, F. B. Y., & Huang, S. S. (2007). Resveratrol neuroprotective effects during focal cerebral ischemia injury via nitric oxide mechanism in rats. *Journal of Vascular Surgery, 46*(2), 346-353.

Tsujimoto, S., Yamamoto, T., Kawaguchi, H., Koizumi, H., & Sawaguchi, T. (2004). Prefrontal cortical activation associated with working memory in adults and preschool children: an event-related optical topography study. *Cerebral Cortex, 14*(7), 703-712.

Udenigwe, C. C., Ramprasath, V. R., Aluko, R. E., & Jones, P. J. H. (2008). Potential of resveratrol in anticancer and anti-inflammatory therapy. [Review]. *Nutrition Reviews, 66*(8), 445-454.

Ungvari, Z., Bagi, Z., Feher, A., Recchia, F. A., Sonntag, W. E., Pearson, K., De Cabo, R., & Csiszar, A. (2010). Resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2. *American Journal of Physiology-Heart and Circulatory Physiology, 299*(1), H18.

Vaibhav, K., Shrivastava, P., Javed, H., Khan, A., Ahmed, M. E., Tabassum, R., Khan, M. M., Khuwaja, G., Islam, F., & Saeed Siddiqui, M. (2012). Piperine suppresses cerebral ischemia–reperfusion-induced inflammation through the repression of COX-2, NOS-2, and NF-κB in middle cerebral artery occlusion rat model. *Mol Cell Biochem*, 1-12.

Valenzano, D. R., Terzibasi, E., Genade, T., Cattaneo, A., Domenici, L., & Cellerino, A. (2006). Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. *Current biology, 16*(3), 296-300.

van de Wetering, K., Burkon, A., Feddema, W., Bot, A., de Jonge, H., Somoza, V., & Borst, P. (2008). Intestinal BCRP/Bcrp1 and MRP3/Mrp3 are involved in the pharmacokinetics of resveratrol. *Mol Pharmacol, 75*, 876-885.

Vellai, T., Takacs-Vellai, K., Zhang, Y., Kovacs, A. L., Orosz, L., & Müller, F. (2003). Influence of TOR kinase on lifespan in C. elegans. *Nature, 426*(620), 10.1038.

Vicenzini, E., Ricciardi, M. C., Altieri, M., Puccinelli, F., Bonaffini, N., Di Piero, V., & Lenzi, G. L. (2007). Cerebrovascular reactivity in degenerative and vascular dementia: a transcranial Doppler study. *European neurology, 58*(2), 84-89.

Vinson, J. A., Teufel, K., & Wu, N. (2001). Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. *Atherosclerosis, 156*(1), 67-72.

Viswanathan, M., Kim, S. K., Berdichevsky, A., & Guarente, L. (2005). A role for SIR-2.1 regulation of ER stress response genes in determining C. elegans life span. *Developmental cell, 9*(5), 605-615.

Walle, T., Hsieh, F., DeLegge, M. H., Oatis, J. E., & Walle, U. K. (2004). High absorption but very low bioavailability of oral resveratrol in humans. *Drug metabolism and disposition, 32*(12), 1377-1382.

Wang, E., Barecki-Roach, M., & Johnson, W. W. (2002). Elevation of P-glycoprotein function by a catechin in green tea. *Biochemical and Biophysical Research Communications, 297*(2), 412-418.

Wang, L., Heredia, A., Song, H., Zhang, Z., Yu, B., Davis, C., & Redfield, R. (2004). Resveratrol glucuronides as the metabolites of resveratrol in humans: characterization, synthesis, and anti-HIV activity. *Journal of pharmaceutical sciences, 93*(10), 2448-2457.

Wang, Q., Xu, J., Rottinghaus, G. E., Simonyi, A., Lubahn, D., Sun, G. Y., & Sun, A. Y. (2002). Resveratrol protects against global cerebral ischemic injury in gerbils. *Brain research, 958*(2), 439-447.

Wang, Z. Y., Agarwal, R., Bickers, D. R., & Mukhtar, H. (1991). Protection against ultraviolet B radiation-induced photocarcinogenesis in hairless mice by green tea polyphenols. *carcinogenesis, 12*(8), 1527.

Weiskopf, R. B., Feiner, J., Hopf, H. W., Viele, M. K., Watson, J. J., Kramer, J. H., Ho, R., & Toy, P. (2002). Oxygen reverses deficits of cognitive function and memory and increased heart rate induced by acute severe isovolemic anemia. *Anesthesiology, 96*(4), 871.

Wenzel, E., & Somoza, V. (2005). Metabolism and bioavailability of trans-resveratrol. *Molecular nutrition & food research, 49*(5).

Wiebe, D. A., Folts, J. D., & Stein, J. H. (2001). Effect of ingestion of purple grape juice on endothelial function in patients with coronary heart disease. *The American journal of cardiology, 88*.

Wightman, E. L., Haskell, C. F., Forster, J. S., Veasey, R. C., & Kennedy, D. O. (2012). Epigallocatechin gallate, cerebral blood flow parameters, cognitive performance and mood in healthy humans: a double-blind, placebo-controlled, crossover investigation. *Human Psychopharmacology: Clinical and Experimental, 27*(2), 177-186.

Willett, W. C., Sacks, F., Trichopoulou, A., Drescher, G., Ferro-Luzzi, A., Helsing, E., & Trichopoulos, D. (1995). Mediterranean diet pyramid: a cultural model for healthy eating. *The American journal of clinical nutrition, 61*(6), 1402S-1406S.

Wollin, S. D., & Jones, P. J. H. (2001). Alcohol, red wine and cardiovascular disease. *The Journal of nutrition, 131*(5), 1401.

Wong, R., Berry, N., Coates, A., Buckley, J., & Howe, P. (2012). Sustained Improvement of Vasodilator Function By Resveratrol in Obese Adults. *Journal of Hypertension, 30*, e70.

Wong, R., Howe, P., Buckley, J., Coates, A., Kunz, I., & Berry, N. (2011). Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure. *Nutr Metab Cardiovasc Dis, 21*, 851-856.

Wu, B., Kulkarni, K., Basu, S., Zhang, S., & Hu, M. (2011). First-pass metabolism via UDP-glucuronosyltransferase: a barrier to oral bioavailability of phenolics. *Journal of pharmaceutical sciences, 100*(9), 3655-3681.

Wu, J. M., Wang, Z. R., Hsieh, T. C., Bruder, J. L., Zou, J. G., & Huang, Y. Z. (2001). Mechanism of cardioprotection by resveratrol, a phenolic antioxidant present in red wine (Review). *International Journal of Molecular Medicine, 8*(1), 3-17.

Xu, Y., Li, S., Chen, R., Li, G., Barish, P. A., You, W., Chen, L., Lin, M., Ku, B., Pan, J., & Ogle, W. O. (2010). Antidepressant-like effect of low molecular proanthocyanidin in mice: Involvement of monoaminergic system. *Pharmacology Biochemistry and Behavior, 94*(3), 447-453.

Xu, Y., Wang, Z., You, W., Zhang, X., Li, S., Barish, P. A., Vernon, M. M., Du, X., Li, G., & Pan, J. (2010). Antidepressant-like effect of trans-resveratrol: Involvement of serotonin and noradrenaline system. *European Neuropsychopharmacology, 20*(6), 405-413.

Yáñez, M., Fraiz, N., Cano, E., & Orallo, F. (2006). (-)-Trans-[epsilon]-viniferin, a polyphenol present in wines, is an inhibitor of noradrenaline and 5-hydroxytryptamine uptake and of monoamine oxidase activity. *European journal of pharmacology, 542*(1-3), 54-60.

Yousuf, S., Atif, F., Ahmad, M., Hoda, N., Ishrat, T., Khan, B., & Islam, F. (2009). Resveratrol exerts its neuroprotective effect by modulating mitochondrial dysfunctions and associated cell death during cerebral ischemia. *Brain Research, 1250*, 242-253.

Zeiher, A. M., Drexler, H., Saurbier, B., & Just, H. (1993). Endothelium-mediated coronary blood flow modulation in humans. Effects of age, atherosclerosis, hypercholesterolemia, and hypertension. *Journal of Clinical Investigation, 92*(2), 652.

Zeiher, A. M., Drexler, H., Wollschlager, H., & Just, H. (1991). Endothelial dysfunction of the coronary microvasculature is associated with coronary blood flow regulation in patients with early atherosclerosis. *Circulation, 84*(5), 1984-1992.

Zern, T. L., Wood, R. J., Greene, C., West, K. L., Liu, Y., Aggarwal, D., Shachter, N. S., & Fernandez, M. L. (2005). Grape polyphenols exert a cardioprotective effect in pre-and postmenopausal women by lowering plasma lipids and reducing oxidative stress. *The Journal of nutrition, 135*(8), 1911-1917.

Zhang, Q., Tang, X., Lu, Q. Y., Zhang, Z. F., Brown, J., & Le, A. D. (2005). Resveratrol inhibits hypoxia-induced accumulation of hypoxia-inducible factor-1α and VEGF expression in human tongue squamous cell carcinoma and hepatoma cells. *Molecular cancer therapeutics, 4*(10), 1465-1474.

Appendix I

Investigation into the subjective perceptions of task difficulty and mental fatigue.

Participants

Fifteen participants (3 male, 12 female; mean age 21.6yrs, range 18-34yrs; all right handed) took part in this investigation designed to ascertain subjective opinion of 'mental fatigue' and 'difficulty' with regards commonly used computerised cognitive tasks.

Tasks

In order, participants completed 2-minutes each of Serial 3s, 7s, 13s and 17s subtractions followed by the 2-minute and then 5-minute version of the Rapid Visual Information Processing (RVIP) task, this was followed by 2-minutes each of simple and choice reaction time, 2-minutes of digit vigilance and, finally, both the 2-Back and 3-Back version of the N-Back task.

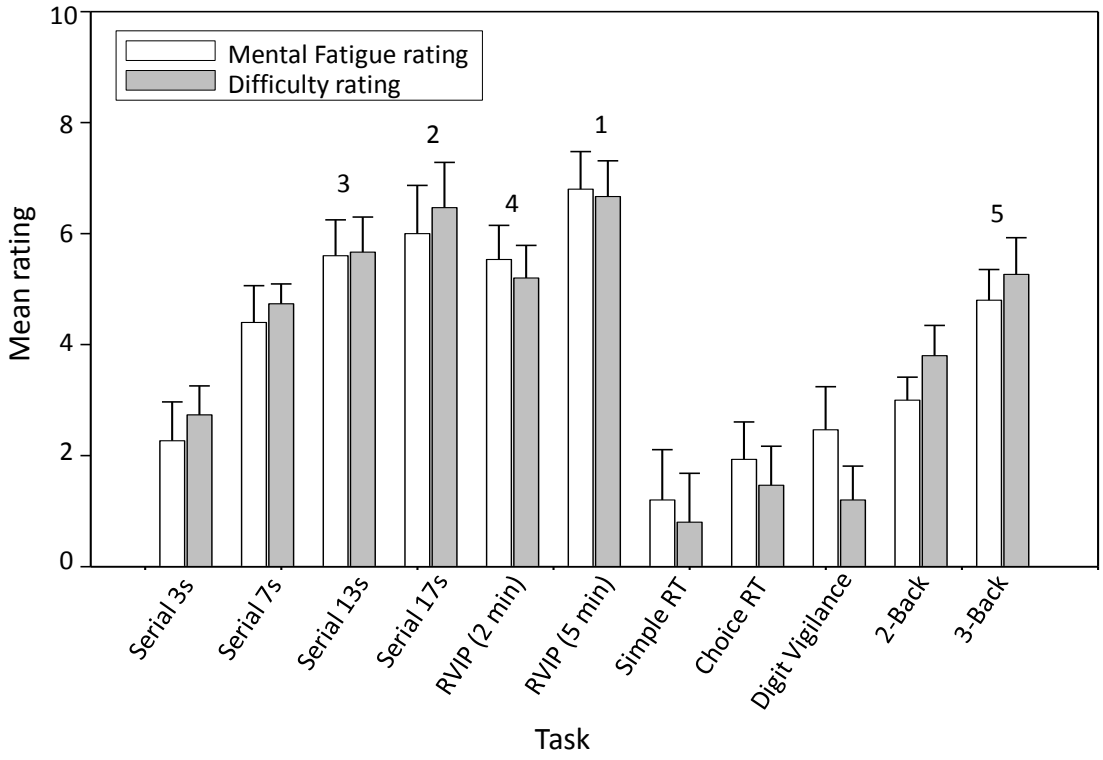
Procedure

Participants arrived at the lab, at a time that was convenient to them, where they were immediately briefed and written informed consent obtained. After verbally explaining the instructions for all tasks, participants completed a 1-minute version of each task once and afterwards discussed the instructions with the researcher in order to ensure that they understood what each task required. Once their understanding was confirmed, participants completed the tasks proper in the order described above.

Participants, at the end of the testing session, were asked to rate all 11 tasks in terms of how 'mentally fatiguing' and 'difficult' they perceived them to be- with a value of 1 being the most and 11 being the least mentally fatiguing/difficult. (These ratings were reverse scored for the purposes of presentation here.)

Results

The results demonstrated that participants rated the same 5 tasks as both the most 'difficult' and the most 'mentally fatiguing': RVIP (5-minute version); Serial 17s subtractions; Serial 13s subtractions; RVIP (2-minute version); and the 3-Back version of N-Back task.



Graph displays the mean ratings (with standard error bars) of 'metal fatigue' and difficulty' for 11 cognitive tasks from 15 young (18-34yrs) males and females. Ratings were assessed by a 1-10 scale with '1' being the most difficult and '10' the least. Ratings were reversed scored for the purposes of presentation and the top 5 most difficult and mentally fatiguing cognitive tasks are highlighted on the graph.

Appendix II

Food consumption questionnaire.

The following questions pertain to your food and beverage consumption over the last 7 days only. Please answer as honestly as you can.

Participant number	
Date	
Questionnaire number (out of 4)	

Q1. How many portions of fruit and vegetables did you eat on an average day in the past week?	
Q2. In the entire previous week, on how many occasions have you eaten a portion of berries or grapes?	
Q3. Approximately how many calories did you consume on an average day in the past week?	
Q4. How many glasses of water did you consume on an average day in the past week?	
Q5. How many cups of tea did you consume on an average day in the past week?	
Q6. How many cups of coffee did you consume on an average day in the past week?	
Q7. Approximately how many units of alcohol did you consume in the past week?	
Q8. Specifically, how many glasses of red wine did you consume in the past week?	
Q9. Did you manage to consume each day's treatment in the past week?	
Q10. If not, please describe which day/s and any other information.	
Q11. Was treatment consumed with breakfast and/or before 9:30am every day in the past week?	
Q12. If not, please describe which day/s and any other information.	
Q13. Have you consumed any medication in the past week? If so, please state the medication, dose, when taken and for what reason.	