# The Psychopharmacological Effects of Blackcurrant Phytochemicals in Humans

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# Abstract

Self-medication of plant based foods and food extracts which have ostensible therapeutic benefits has considerably increased within non-clinical populations over the last two decades. The overarching reason for this rise in consumption is to improve health and cognitive performance. One such group of foods are flavonoid-rich berry fruits. In the literature, there has been a recent rise in evidence showing that the consumption of flavonoid-rich berries can modulate aspects of behaviour, especially memory, in animal models and in aged humans. Physiological parameters such as blood-flow and glucose levels; and biological mechanisms such as monoamine oxidase (MAO) activity and nitric oxide (NO) synthesis, which have the potential to impact human behaviour, are also shown to be manipulated by flavonoid compounds. The dark purple fruits of the blackcurrant (*Ribes nigrum*) are naturally high in flavonoids, however no literature assessing cognitive effects of their consumption is available. The main focus of this thesis was to assess the impact of standardised flavonoid-rich blackcurrant extracts upon cognitive performance and mood in healthy human participants. Two extracts were examined within the thesis, a freeze dried powder extract fortified to contain 30% anthocyanins (Delcyan$^{TM}$) and a fresh from frozen cold pressed juice extract (Blackadder cultivar, Plant and Food Research Ltd). Utilising a series of randomised, between subjects, double bind studies, measures of memory, attention, executive function and psychomotor performance were implemented during the course of the thesis at various post-dose time points. Throughout the investigational chapters of the thesis, physiological parameters and potential mechanisms driving any behavioural changes were measured. Such measures included measures of central and peripheral haemodynamics, MAO inhibition, monoaminergic tone, prolactin secretion and post-prandial glucose profiles.

Single doses of each of the blackcurrant extracts used in this thesis yielded positive results with effects of post-harvest extraction technique evident. Although no clear pattern of behavioural modulation was found after consumption of the blackcurrant extracts, there was some evidence to show increases in attention processes during cognitively demanding paradigms in young participants. No positive effects were evident upon any other cognitive paradigm. Physiological effects of acute blackcurrant supplementation included a modulation of post-prandial glucose profile and hemispheric dependent modulations of cerebral blood flow. Most strikingly, a pharmaceutical level inhibition of both monoamine oxidase isoforms and reductions in blood plasma prolactin were found. The findings of this thesis may have implications for enhancement of cognitive performance, attenuation in natural cognitive decline over the lifespan, and potentially, clinical applications in the treatment of neurological diseases.

# Contents

# Tables and Figures

## Chapter one

***Figures***



## Chapter two

***Figures***



***Tables***

## Chapter three

***Figures***



***Tables***

## Chapter four

***Figures***



***Tables***



## Chapter five

***Figures***



***Tables***

## Chapter six

***Figures***



***Tables***



## Chapter seven

***Figures***



***Tables***

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## Declaration

This work has not been submitted for any other award. Apart from the exceptions where due acknowledgment has been given, in all experimental chapters of this thesis, the author had sole responsibility for the data collection, analysis, and interpretation.

**Name** Anthony William Watson

**Signed**

**Date**

# CHAPTER 1. INTRODUCTION

## 1.1 General introduction

Food is essential for everyday life, providing nutrients to maintain homeostasis. Certain foods also contain bioactive compounds, which can be advantageous for health promotion and disease prevention. Foods that are high in these bioactive compounds have been termed “functional foods” or “super foods”. Plant based functional foods comprise phytochemicals that have the potential to exert a psychological or physiological effect beyond their nutritional capacity. The purported biological effects caused by the consumption of some phytochemicals found in functional foods are wide-ranging and include, but are not restricted to; stimulant properties (Haskell *et al.*, 2007), alleviation from stress (Brattstram, 2009), protection from cardiovascular disease (Francis *et al.*, 2006), cognitive enhancement (Kennedy *et al.*, 2007), neuro-protection (Kim *et al.*, 2010) and protection from carcinogenesis (Bishayee *et al.*, 2011). These effects differ greatly depending upon the compounds present within the foods, their chemical structures and their post-harvest processing. Although many compounds found in functional foods have shown promising results *in vitro,* definitive *in vivo* effects, especially in humans, are lacking. Therefore, a growing discussion regarding potential *in vivo* mechanisms driving purported health promoting properties is emerging in the literature.

Self-medication in healthy non-clinical populations through products derived from functional foods, has significantly increased in popularity over the last decade; to such a level that approximately 20% of the North American population use them daily (Bent, 2008). This is despite a lack of data to support the efficacy of the majority of these products’ purported *in vivo* effects. This increase in consumer demand is driving the search for definitive functional foods with quantifiable, *in vivo* effects upon human health maintenance, disease prevention and behaviour modulation. Berries are such a functional food, which have a growing convergence of scientific evidence suggesting a

modulation of both animal model and human behaviour. These effects are particularly evident in memory and attention paradigms (see section 1.4.1). When compared to other commonly eaten berries, such as raspberries (30mg of flavonoids per 100g of dried berry) and strawberries (10mg/100g dried berry), blackcurrants have especially high levels of the class of phytochemical, flavonoids (80mg/100g dried berry) (Kähkönen *et al.*, 2001).

Given the growing popularity of “super foods”; the emerging research surrounding the potential behavioural enhancing effects of flavonoid-rich foods; and the lack of controlled human trials investigating blackcurrants and their impact upon cognitive performance; the first question that this thesis will attempt to address is whether the consumption of standardised blackcurrant extracts can have a beneficial effect upon human behaviour. The second question is whether the underlying physiological mechanisms of action driving any behavioural effects can be elucidated. The studies that make up this thesis will include investigations of the cognitive enhancement properties of a commercially available powdered blackcurrant extract and a cold pressed blackcurrant juice.

The remainder of this chapter includes a review of the literature across a number of disciplines and, in addition to blackcurrants, encompasses other functional foods that are flavonoid-rich. The inclusion of these foods is necessary due to a lack of research into the effects of blackcurrants, as they provide peer-reviewed evidence from well conducted nutritional intervention trials for an augmentation of psychological and physiological parameters in healthy human participants after the consumption of flavonoid-rich food extracts. The review will begin with a discussion of the potential bioactive compounds in blackcurrants, incorporating their structure and pharmacokinetics. The effect of post-harvest processing upon the levels and structures of phytochemicals in flavonoid-rich foods will also be discussed to outline the impact of

food preparation upon potentially bioactive compounds. The review will then give a synopsis of the modulating effect flavonoid-rich foods upon biological and physiological parameters which could impact human cognitive performance and health. Finally, the impact of foods, rich in flavonoids, upon the cognitive ability of animal models and humans will be discussed.

## 1.2. Phytochemicals in blackcurrants

Phytochemicals are chemical plant compounds that naturally occur in all plants and plant-based foods. A large selection of these phytochemicals consumed in the human diet may have the potential to influence human well-being through a variety of mechanisms. Based upon their chemical structure, there are several major subclasses of phytochemicals including terpenes, thiols, alkaloids, isoprenoids and phenols, with phenols being the largest group. The following section will focus upon polyphenols and phenolic acids, which are abundant in blackcurrants and are theorised to be the compounds behind physiological and psychological modulations discussed in sections 1.4 to 1.5 of this thesis.

### 1.2.1 (Poly)phenols

Polyphenols are organic compounds, of plant origin, which have more than one phenol group. There are thousands of molecules in plants that have a polyphenolic structure (Manach *et al.*, 2005). The structure of natural polyphenols range from simple molecules such as phenolic acids, to large polymerised compounds such as tannins (Bravo, 1998). Polyphenols can be separated into four main sub categories; flavonoids, phenolic acids, lignans and stilbenes. In terms of blackcurrants, flavonoids and phenolic acids make up the majority of the phenolic profile (figure 1.1). Within these sub divisions sit the flavonoids: anthocyanins, proanthocyanidins, flavonols, flavones, flavanones isoflavones and flavanols (Beecher, 2003); and the phenolic acids: hydroxycinnamic acids and hydroxybenzoic acids. These ‘secondary metabolites’ occur

ubiquitously in all plants to varying degrees. They play endogenous roles that can encompass the provision of colour (Koes *et al.*, 1994), protective actions (Kootstra, 1994), and deterrence and attraction of insects and herbivores, including symbiotic hormonal and central nervous system interactions with insects (Wink, 2003). With this in mind, the potential health benefits of polyphenol consumption within mammalian biological systems may be related to their bioactive roles within plants; these have been shown to include antioxidant, anti-allergic, anti-inflammatory and antiviral properties (see Kennedy & Wightman 2011 for a full review). The absorption and, therefore, bioactivity of a polyphenol is dependent upon its molecular size and configuration. The following sub-sections will focus primarily upon the structure of flavonoids and phenolic acids because of their abundance in blackcurrants.

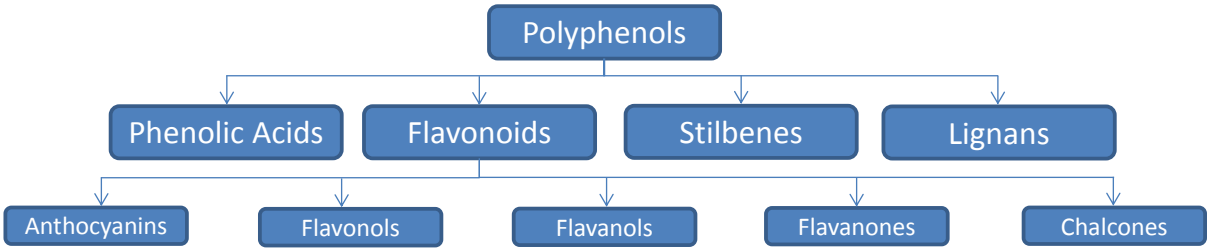



*Figure 1.1 Classifications of the major polyphenols found in blackcurrants.*

### 1.2.1.1 Flavonoids

Flavonoids are organic plant secondary metabolites with a low molecular weight. They are almost universally expressed in plants and include the pigments responsible for the colours in some plants. Derived from 2-phenylchromen-4-one (Mcnaught *et al.*, 1997), flavonoids are an assortment of phenylbenzopyrone structures based on a common three-ring nucleus which can be seen below in figure 1.2. The major subclasses of flavonoids include, flavonols, flavanols, flavones, chalcones and anthocyanidins (Mcnaught *et al.*, 1997). The structural differences of the most common flavonoids can also be seen in figure 1.2.

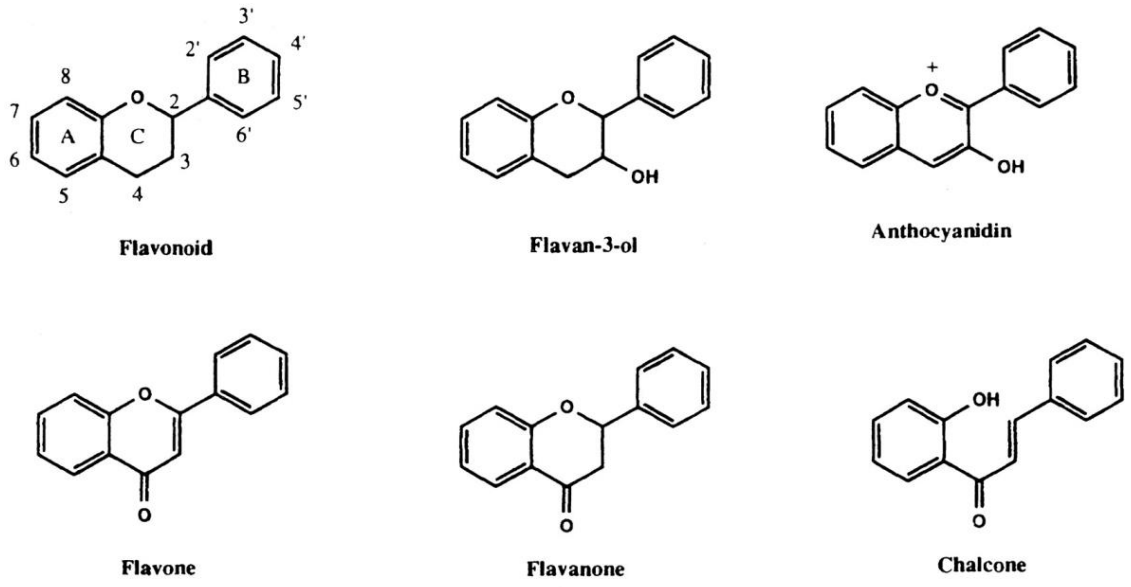



*Figure 1.2 Chemical structures of the most common flavonoid subclasses. (Picture modified with permission from Middleton et al., 2000).*

### *Anthocyanins*

Anthocyanins are the most abundant flavonoid found in the fresh blackcurrant fruit. They are derivatives of the three ring anthocyanidin structure shown in figure 1.2; however, they include pendant sugars, which, as discussed in section 1.3 of this chapter, could greatly increase the bioavailability of the flavonoid. Anthocyanins are responsible for the red, violet, and blue colour of most berries and fruits. Seventeen different anthocyanins exist, each differing in chemical structure to one another (Kong *et al.*, 2003). Below are the structures of the two most abundant anthocyanins in blackcurrants; cyanidin-3-0-glucoside and delphinidin-3-0-glucoside along with their aglycone molecules, cyanidin and delphinidin (figure 1.3).

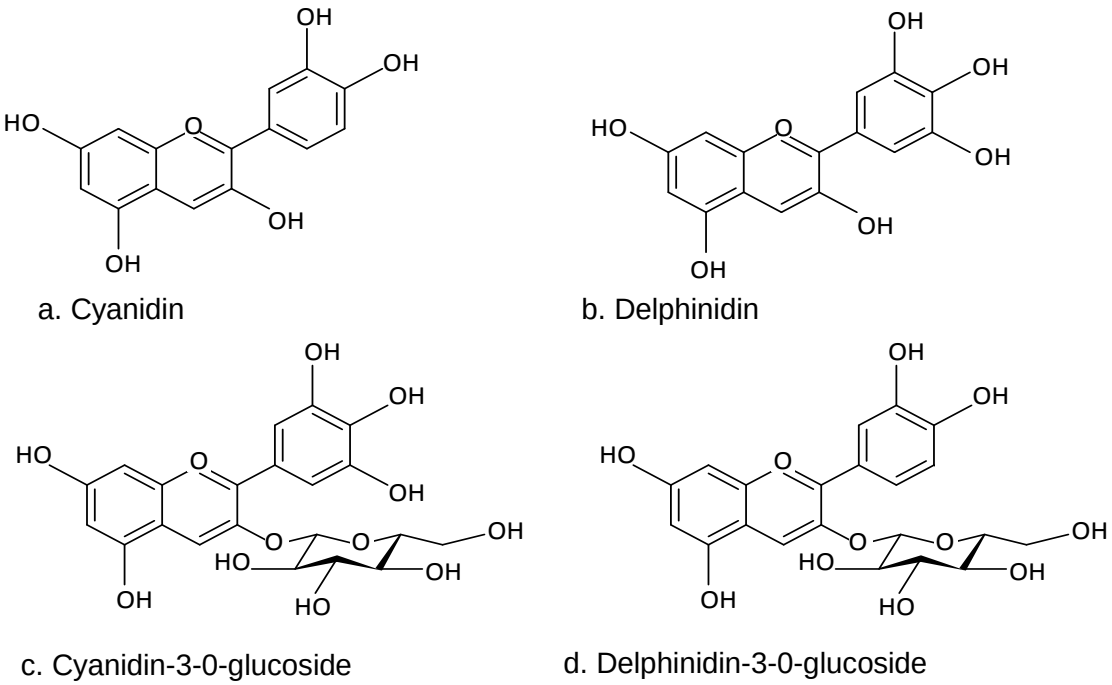
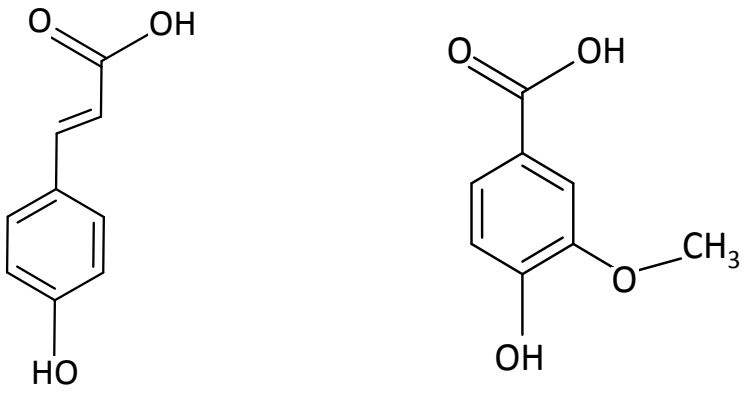



*Figure 1.3 Chemical structure of anthocyanidins cyanidin (a) and delphinidin (b) and their anthocyanin glucoside derivatives cyanidin-3-0-glucoside (c) and delphinidin-3-0-glucoside (d) which are the major phenolic constituents of blackcurrants. Marvin was used for drawing, displaying and characterizing chemical structures. Marvin sketch (version 6.0), 201n (2013), ChemAxon (http://www.chemaxon.com)*

### 1.2.1.2 Phenolic acids

The two major subclasses of phenolic acids, detectable in processed blackcurrants to varying degrees, are hydroxycinnamic acids and hydroxybenzoic acids. The most common hydroxycinnamic acids are ferulic, *p*-coumaric, sinapic and caffeic acids; while the corresponding hydroxybenzoic acids are *p*-hydroxybenzoic, protocatechuic, vanillic, and syringic acids (Mattila *et al.*, 2006), with the structural difference between the two subgroups being a differentiation in the hydroxylations and methoxylations of their aromatic rings (Mattila *et al.*, 2006). Figure 1.4 below provides an example of this, showing the structural differences of the compounds *p*-coumaric acid and vanillic acid, which belong to each of the sub groups.




a. *p*-Coumaric acid b. Vanillic acid

*Figure 1.4 Phenolic acid subgroups (a). an example of a hydroxycinnamic acid (b). an example of a hydroxybenzoic acid. Marvin was used for drawing, displaying and characterizing chemical structures,, Marvin sketch (version 6.0), 201n (2013), ChemAxon (http://www.chemaxon.com)*

### 1.2.2 Foods rich in flavonoids

As discussed in section 1.1, there is evidence to support an augmentation of human behaviour after the consumption of flavonoid-rich foods. However, there are no peer reviewed publications assessing the consumption of blackcurrants upon the cognitive performance of humans. This thesis will, therefore, look at the evidence from cocoa and berries, two flavonoid-rich foods which, in the literature, have evidence to support modulations of cognitive function and physiological parameters that could benefit health, well-being and cognitive performance. The section below outlines these flavonoid-rich foods and their flavonoid constituents. Moreover, this section will also discuss the impact post-harvest processing has upon the quantity and structure of potentially bioactive compounds within these foods; two factors which could affect their ability to impact human behaviour.

#### 1.2.2.1 Cocoa

When ripe, the cocoa pod of the *Theobroma cacoa* plant weighs around 500g and contains approximately 20-60 seeds, known as cocoa beans. The cocoa bean contains around 40% to 60% fat and is rich in polyphenols. Cocoa beans contain high levels of flavan-3-ols, a sub class of flavonoids, which are now widely accepted to be the main active component responsible for the health benefits observed following cocoa

consumption. As with all polyphenols, there is large variability in content of flavan-3-ols as a consequence of cultivar, growing conditions, harvesting and processing; however, flavan-3-ols tend to make up at least 10% of cocoa powder (Hammerstone *et al.*, 2000). Of the flavan-3-ols, the monomers (+)-catechin and (-)-epicatechin are of particular interest. These monomers, along with their enantiomeric forms (-)-catechin and (+)-epicatechin, are often present in nature as subunits of the oligomers (procyanidins). Monomers have been shown to make up around 12% of the total procyanidin content of cocoa based products irrespective of whether measured in unsweetened or dark chocolate, whereas large polymers range from ~28% in dark chocolate to ~34% in unsweetened chocolate (Gu *et al.*, 2004).

#### 1.2.2.2 Berries

Berries are many seeded fleshy fruits (Allaby, 1996). Amongst others, this fruit genus contains grape, blueberry, cranberry, redcurrant and blackcurrant. Berries are polyphenol-rich foods containing up to 5240mg/100g of fruit (crowberry). However, the amount and type of polyphenols depends upon berry species, growing conditions and geological location of cultivation (Kähkönen *et al.*, 2001). The major polyphenols in berries are the flavonoid anthocyanins with up to 3090mg/100g of fruit (Kähkönen *et al.*, 2001) and phenolic acids (Mattila *et al.*, 2006).

***Blueberry***

The blueberry plant [*Cyanococcus vaccinium*] is an erect or bushlike plant found in central America, Europe, Asia and Africa. The fruit is a small berry with a flared crown at the end. When ripe the berries are a purple in colour and are coated in a white bloom “epicuticular wax”. Blueberries contain from 400 to 600mg of total phenolics per 100g (chlorogenic acid equivalents) of fresh fruit, dependent upon cultivar and growing location (Connor *et al.*, 2002). The dark purple colour of the berries is owed to the anthocyanin content which range from 110 to 260 mg/100g of fresh berries dependent

upon the cultivar (Gao & Mazza, 1994). However, processing and storage of blueberries greatly changes the anthocyanin and phenolic acid yield, with a reduction of up to 68% and 47% respectively after the juicing process (Skrede *et al.*, 2000). Cooking of the berries further changes the phenolic content of the berries with reductions of anthocyanins of 20% after the cooking process and an increase in phenolic acids of 15% (Rodriguez-Mateos *et al.*, 2013).

***Blackcurrant***

The blackcurrant [*Ribes nigrum*] is a temperate fruit native to Europe and northern Asia. It is now widely cultivated both commercially and domestically for its abundant berries. Blackcurrant berries are widely commercially available and are consumed in their raw state, cooked, juiced and used as concentrated extracts. The berries of the blackcurrant are dark purple with a glossy skin and contain several small seeds. Blackcurrant berries are known to be naturally high in polyphenols (Hollands *et al.*, 2008). The major anthocyanin constituents of blackcurrants are delphinidin 3-O-glucoside (D3G) (14%), delphinidin 3-O-rutinoside (D3R) (36.59%) cyanidin 3-O-glucoside (C3G) (7.08%) and cyanidin 3-O-rutinoside (C3R) (40.15%) (Slimestad & Solheim, 2002). Other anthocyanins such as petunidin, pelargonidin, peonidin and malvidin glucosides and rutinosides are also found in blackcurrants, but at much lower quantities (Slimestad & Solheim, 2002). Although anthocyanins, their derivatives and conjugates are the main polyphenols found in blackcurrants, accounting for over 90% of their phenolic makeup (Anttonen & Karjalainen, 2006), conjugates of other flavonoids such as myricetin glucoside (22mg/kg), quercetin glucoside (17.8mg/kg) and kaempferol glucoside (4.65 mg/kg); as well as phenolic acids such as *p*-coumaric acid (2.32mg/kg), 3-caffeoylquinic acid (3.37mg/kg) and ferulic acid (4.09mg/kg), can also be found (Anttonen & Karjalainen, 2006). Quantities of these polyphenols are greatly influenced by specifics of cultivar, cultivation techniques, geographical location and post-harvest preparation (Anttonen & Karjalainen, 2006; Hollands *et al.*, 2008). For

example, European cultivars of blackcurrants contain on average 500mg of polyphenols per 100g of fruit, whereas cultivars grown in New Zealand can yield up to 700mg of polyphenols. Further to this, the anthocyanin content of fresh blackcurrants has been shown to be as high as 900mg/100g of fresh fruit as measured by HPLC, whereas ready to drink blackcurrant juice can contain as little as 0.5mg/100g (Hollands *et al.*, 2008).

### 1.3 Pharmacokinetics of flavonoids and phenolic acids

The release of phytochemicals from the food matrix depends upon the plant food source, its processing conditions and the presence of other dietary components. Thereafter, the absorption of the phytochemical separated from the food matrix is dependent upon its molecular size and configuration, lipophilicity, solubility, stability at low pH and the effect of phase one, two and three metabolising enzymes/systems. The available literature on the effect of the plant food matrix on absorption is limited, with very little potential for intra-study comparisons.

In regards to anthocyanins, the bioavailability in humans is extremely low after oral ingestion of berries with less than 0.1% of intact glucoside, rutinoside and acylated forms being found in plasma after oral consumption (Matsumoto *et al.*, 2001; Mazza *et al.*, 2002; Nielsen *et al.*, 2003). Intact glucosides, galactosides and arbinosides of cyanidin, delphinidin, petunidin, pelargonidin, peonidin and malvidin, have been found in the blood and urine of humans after oral ingestion of flavonoid-rich berries, such as blueberries and boysenberries (Kay *et al.*, 2004). However, studies investigating blackcurrants have measured only D3G, D3R, C3G and C3R (Matsumoto *et al.*, 2001; Nielsen *et al.*, 2003; Matsumoto *et al.*, 2005b). The absorption after oral ingestion and subsequent maximal concentrations ($C_{max}$) and time to maximal concentration ($t_{max}$) of anthocyanins quantified in the blood, have been shown to be dependent upon the form of food ingested. For example, Matsumoto *et al*., (2005) supplemented healthy

participants with 33mg (combined total) of isolated D3G, D3R, C3G and C3R compounds per kilo of body weight. D3G, D3R, C3G and C3R were found intact in both plasma and urine with total blood plasma $t_{max}$ at one to two hours post-supplementation, dependent on the anthocyanin, with a gradual decline in plasma concentration to half of $C_{max}$ over the subsequent four hours of measurement. The $C_{max}$ of the total ingested anthocyanins found in blood plasma was less than 1% at ˜40 ng/ml. In a study using a whole food matrix, Mazza *et al.,* (2002) supplemented five adult males with 100g of freeze dried blueberries containing 2.79g of polyphenols, of which 1.2g were anthocyanins. Maximum concentrations of anthocyanins (combined total) were found in plasma four hours after consumption with $C_{max}$ of 13ng/ml. Differences in time to maximum blood plasma concentrations of anthocyanins between these two studies suggest that the absorption rate of anthocyanins and other flavonoids in humans may be affected by not only the chemical structure of the compound, but other factors including chemical interactions within food matrices and intestinal components, as well as structural and compositional characteristics of the source of the flavonoid being studied.

As with circulatory blood levels, urinary excretion of anthocyanins is also low after oral ingestion with between 0.016 and 0.22% of that ingested being excreted (Netzel *et al.*, 2001; Nielsen *et al.*, 2003; Hollands *et al.*, 2008). Animal models have also shown that there is limited excretion of intact anthocyanins in faeces. This poor bioavailability of intact anthocyanins suggests a comprehensive bio-transformation after oral ingestion. Along with pH, metabolism of anthocyanins by microflora may contribute to the bioavailability of anthocyanins. Keppler & Humpf (2005) utilised pig microflora as a model of anthocyanin degradation in the human digestive tract. It was found that anthocyanidin glycosides are hydrolysed extensively by intestinal microflora depending on the sugar moiety. After cleavage of the protective 3-glycosidic linkage, the released aglycones are very unstable under physiological conditions in the intestine at neutral

pH and degrade spontaneously into phenolic acids and aldehydes. A graphical representation of this can be seen in figure 1.5. This would, therefore, reduce the possibility for anthocyanins or anthocyanidins to act locally or systemically in their polymer state because of their deglycosylation and degradation into monomeric phenolic molecules, which is dependent on both pH and microbial metabolism. This was demonstrated in a human *in vivo* study whereby benzoic, hippuric, salicylic and phenylacetic acid were detectable in blood plasma of 20 healthy adults post supplementation of a 20% blackcurrant juice drink containing 31mg of polyphenols (30.4mg of anthocyanins and 616µM phenolic acids), despite not being present in the juice administered. Plasma concentrations of benzoic, hippuric, salicylic and phenylacetic acid peaked 30 minutes after consumption of the drink, levels then diminished after 80 minutes, remerging at 120 minutes and peaking for a second time 180 minutes after ingestion (Jin *et al.*, 2011). This metabolic transformation could indicate quick metabolism of flavonoids in the colon (Kim *et al.*, 1998) then further biochemical or microbial degradation of the phenolic compounds, as suggested by Keppler & Humpf (2005) (figure 1.5). However, anthocyanins have been shown to be quickly absorbed through the stomach, potentially through interactions with gut efflux transporters (Dreiseitel *et al.*, 2009b), and, as discussed above, are detectable in the blood and urine of humans. This outlines absorption of anthocyanins from the stomach into the blood stream, then further transformation of the anthocyanins through a complex stage of events in the gut to smaller phenolic compounds, which are later absorbed and reappear in the blood in a biphasic manner. These multiple peaks in blood plasma phenolics potentially outline several therapeutic windows after the consumption of flavonoid-rich foods and also the potential for a pro-drug effect, where compounds present in the foods are not biologically active *per se*, but are metabolised into an active compound *in vivo*.

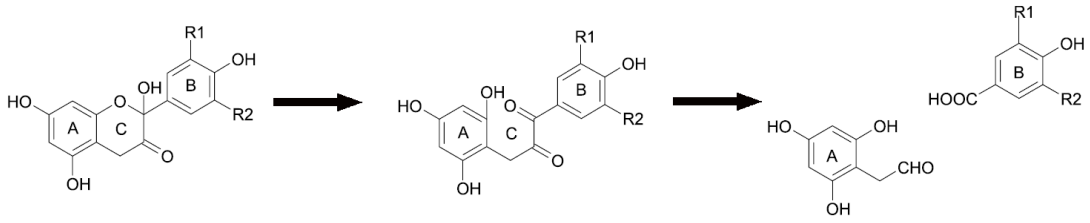



*Figure 1.5 Degradation of an anthocyanidin into an α-diketone and monomeric phenolic compounds (modified with permission from Keppler & Humpf, 2005).*

Despite an increase in evidence regarding the ability of anthocyanins to reach the periphery, a lot less is known about their ability to gain access to the central nervous system; a crucial factor if they are to have a direct effect upon the brain. In order to reach the central nervous system, molecules must cross the blood brain barrier, a selectively permeable endothelial cell layer that actively isolates molecules in the peripheral vasculature from the central nervous system. A recent review by Stevenson *et al.,* (2010) describes how flavonoids have the correct hydrophobicity to cross the blood brain barrier. Although glucoronidation and sulphation restrict their permeability by greatly reducing their hydrophobicity; intact galactoside, glucoside and arabinose forms of anthocyanins have been quantified at femtomolar levels in the cerebellum, cortex, hippocampus and striatum of blueberry fed rats, but not control animals, after eight weeks supplementation of a 2% blueberry diet (Andres-Lacueva *et al.*, 2005). However, blood contamination of the tissue or the possibility of polyphenols sticking to blood vessel walls cannot be ruled out as an explanation for these extraordinarily small amounts being detectable.

The rate of absorption and bioavailability discussed above and the *in vivo* site of availability of potentially psychoactive compounds, has a fundamental effect on their physiological function (Keppler & Humpf, 2005) and, therefore, their ability to modulate human behaviour. If a compound is potent *in vitro* but the *in vivo* bioavailability is low, the direct behaviour modulating effects may be extremely modest or absent. For this reason, research focusing upon the intact compounds found in fruit and vegetables

directly acting upon cells must be treated with caution in terms of its applicability *in vivo*.

## 1.4 Mechanisms of action of flavonoids and phenolic acids

A number of the biological effects of flavonoids could play a key role in the modulation of both acute and chronic human cognitive performance. These include antioxidant and anti-inflammatory properties, modulation of nitric oxide synthesis, enzyme modulation, regulation of cell signalling cascades, gene expression and neurotransmitter modulation. These may facilitate observed physiological effects reported after the consumption of flavonoid-rich foods which, as discussed in section 1.5 of this chapter, include neuro-protection, regulation of haemodynamics and neurotransmitter modulation.

### 1.4.1 Neuro-protection and neuro-signalling

Oxidative stress describes a disparity amongst the accumulation of systemic reactive oxygen species and the body's natural capacity to detoxify the facilitating structures and repair any damage caused. Historically, it has been assumed that the foundations of the neuro-protective actions of flavonoids are based on their ability to scavenge reactive oxygen species or influence intracellular redox species and, therefore, protect against damage associated with oxidative stress. It is now becoming common theory that the effects of flavonoids on the brain are mediated by their capacity to; enhance existing neuronal function, enhance synaptic transmission, stimulate neurogenesis, and protect vulnerable neurons via various mechanisms. As discussed earlier, flavonoids have been found intact in the brains of rodents, so a direct effect upon the brain is not an unreasonable assumption. The ability of flavonoids to affect the memory of humans, discussed later in section 1.6, could be in part related to interactions, on a physiological level, with cells and neurons responsible for normal memory function.

#### 1.4.1.1 Neuro-protection

In the presence of extracellular events, cells send an intracellular response to initiate an appropriate action. The mitogen activated protein kinase (MAPK) pathway is a chain of proteins found in all eukaryotic cells (Robinson & Cobb, 1997). Playing a major role in physiological and pathological cell proliferation, the MAPK pathway transmits a signal from a receptor on the outer surface of the cell to DNA in the nucleus of the cell; directing cellular responses to a diverse array of stimuli, such as mitogens, heat stress, osmotic stress and pro-inflammatory cytokines (for a comprehensive review see Zhang & Liu, 2002). Therefore, MAPK pathways are an ideal therapeutic target for cell survival. A contributing factor to disintegration of neuronal cells is neuronal inflammation. Processes surrounding neuro-inflammation are believed to be heavily implicated in neurodegenerative diseases (Hunot & Hirsch, 2003), as well as in neuronal injury associated with ischemia (Zheng *et al.*, 2003). Increases in interleukin-1β, tumour necrosis factor-alpha (Holmin & Mathiesen, 2000), inducible nitric oxide synthase (iNOS) and nitric oxide (Bal-Price & Brown, 2001) and NADPH oxidase activation (Sun *et al.*, 2008), all contribute to this neuro-inflammation in activated central nervous system cells such as astrocytes and microglia, causing glial-induced neuronal death. Many of these signalling cascades are regulated by MAPK signalling (Bhat *et al.*, 1998), a key mediator of cytokines and iNOS (Marcus *et al.*, 2003). Therefore, regulating upstream MAPK could reduce glial inflammation and associated cell damage, protecting against progressive neuronal loss (Casper *et al.*, 2000) and amyloidogenesis associated with neurodegenerative diseases (Heneka *et al.*, 2005).

The literature demonstrates that phytochemicals found in fruits can regulate MAPK and other signalling pathways. For example, blueberries have been shown to up regulate the expression of protective MAPK/cAMP response element binding (CREB), (Joseph *et al.*, 2003), and to increase neuronal MAPK signalling and extracellular signal-regulated kinases (ERK) and insulin-like growth factor 1 (IGF-1) expression associated

with hippocampal plasticity, learning and memory (Casadesus *et al.*, 2004). Furthermore, naringenin, a citrus fruit flavonoid, has been shown to protect against inflammatory-induced neuronal death in a primary neuronal–glial co-culture system via inhibition of p38/c-Jun N-terminal kinases (JNK) signalling cascades, and also reduced iNOS expression and nitric oxide production in glial cells (Vafeiadou *et al.*, 2009). Flavonoid-rich blueberry extracts have also been shown to attenuate inflammatory responses of brain microglia in animal models via inhibition of nitric oxide, as well as the pro-inflammatory cytokines interleukin-1 β and tumour necrosis factor-alpha (TNFα) (Lau *et al.*, 2007).

***Hippocampal plasticity and signal transduction***

As discussed in section 1.6 of this thesis, the modulation of cognitive performance of research animals after the introduction of flavonoid-rich diets, centres on improvements in memory and learning. This process of learning and memory involves changes in hippocampal synaptic transmission, most pertinently, long term potentiation (LTP) as the mechanism by which memories are stored (Bliss & Collingridge, 1993). This storage of memory primarily depends upon the protein synthesis driven formation of, and changes in, neuronal connections (Rendeiro et al., 2012), such protein synthesis as cAMP response element binding (CREB), which is a key factor in the molecular switch that transforms short-term memory into long-term memory (Brightwell et al., 2007). A recent study (Rendeiro *et al.*, 2013) indicates that flavonoid-rich diets can both promote CREB activation and increase levels of brain-derived neurotrophic factor (BDNF), a neurotrophin responsible for neuronal survival in the hippocampus. This increase in levels of neurotrophins was coupled with improvements in cognitive performance in aged rats. These interactions between flavonoids and the above discussed signalling cascades and protein transcription could, therefore, play a neuro-protective role and lay the basis for an attenuation of cognitive decline found in epidemiological studies and rat models discussed later in this chapter.

### 1.4.2 Haemodynamic modulation

During natural ageing, cerebral blood flow reduces by up to 0.5% per year (Leenders *et al.*, 1990). Preservation of blood flow, including the maintenance of endothelial function and large artery elasticity are inversely associated with the incidence of cardiovascular disease (CVD), dementia and stroke. Furthermore, epidemiological observations demonstrate that diets high in phytochemicals, such as anthocyanins, can significantly reduce incidence of CVD risk factors, dementia and stroke (Curin *et al.*, 2006; He *et al.*, 2006).

*In vitro,* anthocyanins have been shown to induce endothelial dependent relaxation, facilitated by up-regulation of endothelial nitric oxide synthesis (eNOS) (Andriambeloson *et al.*, 1998), inhibition of inducible nitric oxide synthase (iNOS) (Pergola *et al.*, 2006), reduction of adhesion of inflammatory monocytes to endothelial cells (Nizamutdinova *et al.*, 2009) and reduction of oxidative stress in endothelial cells via direct penetration of cell membrane and cytosol (Youdim *et al.*, 2000). In humans, there is a growing body of intervention studies exhibiting the ability of fruit phytochemicals to modulate haemodynamics in the brain (Francis *et al.*, 2006; Kennedy *et al.*, 2010; Wightman *et al.*, 2012) and in the periphery (Grassi *et al.*, 2005b; Matsumoto *et al.*, 2005b; Heiss *et al.*, 2007). The regulation of neuronal blood flow is innately complex with multiple overlapping regulatory systems and key structural components existing, such as auto and neurogenic regulation, flow metabolism coupling and endothelial dependent regulation (see Peterson *et al.*, 2011 for a recent review). Recent human intervention studies focusing upon cerebral blood flow and cognitive activities, relate purported increases or decreases in blood flow to intervention-dependent modulations of ratios in nitric oxide (Kennedy *et al.*, 2010; Wightman *et al.*, 2012). Nitric oxide is a critical regulator of vascular homeostasis. Berry constituents have been shown to up-regulate eNOS *in vitro* (Andriambeloson *et al.*, 1998) and inhibit *in vitro* iNOS (Chen *et al.*, 2001). However, there is no data

published on their effects upon neuronal nitric oxide synthase (nNOS), manipulation of which is essential if changes in neuronal blood flow post-intervention are to been seen (Santizo *et al.*, 2000; Kitaura *et al.*, 2007). Neurotransmitters, and therefore their modulation, play a major role in the regulation of cerebral blood flow. For example, the increase in extracellular levels of adenosine due to neuronal activity are implicated in vasodilation (Blackwell *et al.*, 1967), and noradrenalin, a key neurotransmitter in terms of haemodynamics, causes heart rate to increase and vasoconstriction to occur when released into the bloodstream (Mckim, 2003). Neurotransmitters will be discussed further in the following section.

### 1.4.3 Neurotransmitter modulation

Neurotransmitters are chemicals within the central nervous system and the periphery, which transmit signals from a neuron to a specific cell across a synapse (Mckim, 2003). Since the first neurotransmitter was discovered in 1921, there has been a great deal of research conducted on their structure and function. Over 60 neurotransmitters have been discovered, some directly influencing human behaviour and others protecting the integrity of cell structure (Mckim, 2003). Monoamines are a sub class of neurotransmitters derived from dietary and endogenous amino acids, such as tryptophan, tyramine and phenylalanine (Mckim, 2003). The sub-category of monoamines encompasses a multitude of neuro-modulating chemicals such as the catecholamines: dopamine, noradrenaline and adrenaline; and the tryptamines: serotonin and melatonin (Mckim, 2003). Any abnormalities in monoaminergic tone can cause severe implications to health and well-being, including clinical implications in pathological disorders such as depression (Murphy *et al.*, 2013) and Parkinson's disease (Chaudhuri & Schapira, 2009). The modulation of these neurotransmitters is inherently complex; however, their modulation via pharmaceuticals can be used to alleviate symptoms of clinical conditions such as depression and anxiety (Kopin, 1985), but also pathological diseases such as Parkinson`s (Riederer & Youdim, 1986) and

Alzheimer's disease (Donnelly & Murphy, 1977). More pertinent to this thesis, there is the potential to attenuate age-related cognitive decline by modulation of neurotransmitters via the inhibition of the enzyme monoamine oxidase (MAO) (Alper *et al.*, 1999). MAO are insoluble mitochondrial enzymes (Pombo *et al.*, 2008) that can be found in both peripheral and central tissue. Their main role is to deaminate dietary amines and terminate actions of monoaminergic neurotransmitters, including dopamine, serotonin, adrenaline and noradrenaline. This deamination of neurotransmitters creates amongst other things hydrogen peroxide and ammonia, both of which are highly toxic to cells. Two isoforms of MAO exist: MAO-A and MAO-B, these isoforms differ in substrate preference, tissue distribution and inhibitor specificity. MAO-A preferably catalyses the oxidation of serotonin, and MAO-B is more active towards 2-phenylethilamine and benzylamine. Dopamine, adrenaline, noradrenaline, tryptamine and tyramine are oxidised by both isoforms (Youdim *et al.*, 1988). The ratio of distribution of MAO isoforms in brain tissue is not equal, with approximately three quarters of the enzyme being MAO-B and one quarter MAO-A (Saura Marti *et al.*, 1990). An understanding of the localization of MAO-A and MAO-B is important since tissue and cellular compartmentalization of these enzymes determines, to some degree, the substrates to which they are exposed; and therefore, their action *in vivo*. Inhibition of MAO allows endogenous monoamines and monoamines from the diet to accumulate both centrally and in the periphery and may, therefore, alter the dynamics of regular monoamine transmitters, such as noradrenaline, serotonin, or dopamine (Yamada & Yasuhara, 2004). Anthocyanins and, to a lesser degree, phenolic acids have been shown to be inhibitors of both MAO isoforms *in vitro* at $IC_{50}$ concentrations of 36.9±5.8µM for MAO-A and 36.8±5.2µM for MAO-B (Dreiseitel *et al.*, 2009a). However, these levels are 1000 fold higher than quantities normally found in human plasma following the consumption of an anthocyanin-rich meal (Mazza *et al.*, 2002). For example, concentrations of 34.3µMof cyanidin-3-glucoside are need to competitively inhibit half of the enzyme activity ($IC_{50}$) of MAO-A*in vitro*, whereas

cyanidin-3-glucoside is reported in blood plasma at around 5nMol after oral consumption (Matsumoto *et al.*, 2001). The mean $IC_{50}$ value of phenolic acids was 11.5±7mM for MAO-A with no inhibition discovered for MAO-B. Plasma levels of phenolic acids after blackcurrant consumption have been reported by Jin *et al., (*2011), however from a highly processed drink. Plasma levels of phenolic acids were shown to increase by 40% after consumption of the blackcurrant extract. No units other than percentage were provided.

In regards to blackcurrant extracts *in vivo,* Borman and Schatton., (1996) filed a European patent detailing the inhibitory effect of a single serve undefined blackcurrant drink on human MAO-B activity. They discovered that supplementation of 50g of blackcurrant extract can inhibit peripheral MAO-B activity by up to 92%, one hour post-supplementation. They also recorded inhibition of MAO-A *in vitro* by the same blackcurrant extract. However, it must be noted that this finding was only substantiated in an experiment with one participant.

The manipulation of neurotransmitters via inhibition of MAO isoforms by anthocyanins found in blackcurrants could, in theory, positively affect cognitive ability of not only clinical populations, but also healthy adults. A wide range of pharmaceutical MAO inhibitors are currently available which encompass reversible and irreversible inhibition of MAO-A and MAO-B, or both isoforms. For many decades, these drugs have been providing pharmacological therapy for depressive disorders and neurological diseases. However, Youdim *et al*., (2006) discuss in a recent review that there are still many questions to be answered regarding the actions of MAO and what regulates its activity in the brain. Differing active sites between human and rat brains exist, raising questions about the use of animal models for human MAO trials, a factor which is possibly confounded by arbitrary dosage selection and defects in trial designs.

## 1.5 Physiological effects

The literature outlines several physiological parameters that could be impacted by consumption of flavonoid-rich foods. These include modulations of peripheral and central haemodynamics and peripheral glucose regulation; physiological parameters, which may have the potential to acutely and chronically impact health and behaviour.

### 1.5.1. Glucose regulation

Glucose is the primary source of energy in the brain. After a glucose load, brain extracellular glucose levels increase in a similar pattern to that of blood glucose (Harada *et al.*, 1993).There has, therefore, been considerable interest over the past 30 years regarding glucose and cognition, especially surrounding a possible enhancement in cognitive function after a glucose load. On the most basic level, some of this research has shown glucose to be a modulator of cognitive performance when high demand is placed on the brain (Donohoe & Benton, 2000; Kennedy & Scholey, 2000; Scholey et al.2001; Riby *et al.*, 2004; Scholey & Kennedy, 2004). Glucose regulation is the mechanism whereby blood glucose levels rise sharply immediately after glucose intake, then return to baseline as a consequence of increased insulin secretion, usually within 30 minutes of consumption. In healthy young humans, poor glucose regulation, as defined by glucose levels above normal values after a glucose load, has been shown to be associated with impairments in cognition (Craft *et al.*, 1994; Messier, 1998; Awad *et al.*, 2002). For example, Messier *et al.,* (1999) highlighted that young healthy adult participant with poor glucose regulation show improvements in word recall, paragraph recall or word order recall after a 50g glucose load. However, participants with good glucose regulation showed no cognitive improvement. Donohoe & Benton (2000) report that the quicker blood glucose returned to baseline the better was the performance on paragraph recall. There was, however, no correlation between any other task (vigilance and reaction time) and glucose regulation. Craft *et al.,* (1994) compared the effects of 50g of glucose or 2mg saccharin upon cognitive performance

In young adults (19-28) or older adults (58-77). Measures of declarative memory, working memory, procedural learning and response inhibition were taken 15 minutes after consumption of the study drink. The glucose load was found to enhance memory in young poorer glucoregulators. However, younger males whose blood glucose levels were lowest 60 minutes post glucose ingestion showed reductions in memory outcomes. This glucoregluatory effect upon cognitive performance is more apparent in healthy older adults, especially in aspects of verbal memory executive functioning (Kaplan *et al.*, 2000; Convit *et al.*, 2003; Greenwood *et al.*, 2003; Messier *et al.*, 2003) and in older adults with type two diabetes (Kanaya *et al.*, 2004). Further to this, improving glycaemic control for six months via an oral hypoglycaemic agent in elderly diabetes patients, can improve aspects of dexterity (grooved pegboard), visual attention and task switching (stroop-word naming and trail making-part a) and retrieval memory (cued recall) (Meneilly *et al.*, 1993). It has been suggested that these glucose effects are more readily available in younger adults with poor glucose regulation because of the increased amount of time glucose is raised in the blood stream, therefore, available for utilisation (Craft *et al.*, 1994).

Nakazawa first showed a reduction in glucose transportation as a consequence of exposure to phenolic compounds in 1922 (Nakazawa, 1922). Since then, there has been an increase in evidence to show that flavonoid-rich foods can modulate the profile of post-prandial blood glucose levels. Johnston *et al.,* (2002) supplemented nine healthy adult participants with 25g of glucose naturally occurring in commercial clear apple juice or commercially available cloudy apple juice, and compared their effects to 25g of glucose consumed in water. The authors reported that intestinal glucose uptake, as measured by intravenous cannula, was significantly reduced 15 and 30 minutes, and increased 45 to 120 minutes, after consumption of both apple juice treatments compared to the control. They also found a reduced level of plasma insulin and glucose-dependent insulinotropic polypeptide (GIP) 90 minutes after supplementation

of both apple juice treatments when compared to control, together with higher glucagon-like peptide-1 (GLP-1) plasma concentrations after 90 minutes; but only after supplementation of the cloudy apple treatment. This increase in GLP-1 is likely to be brought about by reduced gastric emptying, facilitated by the inhibition of sodium glucose transporters by phenolic compounds (Manzano & Williamson, 2010). Further to these findings, Bassoli *et al.,* (2008) found that oral supplementation of chlorogenic acid (CA), when combined with a glucose tolerance test, reduces blood glycaemic peak in male albino Wistar rats. Interestingly CA supplemented intravenously did not have any impact upon post- prandial blood glucose levels after a glucose tolerance test in male albino Wistar rats. This finding would suggest that there is an effect of CA in the gut, reducing the uptake of glucose.

A similar blunted post-prandial glucose peak has also been seen after consumption of berries. In a study investigating the effect of cranberry juice, participants received one of five intervention treatments (all 480mls): low calorie cranberry drink (38kcal), normal calorie cranberry drink (280kcal), an isocalorific low calorie corn syrup drink, an isocalorific normal calorie corn syrup drink, or a water control. Consumption of the normal calorie cranberry juice resulted in significantly higher blood glucose concentrations 30 minutes post-prandially. These differences were no longer significant 180 minutes post-supplementation. Plasma insulin was also significantly higher 60 minutes post-prandially after consumption of the normal calorie cranberry juice, but not significantly different 120 minutes post-prandially when compared to control. Interestingly, no effects were found after the consumption of any of the other beverages, indicating a glucose load must be consumed in conjunction with the cranberry phytochemicals for this glucose effect to be observable (Wilson *et al.*, 2008b). The same authors found the same pattern of results in a cohort with type two diabetes (Wilson *et al.*, 2008a). Törrönen *et al*., (2012) supplemented 12 healthy subjects aged 25-69 years with 150g mixed berry purée consisting of equal amounts

(37·5g) of blackcurrants, bilberries and cranberries with 35g sucrose or a control 35g sucrose load in a randomised, controlled cross-over design. Compared to control, the consumption of the mixed berry meals significantly lowered post-prandial blood glucose at 15 and 30 minutes post consumption. Blood glucose levels were then significantly higher at 150 minutes post consumption of the mixed berry meal compared with the control meal. Peak glucose concentrations were reached at 30 minutes after the control meal and 15 minutes later at 45 minutes after consumption of the berry meal. The peak increase from baseline was also 1mmol/l smaller after ingestion of the berry meal. This highlights a slower uptake of glucose into the blood after the consumption of berry purée, suggesting that, the glycaemic response to sucrose consumption can be modulation via co-consumption of a berry purée. As a follow on to this, in the same paper the author supplemented 12 healthy participants with the same berry purée meal (150 g made of bilberries, blackcurrants, cranberries and strawberries) as well as 35g sucrose or a sucrose matched control. This time, fingertip capillary and venous blood samples were taken at baseline and at 15, 30, 45, 60, 90 and 120 min after starting to eat the meal. Compared to the control meal, ingestion of the berry meal resulted in lower capillary and venous plasma glucose and serum insulin concentrations at 15 minutes and higher concentrations at 90 minutes. Post-prandial capillary and venous glucose and insulin concentrations were also reduced, improving the glycaemic response and increasing GLP-1. This suggests that glycaemic control after ingestion of sucrose can be modulated by simultaneous consumption of berries.

The findings above demonstrate a reduction in post-prandial blood glucose levels after consumption of phenolic compounds and phenolic-rich foods for the initial 30 to 45 minutes, with an increased level thereafter. In light of findings by Bassoli *et al.,* (2008) it seems that this modulation of blood glucose profile is due to interactions between phenolic compounds and glucose transport systems in the gut. Indeed, the mechanisms driving this modulation of glucose transport are known, and are believed

to be centralised around direct interactions with the gut endothelial glucose transporters, sodium dependent glucose transporter 1 and glucose transporter 2 in the small intestine (Manzano & Williamson, 2010). Although it is unlikely that modulating the pattern of glucose absorption in young healthy adults will impact memory and central executive functioning as seen in older cohorts, as glucose is the primary fuel of the brain, it is hypothesised that by controlling glucose uptake by phenolic compounds, allowing glucose to be available systemically for longer, a direct modulation of cognitive performance may be attainable during bouts of high cognitive demand.

### 1.5.2 Haemodynamics

#### 1.5.2.1 Peripheral haemodynamics

As discussed in section 1.4.2, there are biological mechanisms controlling blood flow, which can be manipulated after exposure to berry phytochemicals. Epidemiological observations demonstrate that incidence of CVD risk factors, dementia and stroke, can be significantly reduced by long-term diets rich in berry polyphenols (Curin *et al.*, 2006; He *et al.*, 2006). Shorter term intervention studies have reported similar findings after the consumption of flavonoid-rich foods, the findings of which are discussed below.

***Cocoa and peripheral blood flow***

Amongst fruits, cocoa has been the most prolifically investigated for its ability to modulate peripheral blood flow. This body of research highlights a significant impact of flavonoid-rich foods upon human blood flow. Whether investigated acutely two hours post-dose (Heiss *et al.*, 2005; Vlachopoulos *et al.*, 2006; Davison *et al.*, 2008; Faridi *et al.*, 2008b; Berry *et al.*, 2010; Njike *et al.*, 2011) or over periods of eight days to 12 weeks (Engler *et al.*, 2004; Heiss *et al.*, 2007; Davison *et al.*, 2008; Grassi *et al.*, 2008), studies utilising flow-mediated dilation have reported increases in peripheral blood flow following cocoa consumption. There are also demonstrations of an acute increase in peripheral blood flow after consumption of cocoa products, which are superimposed

upon effects of chronic cocoa intake. These benefits are apparent after 30 days of intake, potentially suggesting that the long-term benefit of cocoa consumption may peak beyond 30 days (Heiss *et al.*, 2007; Balzer *et al.*, 2008).

***Berries peripheral blood flow***

Over the last decade, berries have received attention in terms of their modulating effects upon blood flow. For example, grapes and grape extracts have been shown to modulate peripheral blood flow in both clinical and non-clinical populations. In a study of coronary artery disease patients, 15 adults were supplemented with 4ml/kg of body weight of purple grape juice twice daily for 14 days. Flow-mediated vasodilation was measured using high-resolution brachial artery ultrasonography at baseline, and after the 14 day purple grape juice intervention. Compared to baseline, the authors reported an increase in flow-mediated dilation of 6.4%, indicating an improvement in endothelium dependent vasodilation (Stein *et al.*, 1999). Further to this, Coimbra *et al.,* (2005) reported similar results in hypercholesterolemic patients. Sixteen participants received either 250ml of red wine or 500ml of red grape juice per day for 14 days (no phenolic constituent data reported) in a parallel study design (N=8 in each arm), with 24 healthy participants used as baseline controls for vascular reactivity. At baseline, brachial artery flow-mediated dilation was significantly decreased in the patients compared to healthy controls (9.0% in the clinical population vs. 12.1% in the healthy control). After the 14 day intervention, hypercholesterolemic patients showed an increase in brachial artery flow-mediated dilation of 7% after grape juice intervention and 5% after the red wine intervention. The red wine but not the red grape intervention also significantly increased endothelium dependent vasodilation by 5% as measured by nitro-glycerine-mediated dilation. This effect is not only observable in participants with endothelial dysfunction. In an acute study, Agewall *et al.,* (2000) observed an improvement in brachial artery flow-mediated dilation one hour after supplementing healthy volunteers with 250ml red wine. Twelve healthy adults were randomly assigned

to either the red wine group (Merlot red wine containing 12% alcohol) or a dealcoholized red wine (containing less than 0.5% alcohol). Brachial artery diameter and flow mediation were measured at baseline and one hour post-drink consumption. Compared to baseline, brachial artery diameter and blood flow were both increased after consumption of both intervention drinks. However, flow-mediated dilatation was significantly higher after drinking dealcoholized red wine (5·6%) than after drinking red wine (3·9%).

With reference to blackcurrant extracts and peripheral haemodynamics, significant increases in the skin blood circulation have been observed one hour after supplementing eight healthy females with 140mg of blackcurrant polyphenols, containing 50mg of blackcurrant anthocyanins (Matsumoto *et al.*, 2005a). In a later study, the same authors also reported that supplementing 17mg/kg of body weight of pure blackcurrant anthocyanins increased peripheral blood flow, as measured by near infrared spectroscopy, and reduced shoulder stiffness during typing (Matsumoto *et al.*, 2005b). In contrast, no effect was found using flow-mediated dilation on peripheral blood flow after supplementation of a 20% blackcurrant drink when measured supine, one hour and several further time points post-dose (Jin *et al.*, 2011). Although there are methodological differences in measurement techniques in the above mentioned studies, there are also differences between extract types and, therefore, the phenolic profiles of the intervention drinks, most noticeably the amount and type of anthocyanins. Jin *et al.,* (2011) supplemented participants with a cordial-like extract containing 31mg of polyphenols in total (30.4mg of anthocyanins and 616µM phenolic acids) whereas Matsumoto used doses of 33mg of anthocyanins per kg. It is, therefore, likely that the lack of effects presented by Jin *et al.,* (2011) were due to low polyphenol content.

***The impact of cocoa and berries upon blood pressure and heart rate***

As with peripheral blood flow, cocoa consumption has been shown to acutely modulate blood pressure in healthy populations (Grassi *et al.*, 2005a; Grassi *et al.*, 2005b). For example, a recent meta-analysis of 42 acute or short-term chronic studies (under 18 weeks) concluded that consistent acute and chronic benefits of chocolate or cocoa consumption on flow-mediated dilation and blood pressure can be seen. However, the literature reports no acute effects of other fruit extract intake upon blood pressure and heart rate in either healthy or clinical populations (Hooper *et al.*, 2012). Jin *et al.,* (2011) found no effect upon blood pressure or heart rate after acute supplementation of a 20% blackcurrant drink, nor did Matsumoto *et al.,* (2005b) after supplementing nine healthy young adults with 17mg/kg of body weight of blackcurrant polyphenols in the form of a juice drink. Wilson *et al.,* (2008b) also failed to find effects after acutely supplementing 187 healthy young participants with either a control drink or a cranberry juice drink containing 480ml of cranberry juice. In a parallel design, heart rate and blood pressure measurements were taken at baseline and 30, 60,120 and 180 minutes post-supplementation.

In contrast, longer studies have revealed positive effects in clinical populations. For example, un-medicated middle-aged subjects with cardiovascular risk showed a decrease in systolic and diastolic blood pressure after eight weeks supplementation with 150mg of anthocyanins per day for eight weeks (from a bilberry, lingenberry, blackcurrant, strawberry, chokeberry and raspberry purée), when compared to a control group. As might be expected, it was the participants with the highest baseline blood pressure that showed the greatest response to the intervention (Erlund *et al.*, 2008). Further to this, intervention studies supplementing 50ml of pomegranate juice, a fruit containing high levels of anthocyanins and tannins, per day for 14 days to hypertensive participants induced a 5% reduction in systolic blood pressure when compared to placebo (Aviram & Dornfeld, 2001) and a 21% reduction in systolic blood

pressure after supplementing patients with asymptomatic severe carotid artery stenosis for one year. Three years of supplementation did not yield any further changes (Aviram *et al.*, 2004). However, supplementation of 240ml of pomegranate juice for 90 days did not affect systolic blood pressure in ischaemic coronary disease and myocardial ischaemic patients (Sumner *et al.*, 2005). The reasons for the discrepancies between findings is not clear; however, the juices were differentially sourced and Sumner *et al.,* (Sumner *et al.*, 2005) did not report the phytochemical breakdown of the juice, so the interventions are not completely comparable, masking any discrepancies in phytochemical makeup. Naruszewicz *et al.,* (2007) also found reductions in systolic and diastolic blood pressure after supplementing 44 myocardial infarction sufferers with 255mg of chokeberry extract per day for 42 days. The literature would suggest that effects of a fruit intervention, other than cocoa, upon blood pressure and heart rate are unlikely to be seen in healthy participants. This is a cohort where blood pressure and heart rate are tightly regulated by the autonomic nervous system

The literature above shows that peripheral blood flow can be acutely modulated by the consumption of flavonoid-rich cocoa and berries. When FMD is utilised as a measure of endothelial function, the consumption of cocoa has been shown to increase peripheral blood flow and modulate endothelium dependent vasodilatation in healthy populations (Heiss *et al.,* 2005; Vlachopoulos *et al.,* 2006; Davison *et al.,* 2008;). In relation to berry supplementation, studies show a chronic increase in peripheral blood flow after 14 days supplementation of grape extracts in clinical populations (Stein *et al.,* 1999; Coimbra *et al.,* 2005) and one hour post-dose in a healthy cohort (Agewall *et al.,* 2000). There is also evidence to suggest an acute increase in peripheral blood flow following the consumption of a blackcurrant extract when measured using NIRS. However, no effects were observed when peripheral blood flow is assessed via FMD. This null finding is more than likely due to the low dose of polyphenols in the extract used by Jin *et al.,* (2011). Although acute improvements in peripheral endothelial

function are not associated with an acute modulation of cognitive performance, the preservation of large artery elasticity and the preservation of endothelial function are inversely associated with cardiovascular disease (Targonski *et al.,* 2003; Dede *et al.,* 2007). Modulations in blood flow via flavonoid-rich foods could, therefore, potentially impact long term cognitive performance by maintaining healthy blood flow throughout life, attenuating symptoms associated with vascular dementia (Dede *et al.,* 2007).

#### 1.5.2.2 Cerebral haemodynamics

**Cocoa**

An initial pilot study by Francis *et al*., (2006), utilising fMRI, explored the acute effects of flavan-3-ols on cerebral blood flow following either a flavan-3-ol rich cocoa drink (516mg) or matched control (39mg) in healthy young adults. Significant increases in grey matter cerebral blood flow were observed two hours post-consumption using arterial spin labelling, with a return to baseline by six hours post-consumption. Francis *et al.,* (2006) then examined effects of five days' consumption of flavan-3-ol-rich cocoa (172mg) or a matched control (13mg) in healthy young females. fMRI scanning took place during completion of a letter-pair switching task at approximately 90 minutes post-consumption on the fifth day of consumption in each condition. BOLD signal intensity was shown to be significantly increased by flavan-3-ol rich cocoa as compared to control. No significant effects were evident in behavioural paradigms. It must be noted that methylxanthines such as caffeine were not matched between the control and active treatments. As caffeine is known to increase BOLD signal intensity (Chen & Parrish, 2009), this, therefore, cannot be ruled out as the compound driving the reported CNS effects. In a later study, Sorond *et al.,* (2008) assessed the impact, in elderly adults, of short-term flavan-3-ol-rich cocoa consumption on cerebral blood flow velocity and vaso-reactivity as assessed by transcranial doppler. Beat-to-beat arterial pressure and cerebrovascular conductance were also assessed. Seven days' consumption of either flavan-3-ol-rich cocoa (900mg) or flavan-3-ol-poor control

(36mg), matched for calories, macronutrients, micronutrients and the methylxanthines caffeine and theobromine, produced no significant effects on the outcomes measured. When compared to data described by Francis *et al.,* (2006) these results highlight the need for a methylxanthine-matched control for definitive cerebral haemodynamic effects of flavan-3-ols in cocoa products to be quantified.

**Non-flavonoid polyphenol interventions**

Wightman *et al.,* (2012) assessed the impact of the tea polyphenol epigallocatechin gallate (EGCG) on cerebral haemodynamics in healthy young adults. In the crossover intervention study utilising near infrared spectroscopy (NIRS), participants received either 135mg of EGCG, 270mg of EGCG or placebo. After a 45 minute resting absorption period, participants completed a 43 minute cognitive assessment designed to stimulate the pre-frontal cortex. The 135mg intervention caused a reduction in total haemoglobin, a proxy for cerebral blood flow, during cognitive tasks with no differences in hemispheric activity or effect of task cognitive difficulty. No effects were reported after the consumption of the 270mg intervention. The authors attribute this to vasoconstriction properties potentially attributed to inhibition of nNOS. Further to this Kennedy *et al.,* (2010) supplemented 22 healthy adults with 250mg or 500mg of trans-resveratrol, a red grape polyphenol which has been shown to up-regulate levels of both eNOS and nNOS (Tsai *et al.*, 2007). NIRS measurements were recorded during a 45 minute absorption period and during completion of a selection of cognitive tasks that activate the frontal cortex. This within subject's trial showed that supplementation of trans-resveratrol increased pre-frontal cerebral blood flow during cognitive demand in a dose-dependent manner, as compared to placebo. Results also indicated a similar increase in deoxyhaemoglobin towards the end of the 45 minute absorption period and throughout cognitive demand. This decrease in deoxyhaemoglobin during task performance following the resveratrol treatment is not what would be expected during cognitive demand. Neurovascular coupling is a phenomenon which is utilised in

neuroimaging techniques such as BOLD fMRI to assess neuronal activity. It refers to the relationship between active neuronal cells and related changes in cerebral blood flow. Whereby, both oxyhaemoglobin and deoxyhaemoglobing increase in response to cognitive demand. Therefore, it would have been expected that as oxyhaemoglobin increased during cognitive demand after the consumption of resveratrol, deoxyhaemoglobin would also increase due to neurovascular coupling. The authors explained that reductions in deoxyhaemoglobin could be indicative enhanced oxygen extraction (Kennedy *et al.,* 2010).

**Berries**

There are no studies which assess the impact of blackcurrants upon central haemodynamics and only one intervention study has assessed the impact of berries upon cerebral blood flow. Krikorian *et al.,* (2012) supplemented 16 adults aged 68 years and older with mild age-related memory decline with either a grape juice determined by body weight (444ml average) containing, on average, 209mg of polyphenols (96.2mg anthocyanins, 60.61mg phenolic acids, 20.9mg procyanidins), or a sugar matched placebo drink for 16 weeks (N=8 in each arm). Neurological activation was assessed with the use of functional magnetic resolution imaging (fMRI) whilst completing the n-back working memory task. Increased activation on fMRI in the right anterior and posterior cortical regions during cognitive performance was observed. No impact of the intervention was seen on n-back task performance. Increased regional fMRI activation represents greater haemodynamic response, which is associated with increased neuronal activity.

### 1.5.2.3 Summary of haemodynamic effects of polyphenol rich foods

The foregoing literature outlines that polyphenol-rich foods and their isolated components have the ability to modulate blood flow in the brain after acute interventions. These modulations are more pronounced during cognitive demand with

modulations engendering both reduced and increased blood flow. In addition, enhanced oxygen utilisation and potential improvements in neuronal efficiency have been reported, however, these modulations do not manifest into improved cognitive performance. Cerebral blood flow has been reported to reduce by up to 5% per decade during natural ageing (Leenders *et al.*, 1990). Therefore, the chronic modulation of blood flow through nutritional interventions, could potentially aid the preservation of blood flow throughout life, attenuating reductions in blood flow associated with the natural ageing process. On an acute level, increases in cerebral blood flow via a nutritional intervention could be beneficial to acute cognitive performance; increasing the transport of blood borne metabolic substances to the brain and the transportation of waste products from the brain, both of which are necessary for natural brain function (Attwell *et al.*, 2010).

## 1.6 Behavioural effects of flavonoid-rich foods

There is a growing body of literature highlighting cognitive paradigms which are sensitive to berry fruit interventions in animal models. However, evidence regarding their cognitive modulating properties in humans, especially after acute supplementation, is lacking. The following section describes a brief synopsis of the impact of berries upon cognitive abilities in animal models and then reviews the *in vivo* impact of flavonoid-rich food supplementation upon human behaviour. This section provides epidemiological evidence of the impact of flavonoid consumption upon cognitive performance in humans and then goes on to address peer reviewed evidence from well conducted nutritional interventions of the impact of flavonoid-rich food upon cognitive performance.

### 1.6.1 *In vivo* animal models

In a relatively short-term study, Ramirez *et al*., (2005) supplemented 20 male Wistar rats (12 months old) with a dried blueberry extract-supplemented diet containing 3.2mg

of anthocyanins per kilogram of body weight per day, or a non-supplemented diet for 30 days (N=10 in each group). Increases in short-term, but not long-term, memory on inhibitory avoidance tasks; reduced anxiety as measured using the open field maze; and improvements in working memory as measured by the radial maze were found, with results most pronounced for short-term memory outcomes (Ramirez *et al.*, 2005). Further to this, a longer term study supplemented eight, young (six month old) male Lister-hooded rats with a control diet and sixteen aged (eighteen months old) male Lister-hooded rats with either a blueberry supplemented diet or a control diet (N=8 in each condition). Animals in the aged plus blueberry group had their diet supplemented with dried blueberry extract containing 10.5mg of flavonoids per day for 12 weeks. The remaining two groups were fed an iso-calorific diet for 12 weeks. Reversals in age related deficits in spatial working memory (as measured by the cross maze) were found after three weeks of supplementation of the blueberry extract when compared to the control aged group. This improvement continued until the last measurement at 12 weeks where the deficit was reduced to a level similar to the young control animal model. The authors attributed these changes in behaviour to correlations between *ex-vivo* anthocyanin levels in the rat brains and levels of ERK- cyclic AMP-response element binding protein (CREB) and brain-derived neurotrophic factor (Williams *et al.*, 2008), which when up regulated, have been shown to promote cognitive plasticity and positively impact memory (Tully *et al.*, 2003). Strikingly, potentially via ERK and alterations in neutral sphingomyelin-specific phospholipase C activity, Joseph *et al.,* (2003) showed that supplementing the diet of APP+PS1 transgenic mice (Alzheimer's model which show visible amyloid plaque deposition after 6 months of age) with a 2% blueberry diet, eradicated deficits in Y-maze performance at 12 months of age when compared to non-blueberry supplemented controls; suggesting that symptoms of Alzheimer's could be alleviated through diet.

Supplementation of blueberry extracts has also been shown to augment psychomotor performance. Forty, nineteen month old rats were randomly assigned to one of four diet groups; a control diet containing only the base diet, a base diet containing 1.48% strawberry, a base diet containing 0.91% spinach, or base diet containing 1.86% blueberry. Rats were fed the intervention diets for eight weeks. Results showed that the blueberry-supplemented group exhibited increases in psychomotor performance as measured by latency to fall on the rod walk and rotarod. Unlike other similar studies discussed below, no statistically significant effect was found after supplementation of any active treatment on the Morris water maze (Joseph *et al.*, 1999), indicating no impact of the intervention upon spatial memory.

Although blueberries have received the most attention, other flavonoid-rich berries such as blackberries have been shown to augment behaviour in animal models. In a chronic study, 19 month old male Fischer 344 rats were fed either a base diet or a base diet containing 2% blackberry. After eight weeks of supplementation, the 2% blackberry supplemented diet improved motor performance on the wire suspension, the small plank walk and the accelerating rotarod when compared to control diet fed rats; tasks which rely on balance and co-ordination. Further to this, rats in the 2% blackberry-supplemented diet group had significantly better short-term working memory performance, as measured by the Morris water maze, when compared to control fed rats (Shukitt-Hale *et al.*, 2009a). Concord grapes have also been shown to increase psychomotor performance in aged rats (Shukitt-Hale *et al.*, 2006). In addition to these berry studies, Shukitt-Hale *et al.,* (2009b) conducted two intervention studies assessing the effect of two plum extracts upon working memory of aged rats. In the first study, thirty, 19 month old Fischer 344 rats were assigned to a dried plum powder group or a control group. Rats in the dried plum group were supplemented 20g of dried plum extract per kg of body weight, containing approximately 3.30mg of gallic equivalents per day, the powder contained no anthocyanins. Rats were supplemented for eight

weeks before cognitive testing at 21 months of age. In the second study, twenty-eight, 19 month old rats were supplemented with either a reconstituted plum juice drink containing 30.3mg of gallic equivalents, including 0.043mg of anthocyanins per day, or water for eight weeks before cognitive testing at 21 months of age. Testing procedures were the same in both studies. After the eight weeks, increases in spatial working memory, in the Morris water maze were reported after supplementation of the juice extract but not the powder extract when compared to the control group. Although this initially looks like an effect of extraction technique, the total polyphenol content consumed per day was ten times higher in the juice group, instead suggesting an effect of dose upon behaviour. In the most recent study Rendeiro *et al.,* (2013) supplemented four groups of eight male 18 month old Wistar rats with one of four diets for six weeks. The diets were a control diet, a 2% blueberry diet, an anthocyanin diet and a flavanol-enriched diet. Results showed that rats in the supplemented diet groups had increased spatial working memory scores on the cross maze when compared to control. These improvements in cognitive performance were coupled with increased levels of BDNF mRNA expression in the hippocampus when compared to the control group. This elevation was the greatest in the pure anthocyanin fed group.

Research using animal models has, therefore, shown that supplementation of flavonoids, as well as dried fruit or fruit juices can positively impact several aspects of memory. These include spatial and short-term working memory (Ramirez *et al.*, 2005; Williams *et al.*, 2008; Rendeiro *et al.*, 2013), reversal learning and rapid memory acquisition (Wang *et al.*, 2006), long-term reference memory (Casadesus *et al.*, 2004), slow memory acquisition (Joseph *et al.*, 1999) and memory retrieval (Van Praag *et al.*, 2007). The overarching nature of this research is the augmentation of natural cognitive decline in aged animal models. Furthermore, the consumption of polyphenol-rich extracts has been shown to protect rodent cognitive function from a range of neuronal

insults and natural ageing (Bastianetto *et al.*, 2007), as well as to reduce the onset of symptoms of Alzheimer`s disease (Joseph *et al.*, 2003).

### 1.6.2 *In vivo* human studies

#### 1.6.2.1 Epidemiological observations

Epidemiological studies have shown that diets high in polyphenols are linked with an attenuation of cognitive decline associated with natural ageing in humans, when compared to diets lower in polyphenols (Letenneur *et al.*, 2007; Nurk *et al.*, 2009; Devore *et al.*, 2012; Kesse-Guyot *et al.*, 2012). For example, diets high in flavonoid-rich wine (up to 100ml/day), tea (up to 200ml/day) and chocolate (up to 10g/day) were associated with a dose-dependent decrease in cognitive decline in participants aged over 70 years; particularly episodic memory, recall memory, visual attention, visuo-spatial and motor skills, verbal memory and overall memory (Mini Mental State Exam - MMSE) (Nurk *et al.*, 2009). Greater long-term consumption of berries, anthocyanidins, and total dietary flavonoids are also reportedly related to significantly slower rates of cognitive decline, in particular verbal memory and memory span in women aged over 70 years (Devore *et al.*, 2012). Kesse *et al*., (2012) displayed similar findings reporting that an increased intake of catechins, theaflavins, flavonols and phenolic acids were associated with better verbal memory in a 13 year assessment of middle aged adults. Although numerous other factors such as recall bias and other lifestyle factors could be responsible for the preservation of cognitive function shown in epidemiological studies, shorter chronic laboratory controlled studies, which are detailed overleaf, have also highlighted similar findings. Although there are no published peer-reviewed studies investigating the direct effect of blackcurrant supplementation upon human behaviour, information can be drawn from nutritional interventions investigating foods with high polyphenol content.

#### 1.6.2.2 Cocoa

Several peer reviewed intervention studies have assessed the efficacy of cocoa`s purported cognitive enhancing properties, with differing and, at times, conflicting results being discussed. In a randomised, controlled, double-blinded, balanced, crossover trial, 30 healthy young adults consumed drinks containing 520mg, 994mg cocoa flavanols or a matched control. Increases in working memory and/or psychomotor performance (as assessed by the serial threes subtractions task) were reported after both active drinks when compared to placebo and improvements in sustained attention (seen as decreased reaction times during the rapid information processing task) were found after the 994mg drink only, when compared to control. The 994mg drink, however, resulted in more errors during the serial sevens subtractions task. Increases in self-reported mental fatigue were also significantly attenuated but only by consumption of the 520mg drink (Scholey *et al.*, 2010). Interestingly, findings are more pronounced after consumption of the lower flavanol dose, potentially outlining a ceiling effect, or an "inverted u" shaped effect curve if the dosage is too high. Francis *et al.,* (2006) examined the effects of five days consumption of flavan-3-ol-rich cocoa (172mg) or a matched control (13mg) in healthy young females. Despite significant increases in fMRI BOLD signal intensity when compared to control, completion of a letter-pair switching task at approximately 90 minutes post-consumption on the fifth day of consumption in each condition elicited no significant differences in attention switching performance between conditions. More recently, Field *et al.,* (2011) demonstrated improvements to visual contrast sensitivity (as assessed by reading numbers that became progressively more similar in luminance to their background) and the time to detect motion direction, 90 minutes following acute consumption of dark chocolate (720mg cocoa flavan-3-ols, 38mg caffeine) as compared to white chocolate. This study also demonstrated improvements to a visual spatial memory task following dark chocolate. However, given that a crossover design was utilised, the use of a placebo which was not sensorally matched could have inadvertently un-blinded participants, therefore, potentially

impacting the data of this study. In a longer term study, Crews *et al.,* (2008) supplemented 101 healthy adult participants with either 754mg of cocoa proanthocyanins (one dark chocolate bar and one cocoa drink) per day or similar control products matched for appearance taste and energy, but not caffeine content, for six weeks in a parallel fixed dose design. No significant effects were found after six weeks consumption of the cocoa based products in any cognitive paradigms (after an acute load).

Camfield *et al.,* (2012) conducted the only study to measure the cognitive effects of chronic cocoa supplementation in the absence of an acute load, allowing accumulative effects to be measured. Forty to sixty-five year old adults were supplemented with cocoa at low (~0mg), medium (~250mg) and high (~500mg) flavan-3-ol levels for 30 days. At baseline and after 30 days supplementation, participants completed a spatial working memory task whilst Steady State Visually Evoked Potentials (SSVEPs) were recorded with the use of Steady State probe Topography (SST – (Silberstein *et al.*, 1990), a form of electrophysiological brain imaging. After consumption of the medium flavan-3-ol dose, the pattern of posterior-parietal SSVEP amplitude was significantly lower when compared to control. Whereas, latency was decreased in the same region following both the medium and high flavan-3-ol doses. This decrease in latency suggests an increase in neural processing speed. No effects of the intervention upon working memory were seen. The authors suggest that this reduction in latency, but lack of effects upon cognitive performance are indicative of increased neuronal efficiency, allowing the participants to perform at a similar level to baseline, with reduced neuronal activation.

One suggestion for the lack of definitive behavioural effects in the cocoa literature is that the healthy young participants were performing close to ceiling and benefits were, therefore, unlikely to be observed. Further to this, neuropsychological tasks used in the

literature may not be sensitive enough to detect small changes in cognition as a consequence of a nutritional intervention over the time-frame employed. This point is supported by findings from one of only three studies to date to detect an effect of cocoa flavan-3-ols on cognition. Scholey *et al.,* (2010) demonstrated significant improvements to performance and fatigue during an intense 60-minute Cognitive Demand Battery in healthy young adults at 90 minutes post-administration of 520 and 994mg flavan-3-ols compared to matched control (46mg). Therefore, it may be possible that cognitive enhancement after the consumption of flavan-3-ols are only evident during prolonged cognitive demand. It must also be noted that although intervention treatments in the literature are controlled for flavonoid intake, methylxanthines such as caffeine and theobromine, which are known to be cognitively enhancing (Smit *et al.*, 2004), are not consistently matched between treatment groups. Although this give a more ecological outlook on the impact of cocoa upon cognitive performance, these fluctuating levels of methylxanthines could, therefore, be responsible for conflicting findings in the literature.

#### 1.6.2.3 Berries

Only five studies investigating the effect of berry supplementation upon human behaviour have been published, none of which examine blackcurrants. Krikorian *et al.,* (2010a) reported positive effects upon verbal learning, spatial memory and delayed verbal recall after supplementing 12 adults aged 78.2 ± 5 years exhibiting symptoms of age related memory decline with 532ml of concord grape juice per day (phytochemical composition was not disclosed), over a 12 week period; however, effects on spatial and retrieval memory outcomes were not statistically significant. As an addition to this study Krikorian *et al.,* (2010b) supplemented nine adults aged 76.2 ± 5.2 years with age related memory decline, a blueberry juice drink standardised to contain on average 1.26g of polyphenols per day for 12 weeks. Accuracy in the Californian verbal learning task in the blueberry juice group increased compared to baseline after the 12 week intervention. Although indicative of a positive effect of berry consumption upon human memory, it must be noted that the sample size was small and age differences between groups were large. The blueberry study was also an add-on to the earlier grape study, using the initial study's placebo arm, thereby lacking a proper control treatment. Results should, therefore, be interpreted with caution. In a more recent study by the same author, 21 healthy adults aged 68 years and older with mild age related memory decline were supplemented either a grape juice determined by body weight (444ml average) containing, on average, 209mg of polyphenols (96.2mg anthocyanins, 60.61mg phenolic acids, 20.9mg procyanidins) or a sugar matched placebo drink for 16 weeks. After the 16 week supplementation, interference during the recognition memory task was reduced in the concord grape juice arm when compared to control, indicating that participants were better able to discriminate previously learned material from decoy stimuli after consuming the grape juice. The acute effect of the intervention was not assessed. In contrast, a shorter study investigated the effect of six weeks' cranberry juice supplementation in cognitively intact older adults (50-60 years of age). Participants were required to drink two 8oz cranberry drinks per day containing 27%

cranberry juice, no phytochemical data was reported. The study investigated various memory and central executive tasks. No significant effects of the cranberry juice were reported after the six week intervention (Crews *et al.*, 2005).

In terms of intervention studies assessing the effect of berry supplementation on behaviour in a healthy young cohort, the only published study reported no significant positive or negative effects of acute grape juice supplementation upon implicit memory or mood (Hendrickson & Mattes, 1998). It is, however, possible that concomitant consumption of lunch could have obscured an effect of the intervention potentially altering the food matrix, altering the pharmacodynamics of the potentially active compounds. It must also be noted that the memory task employed was implicit and it is, therefore, difficult to compare the findings from this study to others that have explored explicit memory measures. Although no peer reviewed studies of the cognitive effects of blackcurrant in humans have been published, a patent filed in 1992 by Bormann & Shatton (Bormann & Schatton, 1996) examined the effects of blackcurrant on vigilance, tracking and mood. Twenty-four participants (N=8 in each arm) were supplemented with either placebo or 3.4g or 10.2g of blackcurrant extract in a between subjects design. The results highlighted an increase in attention after supplementation. This was evinced as an increase in vigilance as assessed using a modified version of a vigilance task (Miner, 1971) after supplementation with 10.2g extract when compared to placebo. No effects upon mood or tracking performance were observed. However, the authors do not state how the task was modified. The blackcurrant extract employed was also undisclosed and phytochemically un-quantified. In addition, each intervention treatment was supplemented at 13:30, with no information regarding restriction of food intake prior to supplementation or absorption period between consumption of the intervention and commencing of cognitive assessment made available in the patent.

One important factor surrounding the human berry literature cited above is the lack of control for vitamins and minerals such as vitamin-C between intervention treatments. Although no modulations of cognitive performance have been reported in humans after acute consumption of vitamins, chronic supplementation has been shown to improve mood in humans (Brody, 2002; Smith *et al.,* 1999). Further to this, a recent review of the evidence of the cognitive benefits of vitamin-C upon human cognitive performance concluded that there is evidence that maintaining vitamin-C levels throughout life can have a protective effect against age related cognitive decline (Harrison, 2012). Vitamin-C can, therefore, not be ruled out as the compound driving the subtle cognitive effects of berries in aged adults.

#### 1.6.2.4 Summary of the behavioural effects of flavonoid-rich foods

In regards to berry supplementation, the literature outlines some positive benefits of short-term chronic supplementation (six weeks and over), with no acute effects being reported in any population. In humans, these chronic effects include improvements in spatial memory and verbal learning. It must, however, be noted that these nutritional interventions studies contain several methodological issues, such as, small sample sizes and poorly matched control arms. Likewise, acute effects of the intervention are only assessed in one published study, making it impossible to ascertain if the reported effects are due to an accumulative chronic effect, or an acute effect of the intervention on the day of cognitive testing. In animal models, improvements in cognitive performance encompass modulations of; spatial and short-term working memory, reversal learning and rapid memory acquisition, long-term reference memory, slow memory acquisition and memory retrieval, as well as improvements in psychomotor control. Limited acute effects of berry consumption (positive or otherwise) have been reported in the literature to date. The exact reasons are unclear; however, publication bias towards positive findings could be a limiting factor. Although no acute effects of berry consumption upon human performance have been reported, information can be

drawn from controlled trials using nutritional interventions from other flavonoid-rich foods. In the case of this thesis, effects of consumption of cocoa have been drawn upon. In regards to cocoa, acute supplementation of flavan-3-ol-rich cocoa foods in young healthy adults has been shown to increase visuo-spatial attention and attenuation of self-reported fatigue after cognitively fatiguing cognitive tasks. Although there are some positive effects reported, conflicting results are seen in the literature which could be due to methodological limitations such as; varying doses and dosing length, extract types, differing cohorts and varying platforms used to deliver cognitive paradigms. Factors which greatly hinder inter study comparisons.

Given the previously demonstrated modulations of grey matter cerebral blood flow after nutritional interventions outlined in section 1.5.2, it is apparent that blood flow may be modulated by nutritional interventions in areas of the brain where demand is placed during the completion of the specific cognitive task being undertaken. It, therefore, seems surprising that no positive effects upon cognitive performance were identified. One suggestion for the lack of behavioural effects is that the healthy young participants were performing close to ceiling and benefits were therefore unlikely to be observed. A more overarching observation is the choice of cognitive tasks known to activate brain regions being investigated, rather than because task performance outcomes are theorised to be sensitive to the study intervention.

## 1.7 General conclusion and summary of the objectives of the thesis

The foregoing literature review has concentrated on the two main areas of this thesis, the behavioural and physiological impacts of phytochemical compounds found in blackcurrants and other flavonoid-rich fruits. Reference has been made to a number of other dietary manipulations which fall outside of the remit of the experimental focus of the thesis but which may owe demonstration of cognitive enhancing properties to similar mechanisms as blackcurrants.

The evidence in respect to the augmentation or reversal of cognitive decline by flavonoid-rich foods in rodent models is overwhelmingly positive and there is a growing body of literature supporting a similar effect in aged humans. Animal models in particular have highlighted that the cognitive paradigms sensitive to berry fruit interventions are psychomotor performance, short-term memory, spatial memory, working memory and verbal memory; as well as reductions in anxiety with similar findings evident in young human populations after supplementation of other flavonoid-rich foods, such as cocoa.

There is a convergence of research suggesting that foods rich in flavonoids, especially those flavonoids present in berries, exert a number of behavioural effects both in animal models and aged humans. It is, therefore, entirely plausible that blackcurrant extracts that contain high levels of phytochemicals, which potentially have the ability to exert physiological effects great enough to affect human behaviour, could modulate acute cognitive performance and potentially provide novel treatments for pathological diseases and attenuate cognitive decline associated with natural ageing.

It seems from the above that an appropriate starting point for moving forward the research in this domain would ideally incorporate three specific criteria; well-defined extracts with emphasis based on their post-harvest preparation and their phytochemical composition; objective computer-based measures of cognitive functioning and; rigorous methodological design incorporating paradigms shown to be sensitive to similar nutritional interventions in animal models and human trials as well as to changes in monoaminergic tone.

In regards to the first of these criteria, two extracts will be utilised. The first is a commercially available standardised, freeze-dried, anthocyanin-enriched powdered

blackcurrant extract ((Delcyan$^{TM}$), Just The Berries, Palmerston North. New Zealand)) and the second will be a fresh from frozen, cold-pressed crude blackcurrant juice extract created from the Blackadder cultivar (Plant & Food Ltd. New Zealand). When used alongside each other in an intervention study, extracts will be standardised for total polyphenol level only, therefore, they will differ in phenolic profiles, allowing for a direct comparison between different extract types and placebo.

With regards to the psychometric instruments, the Computerised Mental Performance Assessment System (COMPASS) will be utilised throughout the thesis. This is a purpose designed software application for the flexible delivery of randomly generated parallel versions of standard and novel cognitive assessment tasks, which has previously been shown to be sensitive to a range of nutritional interventions (e.g. Haskell *et al.*, 2008; Kennedy *et al.*, 2010; Kennedy & Haskell, 2011; Moss & Oliver, 2012; Asamoah *et al.*, 2013; Stonehouse *et al.*, 2013).

In relation to rigorous methodological design, studies will be designed to incorporate paradigms which have been shown previously to be sensitive to flavonoid intake in animal models and humans, and to modulations in monoaminergic tone. These will include attention, several aspects of memory, psychomotor performance, mood and central executive tasks. Cognitive measurements will be observed during times when the purported active constituents and associated metabolites of the berry juice are available in the peripheral circulatory system, with multiple time point experiments being used to accommodate the biphasic nature of flavonoid and phenolic acid absorption.

Finally, the thesis will examine the dose effect and time course of the purported monoamine oxidase inhibiting effect, and the effect upon circulating monoamines, associated metabolites and hormones.

It is necessary to acknowledge that the double-blind placebo-controlled, randomised intervention studies that will make up the majority of this thesis, are not intended to answer all of the fundamental questions pertaining to the consumption of blackcurrants and their effects upon human behaviour. The chronic and accumulative effects of the consumption of blackcurrant extracts will not be assessed. Whilst this is a major factor surrounding the augmentations of flavonoids in animal models and in the long-term, questions of this nature are of upmost importance, it is necessary to start with the basic acute effects, and provide a foundation for future scientific research which will be required to assess more long-term questions.

The objective of the studies making up this thesis is to investigate the potentially beneficial behavioural effects of acute supplementation of standardised blackcurrant extracts, and the mechanisms underlying such effects. The main aims of the thesis are:

1) To assess the effect of standardised blackcurrant extracts upon cognitive performance in healthy adults
2) To examine the physiological and haemodynamic effects of blackcurrant extracts
3) To examine underlying mechanisms of any effects, in particular the potential *in vivo* monoamine oxidase inhibitory properties of blackcurrants and other associated biomarkers

# CHAPTER 2. ACUTE SUPPLEMENTATION WITH BLACKCURRANT EXTRACTS MODULATES COGNITIVE FUNCTIONING AND INHIBITS MONOAMINE OXIDASE-B IN HEALTHY YOUNG ADULTS

## 2.1 Introduction

There is a growing body of evidence supporting modulation of mammalian behaviour, in particular an improvement in cognitive ability of research animals following the ingestion of flavonoid-rich berries (Casadesus *et al.*, 2004; Williams *et al.*, 2008). This literature surrounding berries and mammalian behaviour has mainly focused upon the slowing or reversal of natural cognitive decline (Joseph *et al.*, 1999; Casadesus *et al.*, 2004; Williams *et al.*, 2008). As discussed in section 1.4, several mechanisms of action have been proposed and investigated in an attempt to underpin and explore improvements to memory in animal models, ranging from anti-inflammatory and antioxidant responses, to improvements in neural signalling.

Peer-reviewed intervention studies have highlighted positive effects of short-term chronic berry consumption upon cognitive functions of aged adults. Most notably, improvements in verbal learning, spatial memory and delayed verbal recall after supplementation of concord grape juice (Krikorian *et al.*, 2010a); and increased verbal memory after supplementation of blueberry (Krikorian *et al.*, 2010b) have been observed. Increases in recalled words in the paired learning task have also been reported by the same author after supplementation of concord grape juice (Krikorian *et al.*, 2012). In contrast, a shorter study investigating the effect of six weeks cranberry juice supplementation found no significant effects upon various memory and central executive tasks (Crews *et al.*, 2005). The only published intervention study focusing upon the effects of berries upon cognitive processing in healthy young adults reported that an acute intervention assessing the effect of grape juice consumed with a standardised lunch did not have a significant effect on cognitive function or mood

(Hendrickson & Mattes, 1998). Conversely, the few reported studies that have investigated the impact of berry extracts *in vivo* (Bormann & Schatton, 1996; Matsumoto *et al.*, 2005b) and *in vitro* (Dreiseitel *et al.*, 2009a) upon biological mechanisms with potential to affect the behaviour of healthy young adult human participants, have revealed promising results. Most prominently, anthocyanins, their aglycones, and phenolic acids, have been shown to have monoamine oxidase inhibiting properties. The average $IC_{50}$ effects *in vitro* were shown to be 36.9±5.8μM for MAO-A and 36.8±5.2μM for MAO-B (Dreiseitel *et al.*, 2009a). However, these quantities were much greater than normally found in human plasma following the consumption of an anthocyanin rich meal (Mazza *et al.*, 2002).

In 1992, Borman & Schatton filed a European patent detailing the inhibitory effect of a single serve of undefined blackcurrant drink on human monoamine oxidase-B (MAO-B) activity (Bormann & Schatton, 1996). They also record inhibition of MAO-A *in vitro*. Although the *in vivo* findings of the patent are demonstrated in only one human subject and are not peer-reviewed to scrutinise their scientific integrity, these “pilot” findings are of great interest in terms of the potential of a berry extract to modulate human behaviour. In the same patent, Bormann and Schatton also discussed improvements to attention and mood state. In a parallel study 24 (n=8 in each study arm) healthy human participants were supplemented with 3.2g or 10.2g of an unnamed blackcurrant extract or an inert placebo. The 10.2g dose was reported to engender improvements in vigilance and mood. However, no absorption period or fasting regime were reported. As discussed in section 1.4, MAO inhibitors have been used for several decades for the treatment of mood disorders and amelioration of neurodegenerative disease symptoms, including those of Parkinson’s disease (Youdim & Bakhle, 2006). MAO-B constitutes about 80% of total MAO activity in the brain (Johnston, 1968) and unlike most neuronal cells and their associated neurotransmitters and enzymes, MAO-B does not exhibit age-related reductions in activity or presence. In fact, brain MAO-B activity

has been shown to increase with natural ageing (Sparks, 1991) and during neurodegenerative disease, specifically in areas of cell loss in Parkinson's disease. A major by-product of monoamine degradation is hydrogen peroxide ($H_2O_2$). $H_2O_2$ is the major source of central nervous system oxidative stress and is, therefore, implicated in reductions in cell function and cell death via oxidative stress and the pathogenesis of neurodegenerative diseases (Ciccone, 1998). Inhibiting this degradation of central monoamines through inhibition of central MAO could, therefore, reduce the oxidative stress caused by the oxidative metabolism of monoamines (Aluf *et al.*, 2013) and allow monoamines, and their precursors, to accumulate and potentially alter the dynamics of regular monoamine transmitters (Youdim & Bakhle, 2006).

As outlined in chapter one, the major anthocyanin constituents of blackcurrants are D3G, D3R, C3G and C3R. There is also an abundance of other less prolific phenolic structures present in smaller quantities. These have the potential to exert physiological changes capable of modulating human behaviour, such as the rate and pattern of glucose uptake from the small intestine (Bassoli *et al.*, 2008; Manzano & Williamson, 2010; Cropley *et al.*, 2012)

It must also be noted that blackcurrants contain up to 175mg of vitamin-C per 100g of fruit (Hägg *et al.*, 1995) which, following chronic supplementation, has been shown to improve mood in humans (Smith *et al.*, 1999; Brody, 2002) and cognition in mice (Arzi *et al.*, 2004). Extracts used in the intervention chapters of this thesis were not matched for vitamin-C, however, there are no reports of acute behavioural effects in humans or any data relating to its impact upon MAO activity.

The primary outcome in published flavonoid intervention studies has been an improvement in memory performance after chronic supplementation of aged cohorts. Data depicting the impact of flavonoid supplementation upon cognitive outcomes in

young healthy participants is lacking. With reference to the above literature, the flavonoid-rich berries of the blackcurrant plant could potentially modulate behaviour through various mechanisms, particularly via inhibition of central monoamine oxidase, which could modulate the levels of neurotransmitters such as serotonin and dopamine that are responsible for the regulation of attention and cognitive flexibility. Patent data can also be drawn upon, outlining improvements in attention processing after acute blackcurrant supplementation. The primary objective of the current study will therefore focus upon the impact of two standardised blackcurrant extracts upon attention functioning and mood. The extracts to be used will be a crude, cold pressed blackcurrant juice and an anthocyanin-enriched, commercially available blackcurrant powder. The two blackcurrant extracts were matched for quantities of polyphenols and sugars but have differing phenolic profiles. As anthocyanins are hypothesised to be one of the major active compounds in blackcurrants, cognitive assessments will be administered whilst anthocyanin levels are at maximal concentration (Cmax), between 60 and 120 minutes post-supplementation.

The second aim of the current study is to ascertain if the extracts employed inhibit the activity of platelet MAO-B and blood parameters associated with peripheral MAO-A inhibition, as well as establishing whether any inhibition is affected by the differing phenolic profiles of the blackcurrant extracts. As the MAO activity in human blood platelets has been shown to be primarily MAO-B (Donnelly & Murphy, 1977) and has a high correlation with brain MAO-B activity (Benchet al., 1991), platelets will be isolated so peripheral MAO-B can be quantified. As MAO-A is mostly active in the digestive system and the CNS, it is difficult to directly measure MAO-A activity in humans without tissue biopsy. Instead 3,4-Dihydroxyphe-nylglycol (DHPG), the major MAO-A-mediated deaminated metabolite of noradrenaline (Kopin, 1985), will be used as an indirect measurement. This method has been used in a plethora of pharmacological MAO inhibitor studies (Koulu *et al.*, 1989; Zimmer, 1990; Dingemanse *et al.*, 1992).

Monoaminergic tone will also be measured through blood plasma monoamine content and associated metabolites, general dopaminergic tone will be assessed through the measurement of plasma prolactin levels. Prolactin is a polypeptide hormone predominantly secreted from lactotroph cells of the anterior pituitary gland (Fitzgerald & Dinan, 2008). The predominant control of prolactin secretion into plasma is via hypothalamic inhibition of lactotroph activity (Ben-Jonathan & Hnasko, 2001). The most important hypothalamic prolactin inhibiting factor is dopamine, where it is secreted into the portal vessels of the hypothalamus in high enough quantities to inhibit lactotroph prolactin secretion (Fitzgerald & Dinan, 2008). However, it must be noted that because the pituitary gland is placed both within and outside of the blood brain barrier it is readily exposed to circulating hormones (Ho *et al.*, 1985). Although the majority of circulating dopamine is biologically inactive conjugate dopamine sulphate (Claustre *et al.*, 1990), this placing of the pituitary makes it difficult to demonstrate a direct increase in central dopaminergic activity through the measurement of peripheral prolactin activity. However, it indicates the effect of the study treatment on general dopaminergic tone and potentially modulations of cerebral MAO activity. Finally, the study will assess the effect of the extracts, with differing phenolic profiles on post-prandial blood glucose levels, heart rate and blood pressure.

## 2.2 Materials and methods

### 2.2.1 Design

The study investigated the effects of two blackcurrant drinks, with balanced polyphenol content, on human cognitive function, mood and defined biochemical parameters. The study followed a double-blind, counterbalanced, placebo controlled, repeated measures design. Participants were randomly allocated to treatment orders as selected through a Williams Latin Square (Williams, 1949).

### 2.2.2 Participants

Thirty six participants were recruited, of which thirty-five participants (17 male, 18 female) aged 24 years ± 3.9, with a mean body mass index of 24 ± 4.7 kg/$m^2$ completed the study. Full demographics can be seen in table 2.1.

*Table 2.1 Mean participant characteristics*

| Measure | Average measurement | SD | Range |
|---|---|---|---|
| Age (years) | 24.8 | 3.93 | 18-34 |
| Height (m) | 1.72 | 0.12 | 1.52-1.97 |
| Mass (kg) | 70.93 | 17.82 | 44-116 |
| BMI ($m^2$) | 23.67 | 3.96 | 17-34 |

Participants were recruited from Auckland, New Zealand using opportunity sampling and received $120NZ to recompense them for any expense they may have incurred to participate in the trial. Before participants were enrolled in the study they attended a 90 minute training session. During this training session participants gave their signed consent to participate in the study and were screened for any contraindications to the study with the use of an exclusion questionnaire (see appendix I for an example) and a case report form (see appendix II for an example). In brief, all participants reported themselves to be healthy, not pregnant, non-tobacco users. Participants were not using dietary supplements or over the counter or recreational drugs (excluding the contraceptive pill), did not have any sensitivities to any of the study treatments and had a body mass index below 35kg/$m^2$. Participants then completed three repetitions of the study day tasks to ensure they met the required minimum standards (internally set) to

participate in the study and to minimise practice effects. The study received ethical approval from the New Zealand Regional Northern X Ethics board. (Application number NTX/10/07/066) and was conducted according to the Declaration of Helsinki (1964). All participants gave their written informed consent before their inclusion in the study.

### 2.2.3 Treatments

Participants received three treatment drinks in an order dictated by random allocation to a counterbalancing (Williams Latin Square) order with at least one week washout between visits. Extracts were assessed for the phytochemical constituents by Dr David Stevenson at Plant and Food Research Ltd using the method described by Schrage *et al.,* (2010). The phytochemical content of each treatment can be seen in table 2.2 ans 2.3. Anthocyanin stability of the Blackadder juice extract at -20 degrees was confirmed via HPLC. Over the eight week period no significant loss due to storage was seen. Drinks were standardised at ~500mg of polyphenols per 60kg individual as this is the average world weight of an adult (Walpole *et al.*, 2012). Intervention drinks contained either 0mg of polyphenols (control) or 525±5mg of polyphenols per 60kg of bodyweight from an anthocyanin enriched blackcurrant extract, (1.66g of Just the Berries, New Zealand (Delcyan$^{TM}$)) or from 142ml of a cold pressed blackcurrant fruit juice, (Blackadder cultivar, cultivated and processed in 2010 by Plant and Food Research Ltd, New Zealand (Blackadder Juice)). The Blackadder juice was frozen in 50ml aliquots at -20°C until the day of use. The naturally occurring sugars in the Blackadder juice were quantified by Helen Boldingh, Plant and Food Research Ltd. The same levels were supplemented to the control and Delcyan$^{TM}$ treatments to ensure caloric equality. In each case all drinks contained; 0.78g of glucose, 0.13g of fructose, 0.09g of Splenda® sweetener and 3.34µl blackcurrant flavouring (NI #12220, Formula foods NZ) per kilogram of bodyweight. The total volume of the drink was then made up to 200ml (for a 60kg person) with cold drinking water. All drinks quantities were calculated per kilo of body weight resulting in differing volumes. Drinks were coded and prepared

fresh each morning by a third party who had no further part in the running of the study. No member of the investigation team was aware of the coding of the drinks until a blind-data review was completed.

*Table 2.2 Phytochemical constituents of Blackadder juice and Delcyan™ extracts (mg/100ml of raw juice and mg/g of raw powder) and the constituents supplemented per kilo of bodyweight (mg/kg).*

| Compound | Blackadder juice mg/100ml | Blackadder juice mg/kg | Delcyan™ extract mg/g | Delcyan™ extract mg/kg body weight |
|---|---|---|---|---|
| Caffeoyl quinate | 6.3 | 0.15 | 0.1 | 0.002 |
| Caffeic acid glucoside | 1.9 | 0.04 | 0.2 | 0.005 |
| pCoumaroyl quinate | 3.6 | 0.09 | 0.4 | 0.011 |
| EGC | 8.6 | 0.20 | 0 | 0 |
| Delphinidin glucoside | 24.1 | 0.57 | 44.6 | 1.23 |
| Delphinidin rutinoside | 115.9 | 2.74 | 107.4 | 2.97 |
| Cyanidin glucoside | 13.6 | 0.32 | 28.8 | 0.79 |
| Cyanidin rutinoside | 150.9 | 3.57 | 149 | 4.12 |
| Myricetin rutinoside | 15.6 | 0.37 | 4.5 | 0.12 |
| Myricetin glucoside | 2.1 | 0.05 | 0 | 0 |
| Quercetin rutinoside | 3.4 | 0.08 | 1.2 | 0.03 |
| Quercetin glucoside | 1.9 | 0.04 | 2.3 | 0.06 |
| Quercetin pentoside | 1.4 | 0.03 | 5.5 | 0.15 |
| Myricetin | 0.2 | 0.00 | 0.6 | 0.01 |
| Vitamin C | 168 | 3.97 | 0 | 0 |

*Table 2.3 Anthocyanins and other phenolic compounds in each of the treatment conditions. (mg per kilo of body weight, average dose used in the intervention and dose range)*

| Treatment | Anthocyanins (mg/kg) | Anthocyanin average dose (mg) | Dose range | Other polyphenols (mg/kg) | Other polyphenols average dose (mg) | Dose range | total polyphenols (mg/kg) | Total polyphenols average dose | Dose range |
|---|---|---|---|---|---|---|---|---|---|
| Placebo | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| Blackadder | 7.786333 | 552.3336 | 344-906 | 0.66 | 46.93 | 29-77 | 8.44 | 599.3 | 373-983 |
| Delcyan™ | 8.05 | 571.03 | 356-937 | 0.28 | 19.86 | 12-32 | 8.32 | 590 | 368-968 |

### 2.2.4 Cognitive and mood measures

All cognitive measures and mood scales were delivered using the Computerised Mental Performance Assessment System ((COMPASS) as developed by Northumbria University), a purpose designed software application for the flexible delivery of randomly generated parallel versions of standard and novel cognitive assessment tasks, which has previously been shown to be sensitive to a range of nutritional interventions (Haskell *et al.*, 2010; Kennedy *et al.*, 2010; Kennedy & Haskell, 2011). For the purpose of behavioural analysis, three tasks were selected with the intention that attention performance and cognitive flexibility could be assessed. Seven repetitions of the digit vigilance task, Stroop task and rapid visual information task were completed in a fashion similar to that of the cognitive demand battery (Kennedy & Scholey, 2004) where subsequent repetitions of a ten minute battery are shown to incrementally induce mental fatigue.

**2.2.4.1 Digit vigilance***:* The digit vigilance task is a measure of vigilance involving accurate selection of target stimuli. It focuses on alertness and vigilance while placing minimal demands on two other components of attention: selectivity and capacity. A single target digit was randomly selected and constantly displayed to the right of the screen. A series of single digits were presented in the left of the screen at the rate of 80 per minute. The participant was required to press the target button on the computer keyboard as quickly as possible every time the digit in the series matched the target digit. The task lasted two minutes and there were 30 stimulus-target matches. Task outcomes were accuracy (%) and reaction time for overall responses (msec).

**2.2.4.2 Stroop:** The Stroop test is a measure of attention, inhibition and cognitive flexibility. Participants were presented with a colour name. The colour name presented was written in a coloured ink which could be the same as the colour name or different. Participants had to respond to the colour of the ink using the peripheral mouse and corresponding colour response buttons. Participants were presented with 60 stimuli. Task measures were accuracy (percent correct), incorrect responses and reaction time (msec).

**2.2.4.3 Rapid visual information processing** *(RVIP):* The RVIP task is a measure of sustained attention and working memory. The participant was required to monitor a continuous series of digits for targets of three consecutive odd or three consecutive even single digits. The single digits were presented at the rate of 100 per minute and the participant responded to the detection of a target string by pressing the space bar on the computer keyboard as quickly as possible. The task was continuous and lasted for 5 minutes, with 8 correct target strings being presented in each minute. The task was scored for percentage of target strings correctly detected, average reaction time for correct detections (msec), and number of incorrect responses (false alarms).

**2.2.4.4 Logical reasoning***:* The logical reasoning test requires the participant to think logically and analytically and is a measure of cognitive flexibility. A series of statements referring to the relationships between two letters appeared on the screen one at a time (e.g. “a precedes b: ba”). Participants were required to decide if each statement correctly described the order of the 2 letters that followed it by pressing the designated ‘YES’ or ‘NO’ button on the computer keyboard. There were 24 stimuli. Mean reaction times were measured in msec, and accuracy of responses were recorded as percentages.

**2.2.4.6 Bond-Lader visual analogue scales***:* Bond-Lader visual analogue mood scales (Bond & Lader, 1974) as used in a number of nutritional intervention studies (Kennedy *et al.*, 2006; Haskell *et al.*, 2008; Haskell *et al.*, 2010) were employed. The reliability and validity of these visual analogue scales has been demonstrated (Ahearn, 1997). The scales comprise a total of sixteen 100mm lines anchored at either end by antonyms (e.g. alert-drowsy, calm-excited) on which participants mark their current subjective position. Scores from the 16 Bond-Lader visual analogue scales were combined as recommended by the authors to form three mood factors: ‘alert’, ‘calm’ and ‘content’ (Bond & Lader, 1974).

**2.2.4.7 Visual analogue scales***:* Following each repetition of the attentional demand battery, participants were asked to subjectively rate how mentally fatigued they felt and how difficult they found the cognitive tasks. The electronic visual analogue scales were anchored “Not at all” on the left hand side of the scale and “extremely” on the right, with higher scores representing more mental fatigue/higher difficulty.

### 2.2.5 Blood analysis

Venous blood samples (2x5ml) were collected by a qualified phlebotomist at baseline and 150 minutes after supplementation of the intervention treatments. Samples were collected in 5ml BD vacutainers© (Becton, Dickinson and company, Plymouth, New Zealand). Both receptacles were treated with anticoagulants, one with lithium heparin (LH) and one with ethylenediaminetetraacetic acid (EDTA). Whole blood samples treated with LH were immediately centrifuged at 5000rpm for 10 minutes at 4ºC. Plasma was then extracted and aliquoted into 1ml eppendorf© tubes. One aliquot was spiked with 5% triflorocetic acid for the purpose of measuring plasma anthocyanin content. Plasma samples were stored at -80°C until analysis was performed.

Whole blood samples treated with EDTA were used to isolate blood platelets using the method reported by Corash *et al.,* (1980) as modified by Snell *et al.,* (2002). Three and a half millilitres of whole blood and 2ml of phosphate buffered saline solution (PBS) containing 2g of glucose per litre of solution (PBS solution) were added to a 25ml Falcon tube (Becton and Dickinson, AU) and gently inverted to mix the blood and buffer solution. The solution was then centrifuged at 600g at 22ºC for three minutes without the use of a centrifuge brake. The supernatant was then removed from the red cell pellet with the use of a pipette and placed on ice. The volume of the residual red cell pellet was restored to 7ml with PBS solution, gently mixed and centrifuged again at 600g at 22°C for three minutes without the use of a centrifuge brake, the supernatant portion was again removed and pooled with the first supernatant fraction. This procedure was performed five times. The pooled supernatant fraction was then centrifuged at 2000g at 4°C for 10 minutes. The supernatant fraction was then poured off to leave a platelet pellet. The pellet was stored at -80ºC until the MAO-B analysis was performed.

#### 2.2.5.1 Platelet monoamine oxidase-B (MAO-B) activity analysis

Due to technical difficulties, only 8 of the 35 participants provided sufficient blood samples for all the study time points for MAO-B analysis to be conducted.

The isolated platelet pellet was slowly thawed on ice, re-suspended in 1ml of PBS solution, sonicated with a probe sonicator (Microson ultrasonic cell disruptor, model XL2005) for 15 seconds on ice and centrifuged (Hitachi Himac preparative ultracentrifuge model CP100MX) for 10 minutes at 36,000g at 4 °C. Sonication and centrifugation were then repeated after which the supernatant was removed and the pellet consisting of lysed platelets was re-suspended in sodium phosphate buffer (reaction buffer) (as supplied in the Amplex® Red Monoamine oxidase-B Assay Kit (A12214 Invitrogen). The protein concentration of the lysed platelet solution was determined against a bicinchoninic acid standard curve by using the Pierce BCA protein assay (ThermoFischer Scientific New Zealand Ltd) as per manufacturer's instructions. Each sample was measured in triplicate and the average protein concentration was used. The lysed platelet solution was re-suspended in PBS to a final concentration of 150µg/ml of protein.

Determination of MAO-B activity was conducted using the Amplex® Red monoamine oxidase-B assay kit (A12214 Invitrogen), as per manufacturer's instructions. One hundred microlitres of the diluted lysed platelet solution was added to a black flat bottom 96 well plate (Greiner bio-one, REF 655101) in triplicates. Two microlitres of the MAO-A inhibitor clorgyline were then added to each well that contained platelet membranes and incubated for 30 min at room temperature. During the incubation, 100µl of $H_2O_2$ standards and the negative control were then added to the micro-plate in triplicate. After the 30 minute incubation, 100µl of the Amplex® Red working solution were added to each well. The microplate was immediately placed into the microplate reader (FLUOstar Omega Plate reader, BMG labtech), and set to incubate at 37°C with

an excitation wavelength of 530-560nm and an emission wavelength of 590nm. The micro-plate reader was programmed to take a reading every 5 min for one hour (13 readings in total). The 30 minute reading was used to compare platelet MAO-B activity between treatments. A full methodology can be found in appendix III.

#### 2.2.5.2 Glucose

Blood glucose was measured from a finger prick puncture with the use of an Accu-Check (Roche Healthcare, NZ) blood glucose monitor at baseline, 60 minutes and 150 minutes post-supplementation. The Accu-Check reader has a reported coefficient of variance (CV) of less than 5%.

#### 2.2.5.3 Anthocyanin analysis

Blood plasma anthocyanin analysis was conducted by Dr Janine Cooney at Plant and Food Research Ltd. Anthocyanins; D3G, D3R, C3G and C3R were identified by LC-MS using a 5500 QTrap triple quadrupole/linear ion trap (QqLIT) mass spectrometer equipped with a TurboIon-Spray$^{TM}$ interface (AB Sciex, Concord, ON, Canada) coupled to an Ultimate 3000 UHPLC (Dionex, Sunnyvale, CA, USA). Compound separation was achieved on a Zorbax SB-C18 Rapid Resolution HD 2.1x100mm ID 1.8 micron column (Agilent Technologies, Santa Clara, CA, USA) maintained at 70ºC. Solvents were (A) 5:3:92 acetonitrile/formic acid:water v/v/v and (B) 99.9:0.1 acetonitrile/formic acid v/v and the flow rate was 600µL/min. During the initial mobile phase, 100% A was held isocratically for 0.5 min, then ramped linearly to 10% B at 5 min, followed by another linear ramp to 90% B at 5.1 min and held for 1.9 min before resetting to the original conditions. Sample injection volume was 20µL. MS data was acquired in the positive mode using a multiple reaction monitoring (MRM) method in order to maximise sensitivity by screening out any chemical noise from other compounds present. This method monitors the distinctive daughter ions formed by fragmenting the targeted precursor ions $(M)^+$ for the 4 major blackcurrant anthocyanins, D3G, D3R, C3G and C3R; D3G: MRM *m/z* 465 > *m/z* 303; D3R: MRM *m/z* 611 > *m/z* 303; C3G: MRM *m/z*

449 > *m/z* 287 and C3R: MRM *m/z* 595 > *m/z* 287 in addition to malvidin-3-*O*-galactoside (Mv-gal: MRM *m/z* 493 > *m/z* 331), the internal standard. The turbo spray voltage, temperature, gas one, gas two and curtain gas pressure were set at 5500V, 700º C, 20 psi, 20 psi and 20 psi, respectively.

#### 2.2.5.4 Catecholamine Analysis

Eight catecholamines were analysed from blood plasma using LCMS. These were; serotonin, dopamine, phenylethylamine, adrenalin, noradrenalin, normetanephrine, 3,4-dihydroxyphenylglycol (DHPG) and homovanillic acid (HMV).

***Materials***

Formic acid (Riedel-de Haën), ammonium formate and acetic anhydride (Fluka), and Hunig's base were purchased from Sigma Aldrich (Auckland, New Zealand). Optima LC/MS grade acetonitrile (Fisher Scientific) was purchased from ThermoFisher (Auckland, New Zealand). Water was of Milli-Q grade. Analytical standards, dopamine, normetanephrine, noradrenalin, adrenalin, 3,4-dihydroxyphenylglycol (DHPG), serotonin and homovanillic acid (HMV) were purchased from Sigma-Aldrich and phenylethylamine (PEA) from Acros Organics (Geel, Belgium). Deuterated acetic anhydride [d6] was purchased from Sigma-Aldrich and deuterated dopamine [d4] from CDN Isotopes (Quebec, Canada). Phree ™ Phospholipid removal plates were purchased from Phenomenex (Torrance, CA, USA).

***Standard Preparation***

Individual stock standards (1000 □g/mL) (PEA, dopamine, serotonin, normetanephrine, noradrenalin, adrenalin, DHPG and HMV) were prepared in 0.1% formic $acid_{aq}$, and used to create a mixed catecholamine standard of all compounds (10 □g/mL). A separate labelled internal standard for spiking and recovery was also prepared [(IS) dopamine [d4] 10 □g/mL]. 100 □L of each of these standards was derivatized separately, as described for the samples, to prepare a derivatised mixed standard, and a derivatised (IS). The derivatised mixed standard was used to prepare calibration standards in the range 0.02 ng/mL to 1 ng/mL.

To facilitate quantitation and correct for matrix effects during analysis, labelled internal standards for each analyte were prepared (d-IS) by derivatizing 100 □L of the mixed catecholamine standard (10 □g/mL), as described for the samples, with the exception that deuterated acetic anhydride [d6] was used in place of unlabelled acetic anhydride.

All calibration standards were spiked with 10 □L of the derivatized [(IS) dopamine [d4] 100 ng/mL; final concentration 1 ng/mL] and 100 µL of the derivatized labelled internal standard catecholamine mixture [(d-IS) 10 ng/mL; final concentration 1 ng/mL] and prepared at a final volume of 1 mL.

***Sample Preparation***

Plasma samples were treated to remove proteins and phospholipids and derivatised in two stages to acetylate alcohol and amine functional groups and alkylate free carboxylic acids with hunig's base prior to LC-MS analysis. A double derivatization was found to be necessary to acetylate the less reactive alkyl hydroxyl groups. Briefly, each plasma sample (200 µL) was added to an individual well of a Phree ™ Phospholipid removal plate already containing cold 600 µL acetonitrile, 100 µL acetic anhydride and 10 µL 100 ng/mL dopamine-d4 [internal standard (IS); final concentration 1 ng/mL]. The Phree ™ plate was centrifuged at 500 g for 30 min, a further 200 µL acetonitrile added to each well, and the plate centrifuged at 500 g for a further 10 min. The filtrate was transferred to a 2 mL micro tube, 100 µL acetic anhydride added and heated at 50 $^{o}$C. After 30 min 20 µL Hunig's base was added to each sample, vortexed then heated for a further 60 min. Samples were then evaporated to near dryness with nitrogen at 40 $^{o}$C. Samples were re-derivatised; 100 µL acetonitrile, 100 µL acetic anhydride and 10 µL Hunig's base heated for 40 min at 50 $^{o}$C. Finally, to each sample 100 µL of the derivatized labelled internal standard catecholamine mixture [(d-IS) 10 ng/mL; final concentration 1 ng/mL] was added, and the sample made up to 1 mL with water and transferred to an autosampler vial ready for analysis.

***LC-MS Analysis***

Analysis of Catecholamines was performed using an AB Sciex Qtrap 5500 equipped with a Turbo V electrospray source (ESI) (AB Sciex, Foster City, California, USA), coupled to a Dionex UltiMate 3000 HPLC system, which consisted of two UltiMate 3000 RS pumps, an UltiMate 3000 RS autosampler and an UltiMate 3000 RS column compartment (Dionex, Olten/Switzerland) and controlled with Analyst 1.5.2 software. The analytical column used was a 150 by 2.1 mm Atlantis® T3 column, (3 µm particle size; Waters Corp., Milford, MA, USA), maintained at 40 $^{o}$C. Solvents were (A) MilliQ water +0.03 % ammonium formate + 0.1 % formic acid and (B) acetonitrile + 0.1 % formic acid and the flow rate was 0.6 mL/min. The initial mobile phase, 98% A, was held for 4 min then ramped linearly to 70 % A at 11 min, 20 % A at 14 min, and 0 % A at 14.5 min and held for 5 min before resetting to the original conditions. Sample injection volume was 100 µL.

The ESI conditions were: gas 1, nitrogen (40 psi); gas 2, nitrogen (50 psi); ion spray voltage, 2500 V; ion source temperature, 700 $^{o}$C; curtain gas, nitrogen (50 psi). MS/MS data was acquired in the positive mode using the most intense selected reaction monitoring (SRM) transition of each compound. In some cases the ammonium adduct was the most abundant ion observed for Q1. Scheduled SRM mode was used with a 2 minute window. A detailed description of analyte specific MS parameters is given in the table below. Quantitation was performed using the internal standard ratio method using MultiQuant software.

*Table 2.4: MRM Transitions used for Catecholamines and their Isotopically Labelled Internal Standard Analogues*

| Q1 | Q3 | Time | Name | DP | EP | CE | CXP |
|---|---|---|---|---|---|---|---|
| 164 | 105 | 10.6 | PEA 105 | 30 | 10 | 25 | 10 |
| 167 | 105 | 10.6 | PEA [d3] | 30 | 10 | 25 | 10 |
| 280 | 137 | 11.1 | Dopamine | 70 | 6.14 | 35 | 15 |
| 289 | 139 | 11.1 | Dopamine [d9] | 70 | 6.14 | 35 | 15 |
| 284 | 141 | 11.1 | Dopamine [d4] | 70 | 10 | 37 | 15 |
| 293 | 143 | 11.1 | Dopamine [d4] [d9] | 70 | 10 | 37 | 15 |
| 261 | 160 | 11.1 | Serotonin | 10 | 5 | 25 | 1 |
| 267 | 161 | 11.1 | Serotonin [d6] | 10 | 5 | 25 | 1 |
| 250 | 166 | 11.6 | Normetanephrine | 50 | 9 | 25 | 15 |
| 256 | 168 | 11.5 | Normetanephrine [d9] | 50 | 9 | 25 | 15 |
| 355 | 194 | 11.6 | Noradrenalin | 10 | 10 | 30 | 1 |
| 367 | 199 | 11.5 | Noradenalin [d12] | 10 | 10 | 30 | 1 |
| 292 | 250 | 12.5 | Adrenalin | 170 | 10 | 20 | 1 |
| 301 | 257 | 12.5 | Adrenalin [d12] | 170 | 10 | 20 | 1 |
| 356. | 237 | 13.4 | DHPG | 90 | 13 | 20 | 20 |
| 368 | 244 | 13.3 | DHPG [d12] | 90 | 13 | 20 | 20 |
| 308 | 224 | 13.9 | HMV | 110 | 10 | 25 | 20 |
| 311 | 225 | 13.9 | HMV [d3] | 110 | 10 | 25 | 20 |

#### 2.2.5.5 Prolactin analysis

Prolactin was measured by diagnostic Medlab, Auckland, New Zealand. Prolactin was analysed in 300µL of blood plasma collected in LH treated vacutainers. Due to technical issues, only 20 sets of blood samples were available for prolactin analysis (8 control, 7 Delcyan$^{TM}$, 5 Blackadder juice). For the above reasons, prolactin analysis was between subjects.

### 2.2.6 Study day testing procedures

Each participant was required to attend a total of three study days which were conducted at least seven days apart to ensure a sufficient wash out between conditions. During the week before, and throughout their participation in the study, participants were asked to abstain from berry consumption. Cognitive testing took place in a laboratory with participants visually and auditorily isolated from each other. On arrival at their first session, participants were randomly allocated to a treatment regime using a Latin square design that counterbalanced the order of treatments across the three active days of the study. On all three study days participants arrived at the lab in the morning (8:30 am), after an overnight fast, and firstly gave a 10ml venous blood sample. Heart rate, blood pressure and blood glucose were then measured. Participants then completed one repetition of the ten minute baseline cognitive

assessment battery comprising of the digit vigilance task, the Stroop task, the RVIP task, mood scales and the logical reasoning task. This constituted the baseline measure for that day. Participants were then supplemented with one of the study treatments in the form of a drink, which they were given five minutes to consume. Drinks were served chilled and in a dark brown 300ml plastic bottle with a straw to minimise the possibility of the participant recognising subtle differences in taste, look and mouth-feel between the treatments. After a 60 minute resting absorption period, in which participants read in a waiting area, participants' blood pressure and heart rate were measured again and a second blood glucose reading was taken by finger prick. Participants then completed the post-dose cognitive assessment. This paradigm consisted of seven repetitions of the attention tasks (digit vigilance, Stroop RVIP) and mood scales. This lasted 70 minutes and was followed by the logical reasoning central executive task. Participants then gave a third blood pressure reading and a third blood glucose reading before providing a second and final venous blood sample. A diagram of the study visit running order can be seen in figure 2.1

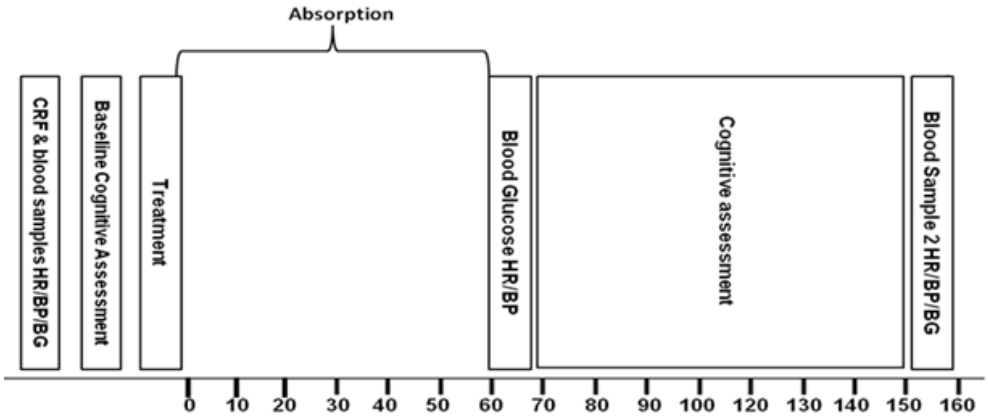



*Figure 2.1 Study day running order. Scale depicts minutes post-supplementation. CRF=Case report form, HR=heart rate ,BP=blood pressure, BG=blood glucose.*

## 2.3 Statistics

Mood, cognitive scores and the physiological measures were analysed as 'change from baseline' using the SPSS 18 statistics package. The null hypothesis was rejected with

a p value <0.05. Baseline differences were calculated for all measures using a one way (treatment) ANOVA.

Two way repeated measures ANOVAs (General linear model) (Treatment [placebo, Delcyan$^{TM}$, juice] X completion [1 to 7] for cognitive battery and visual analogue scale outcomes OR Treatment [placebo, Delcyan$^{TM}$, Juice] X completion [1 to 2] for blood glucose and Bond-Lader) were conducted. Logical reasoning performance, platelet Monoamine Oxidase B activity and plasma anthocyanins levels were analysed by one-way (treatment) repeated measures ANOVA. Blood plasma prolactin was analysed using a one way (treatment) between subjects ANOVA. In all instances Mauchly`s test of sphericity was used to assess equality of the variances of the differences between factors. Where sphericity had been violated, Huynh-Feldt corrections for non-sphericity were implemented. Pairwise comparisons were conducted on all outcomes with a p value <0.5 on the initial ANOVA to ascertain any differences between treatments for the whole session and at each task repetition. Partial Bonferroni corrections were applied to protect for error against multiple comparisons, therefore, the p value was multiplied by the number of treatments being compared to control. All post hoc p values are reported after corrections for multiple comparisons have been applied.

## 2.4 Results

Prior to analysis of change from baseline data, mean pre-dose scores for each outcome were subjected to a one way repeated measures ANOVA to assess on-day differences. The only significant baseline difference found between treatments was for homovanillic acid [F (1.622,25.95)=4.768. p=0.025] with higher levels of homovanilic acid in the baseline Blackadder juice group when compared to control (p=0.026). Therefore, results for this variable should be interpreted with caution.

A one way ANOVA was conducted on control change from baseline scores for the outcome fatigue to ensure the sustained attention cognitive paradigm was indeed mentally fatiguing. A significant effect of repetition was observed [F(6,192)=16.436. p<0.0001] with each subsequent repetition of the tasks causing an increased rating of self-reported mental fatigue. A graphical representation can be seen in figure 2.2.

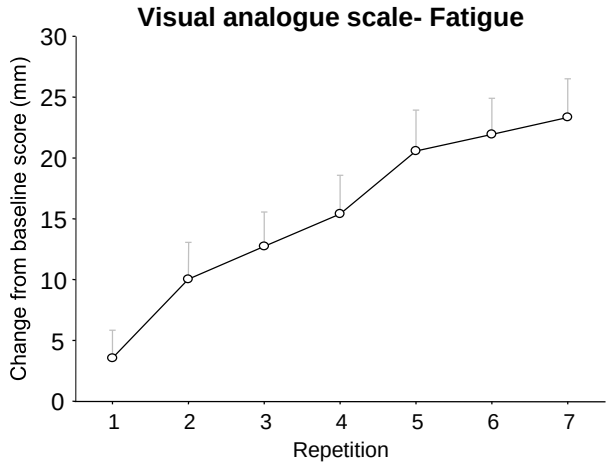



*Figure 2.2 Mean change from baseline scores for the visual analogue outcome fatigue post supplementation of the control treatment, depicting increasing self-reported fatigue with each repetition of the cognitive battery.*

### 2.4.1 Cognitive performance

Mean pre-dose baseline and change from baseline scores for each behavioural condition are presented in tables 2.5 and 2.6 and significant differences are presented in figure 2.3. Unchanged “raw” data tables can be found in appendix x .Only ANOVA

results for behavioural measures which generated significant effects are reported below.

*Table 2.5 Mean pre-dose baseline and change from baseline scores, standard deviations and ANOVA outcomes for Bond-Lader mood scales following supplementation of the control, Delcyan™ and Blackadder juice drinks.*

| Measure | N | Treatment | Baseline | | Repetition 1 | | Repetition 2 | | Effect of Treatment | Treatment*repetition interaction |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | | |
| Bond-Lader calm | 33 | Control | 58.71 | 15.05 | -0.65 | 11.02 | 4.52 | 20.13 | F=1.15 p>0.1 | F=0.71 p>0.1 |
| | | Delcyan™ | 61.06 | 16.35 | -3.23 | 11.31 | 2.45 | 14.53 | | |
| | | Juice | 60.11 | 15.08 | 2.24 | 14.32 | 4.44 | 18.43 | | |
| Bond-Lader content | 33 | Control | 69.33 | 10.20 | -1.93 | 10.08 | -10.38 | 13.78 | F=1.51 p>0.1 | F=0.98 p>0.1 |
| | | Delcyan™ | 70.16 | 11.41 | -0.40 | 5.58 | -6.42 | 10.13 | | |
| | | Juice | 69.01 | 11.09 | 0.08 | 5.79 | -6.25 | 12.99 | | |
| Bond-Lader alert | 33 | Control | 61.31 | 12.60 | -1.81 | 10.83 | -22.49 | 15.95 | F=2.61 p>0.1 | F=2.60 p=0.082 |
| | | Delcyan™ | 62.99 | 15.32 | -0.62 | 6.51 | -14.16 | 13.99 | | |
| | | Juice | 62.83 | 13.15 | -0.76 | 11.45 | -18.37 | 18.54 | | |

*Table 2.6 Mean pre-dose baseline and change from baseline scores, standard deviations and main ANOVA outcomes for each cognitive condition following supplementation of the control, Delcyan™ and Blackadder juice drinks.*

| Measure | N | Treatment | Baseline | | Repetition 1 | | Repetition 2 | | Repetition 3 | | Repetition 4 | | Repetition 5 | | Repetition 6 | | Repetition 7 | | Effect of treatment | Treatment*repetition interaction |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | | |
| Digit vigilance accuracy (%) | 33 | Control | 97.03 | 4.30 | -0.82 | 4.75 | -2.55 | 6.32 | -4.85 | 9.69 | -6.89 | 6.49 | -7.34 | 9.36 | -8.99 | 8.00 | -7.35 | 7.14 | F=1.03 p>0.1 | F=0.95 p=0.1 |
| | | Delcyan™ | 97.50 | 3.22 | -2.63 | 6.38 | -4.69 | 8.61 | -4.59 | 7.74 | -5.84 | 8.85 | -5.99 | 8.68 | -5.89 | 7.54 | -6.84 | 9.24 | | |
| | | Juice | 96.45 | 3.83 | 0.02 | 3.78 | -2.54 | 5.21 | -3.84 | 8.39 | -5.94 | 9.55 | -4.99 | 5.57 | -6.27 | 8.21 | -4.52 | 6.63 | | |
| Digit vigilance false alarms (Number) | 33 | Control | 0.91 | 1.34 | 0.42 | 1.68 | 0.36 | 1.58 | 0.94 | 1.39 | 1.58 | 1.89 | 1.61 | 1.84 | 1.94 | 1.90 | 1.61 | 1.92 | F=0.32 p>0.1 | F=0.98 p>0.1 |
| | | Delcyan™ | 0.80 | 0.80 | 0.33 | 1.27 | 0.70 | 1.45 | 1.12 | 1.95 | 1.00 | 1.90 | 1.55 | 1.79 | 1.12 | 1.56 | 1.30 | 2.01 | | |
| | | Juice | 0.86 | 1.14 | 0.24 | 1.37 | 0.88 | 1.67 | 1.15 | 2.31 | 1.55 | 1.82 | 1.39 | 1.50 | 1.73 | 2.18 | 1.36 | 1.83 | | |
| Digit vigilance reaction time (msec) | 33 | Control | 414.1 | 29.28 | 20.41 | 24.68 | 28.34 | 22.78 | 31.30 | 25.15 | 45.13 | 25.95 | 38.98 | 26.07 | 47.04 | 27.50 | 45.04 | 24.73 | F=3.48 p=0.037 | F1.82 p>0.043 |
| | | Delcyan™ | 413.5 | 28.64 | 15.70 | 19.23 | 19.11 | 25.51 | 36.27 | 30.17 | 31.88 | 25.62 | 34.79 | 24.12 | 32.85 | 23.30 | 39.97 | 24.72 | | |
| | | Juice | 422.1 | 31.03 | 4.43 | 28.43 | 16.01 | 31.00 | 25.34 | 34.69 | 24.73 | 29.66 | 28.96 | 34.31 | 32.58 | 35.92 | 28.62 | 29.62 | | |
| RVIP accuracy (%) | 32 | Control | 65.86 | 15.26 | -1.25 | 11.47 | -6.72 | 11.84 | -3.59 | 12.76 | -7.58 | 12.32 | -8.59 | 16.82 | -8.13 | 11.55 | -10.63 | 14.84 | F=5.88 p=0.005 | F=1.07 p>0.1 |
| | | Delcyan™ | 64.53 | 17.59 | -0.23 | 9.38 | -1.56 | 10.04 | 1.95 | 10.79 | -1.72 | 11.80 | -1.72 | 10.73 | -1.56 | 10.79 | -2.03 | 16.08 | | |
| | | Juice | 70.47 | 14.60 | -5.31 | 12.08 | -6.56 | 12.84 | -7.58 | 14.11 | -10.70 | 13.17 | -10.55 | 13.81 | -9.84 | 13.51 | -8.83 | 14.31 | | |
| RVIP false alarms | 32 | Control | 4.00 | 4.70 | -1.03 | 3.76 | -0.94 | 3.43 | -1.28 | 3.21 | -0.25 | 3.56 | -0.75 | 4.13 | -0.97 | 3.36 | -0.72 | 3.39 | F=0.57 p>0.1 | F=1.10 p>0.1 |
| | | Delcyan™ | 2.88 | 4.88 | -0.25 | 3.41 | -0.19 | 3.76 | -0.25 | 4.44 | -0.38 | 4.29 | 0.03 | 3.95 | -0.25 | 4.54 | 0.28 | 5.29 | | |
| | | Juice | 2.81 | 3.35 | -0.88 | 2.55 | -0.41 | 2.87 | 0.38 | 2.09 | 0.66 | 3.74 | -0.13 | 2.78 | -0.72 | 3.20 | -0.25 | 2.86 | | |
| RVIP reaction time (msec) | 32 | Control | 484.4 | 43.93 | -0.87 | 29.83 | 7.16 | 31.80 | 12.71 | 31.23 | 9.43 | 32.70 | 10.89 | 35.65 | 8.75 | 40.32 | 19.71 | 44.98 | F=0.04 p>0.1 | F=1.01 p>0.1 |
| | | Delcyan™ | 479.8 | 35.20 | 7.71 | 34.25 | 14.39 | 37.04 | 8.41 | 31.31 | 10.65 | 35.15 | 14.34 | 37.62 | 4.54 | 37.58 | 12.49 | 35.15 | | |
| | | Juice | 484.6 | 40.58 | -1.38 | 26.74 | 15.88 | 32.19 | 11.08 | 34.31 | 14.36 | 40.99 | 11.96 | 35.43 | 7.52 | 35.99 | 10.27 | 43.18 | | |
| Stroop accuracy (%) | 35 | Control | 98.88 | 2.14 | -0.16 | 2.28 | 0.14 | 1.61 | 0.15 | 2.43 | -0.73 | 2.86 | -0.67 | 2.73 | -0.51 | 2.53 | -0.29 | 2.29 | F=0.014 p>0.1 | F=0.94 p>0.1 |
| | | Delcyan™ | 99.14 | 1.61 | -0.31 | 1.91 | -0.31 | 2.05 | 0.07 | 1.90 | 0.04 | 1.59 | -0.42 | 1.83 | 0.09 | 1.69 | -0.72 | 2.27 | | |
| | | Juice | 98.90 | 2.77 | -0.57 | 2.97 | -0.26 | 2.11 | 0.03 | 2.06 | 0.00 | 2.49 | -0.27 | 2.51 | -0.28 | 2.29 | -0.50 | 3.01 | | |
| Stroop reaction time (msec) | 35 | Control | 797.4 | 105.9 | -9.03 | 51.78 | -4.23 | 49.64 | -11.26 | 54.52 | 14.94 | 60.77 | 10.51 | 59.67 | 53.80 | 174.7 | 18.60 | 64.57 | F=0.33 p>0.1 | F=0.57 p>0.1 |
| | | Delcyan™ | 801.5 | 124.4 | -15.86 | 49.59 | -2.31 | 57.42 | 26.43 | 157.3 | -1.57 | 57.53 | -11.39 | 120.66 | 6.00 | 60.99 | -3.26 | 72.49 | | |
| | | Juice | 788.5 | 115.1 | -15.69 | 70.97 | -7.91 | 81.20 | 5.31 | 102.1 | 25.00 | 121.7 | -2.43 | 70.25 | 19.66 | 95.64 | 7.77 | 79.30 | | |
| VAS fatigue (mm) | 33 | Control | 35.06 | 19.23 | 3.55 | 13.13 | 10.03 | 17.41 | 12.73 | 16.19 | 15.39 | 18.22 | 20.58 | 19.31 | 21.94 | 17.08 | 23.33 | 18.22 | F=1.27 p>0.1 | F=1.70 p=0.063 |
| | | Delcyan™ | 34.42 | 19.97 | 3.15 | 10.48 | 7.03 | 12.57 | 8.30 | 12.93 | 10.64 | 13.55 | 14.39 | 14.88 | 15.03 | 15.68 | 15.30 | 16.53 | | |
| | | Juice | 32.61 | 17.69 | 5.18 | 15.47 | 7.52 | 15.99 | 14.03 | 17.30 | 14.91 | 17.48 | 18.91 | 15.99 | 18.97 | 16.70 | 20.97 | 16.54 | | |
| VAS difficulty (mm) | 33 | Control | 48.97 | 15.48 | 2.94 | 14.29 | 10.97 | 20.25 | 17.24 | 14.14 | 20.76 | 18.76 | 25.03 | 17.67 | 25.55 | 18.86 | 29.24 | 17.73 | F=0.51 p>0.1 | F=1.46 p>0.1 |
| | | Delcyan™ | 40.12 | 18.97 | 4.91 | 10.85 | 12.88 | 14.57 | 15.00 | 12.78 | 17.61 | 14.83 | 21.24 | 15.25 | 22.45 | 15.95 | 23.12 | 16.04 | | |
| | | Juice | 39.85 | 20.63 | 7.70 | 18.05 | 15.67 | 21.86 | 17.79 | 18.51 | 20.58 | 17.26 | 24.82 | 19.43 | 26.73 | 21.01 | 27.48 | 20.04 | | |
| Logical reasoning reaction time | 33 | Control | 3977 | 1330 | -136.7 | 797.52 | | | | | | | | | | | | | F=0.35 p>0.1 | |
| | | Delcyan™ | 3834 | 1132 | -108.9 | 964.06 | | | | | | | | | | | | | | |
| | | Juice | 3963 | 1266 | -257.7 | 750.06 | | | | | | | | | | | | | | |
| Logical reasoning accuracy (%) | 33 | Control | 86.48 | 15.88 | 1.39 | 22.97 | | | | | | | | | | | | | F=0.07 p>0.1 | |
| | | Delcyan™ | 86.48 | 15.85 | 0.18 | 8.34 | | | | | | | | | | | | | | |
| | | Juice | 86.30 | 16.93 | 1.52 | 11.25 | | | | | | | | | | | | | | |

### 2.4.1.1 Digit vigilance

There was a significant treatment × repetition interaction on digit vigilance reaction time [F (12,384)=1.82 p=0.04] without any effect upon accuracy. Pairwise comparisons revealed a positive modulation of reaction time after supplementation of the juice treatment at repetition 1 (p=0.03), 4 (p=0.01) and 7 (p=0.04). See Figure 2.3a. There were no effects on any digit vigilance outcomes after supplementation with Delcyan™.

### 2.4.1.2 RVIP

There was a significant main effect of treatment on RVIP accuracy [F (2,62)=5.87, p=0.005]. Pairwise comparisons showed an attenuation in the reduction of RVIP accuracy after supplementation of the Delcyan™ extract treatment when compared to control (p=0.01), without any significant effect on reaction time or false alarms. See Figure 2.3b. There were no effects on any RVIP outcomes after supplementation with juice.

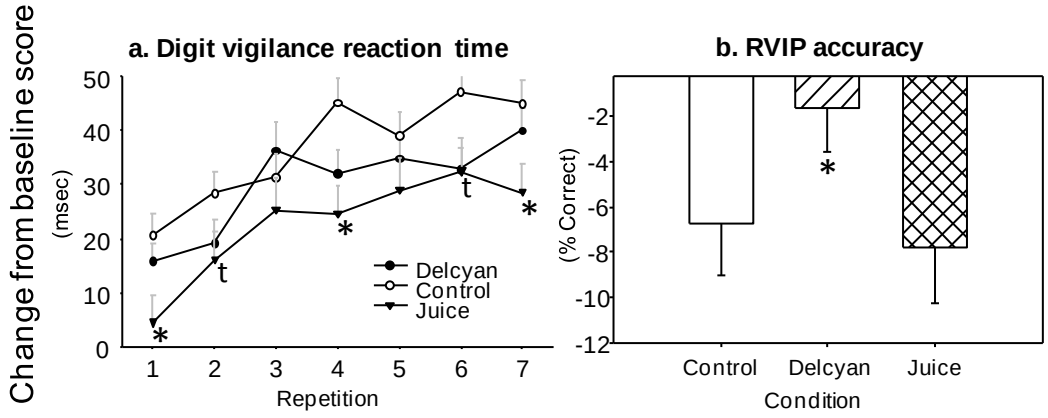



*Figure 2.3 Mean change from baseline scores following Control, Delcyan™ and Juice treatments for digit vigilance reaction time (millisecond (msec)) and RVIP accuracy (% correct). Significant differences compared to control are indicated (*p<0.05).*

### 2.4.2 Physiological parameters

Mean pre-dose baseline and change from baseline scores for each physiological measure are presented in table 2.7 and significant differences are presented in figure 2.4. Unchanged "raw" data tables can be found in appendix X. Only ANOVA results for

physiological measures which generated significant treatment related effects are reported below.

*Table 2.7 Mean pre-dose baseline and change from baseline scores, standard deviations and ANOVA outcomes for each physiological parameter following supplementation of the control, Delcyan™ and Blackadder juice drinks.*

| Measure | N | Treatment | Baseline | | Repetition 1 | | Repetition 2 | | Effect of Treatment | Treatment* repetition interaction |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | | |
| Heart rate (BPM) | 35 | Control | 69.6 | 11.76 | -2.26 | 9.12 | -7.46 | 10.12 | F=1.00 p>0.1 | F=0.72 p>0.1 |
| | | Delcyan™ | 72.14 | 12.07 | -4.2 | 7.26 | -8.14 | 7.87 | | |
| | | Juice | 72.46 | 11.89 | -4.09 | 9.81 | -10.2 | 11.2 | | |
| Diastolic blood pressure (mmHg) | 35 | Control | 76.83 | 7.64 | -2.17 | 6.88 | 0.71 | 10.6 | F=0.04 p>0.1 | F=1.64 p>0.1 |
| | | Delcyan™ | 76.89 | 8.31 | -1.91 | 6.22 | -0.11 | 6.76 | | |
| | | Juice | 74.63 | 7.32 | 0.54 | 6.13 | 0 | 8.32 | | |
| Systolic blood pressure (mmHg) | 35 | Control | 121.3 | 12.62 | -3.09 | 9.3 | -0.29 | 10.7 | F=0.16 p>0.1 | F=1.53 p>0.1 |
| | | Delcyan™ | 122.7 | 13.65 | -2.2 | 9.77 | -0.6 | 11.37 | | |
| | | Juice | 122.9 | 11.74 | -1.86 | 11.77 | -3.51 | 14.41 | | |
| Glucose (mmoL) | 35 | Control | 4.71 | 1.22 | -0.2 | 0.8 | -0.43 | 0.42 | F=8.89 p<0.001 | F=2.45 p=0.09 |
| | | Delcyan™ | 4.68 | 1.13 | 0.02 | 0.62 | -0.39 | 0.39 | | |
| | | Juice | 4.71 | 1.15 | 0.33 | 0.82 | -0.21 | 0.47 | | |
| Prolactin (IU/L) | 8 | Control | 271.6 | 70.52 | -103.2 | 79.85 | | | F=1.6 p>0.1 | |
| | 7 | Delcyan™ | 315.4 | 135.9 | -121.3 | 116.36 | | | | |
| | 5 | Juice | 353.4 | 105.3 | -196.2 | 77.42 | | | | |
| MAO-B (nmol $H^2O^2$) | 8 | Control | 258 | 112.3 | 11.32 | 68.28 | | | F=15.22 p<0.001 | |
| | | Delcyan™ | 220.6 | 131.2 | -78.06 | 131.88 | | | | |
| | | Juice | 273.1 | 114.1 | -267.4 | 109.82 | | | | |
| Total anthocyanins (nM/L) | 17 | Control | 0 | 0 | -0.02 | 0.35 | | | F=105.54 p<0.001 | |
| | | Delcyan™ | 0 | 0 | 22.03 | 7.62 | | | | |
| | | Juice | 0 | 0 | 15.16 | 4.6 | | | | |
| Phenylethylamine (ng/ml) | 17 | Control | 0.05 | 0.18 | 0.01 | 0.04 | | | F=0.96 p>0.1 | |
| | | Delcyan™ | 0.04 | 0.14 | 0.02 | 0.08 | | | | |
| | | Juice | 0.09 | 0.35 | 0.01 | 0.02 | | | | |
| Dopamine (ng/ml) | 17 | Control | 0.02 | 0.02 | 0 | 0.02 | | | F=0.39 p>0.1 | |
| | | Delcyan™ | 0.02 | 0.01 | 0 | 0.01 | | | | |
| | | Juice | 0.02 | 0.01 | 0 | 0.01 | | | | |
| Serotonin (ng/ml) | 17 | Control | 1.9 | 1.34 | -0.15 | 2.25 | | | F=0.75 p>0.1 | |
| | | Delcyan™ | 2.37 | 2.06 | -0.73 | 1.26 | | | | |
| | | Juice | 2.23 | 2.3 | -0.62 | 2.03 | | | | |
| Normetanephrine (ng/ml) | 17 | Control | 0.05 | 0.02 | 0 | 0.02 | | | F=12.19 p<0.001 | |
| | | Delcyan™ | 0.06 | 0.03 | 0 | 0.02 | | | | |
| | | Juice | 0.05 | 0.02 | 0.03 | 0.02 | | | | |
| Noradrenaline (ng/ml) | 17 | Control | 0.3 | 0.1 | 0.15 | 0.16 | | | F=0.97 p<0.1 | |
| | | Delcyan™ | 0.35 | 0.1 | 0.11 | 0.15 | | | | |
| | | Juice | 0.33 | 0.11 | 0.09 | 0.17 | | | | |
| Adrenaline (ng/ml) | 17 | Control | 0.02 | 0.02 | 0.01 | 0.03 | | | F=2.189 p>0.1 | |
| | | Delcyan™ | 0.02 | 0.03 | 0 | 0.01 | | | | |
| | | Juice | 0.02 | 0.02 | -0.01 | 0.02 | | | | |
| DHPG (ng/ml) | 17 | Control | 1.61 | 0.36 | 0.2 | 0.41 | | | F=21.30 p=0.001 | |
| | | Delcyan™ | 1.6 | 0.34 | 0.2 | 0.51 | | | | |
| | | Juice | 1.63 | 0.37 | -0.58 | 0.34 | | | | |
| Homovanillic acid (ng/ml) | 17 | Control | 21.42 | 12.21 | -4 | 14.9 | | | F=0.55 p>0.1 | |
| | | Delcyan™ | 27.45 | 16.7 | -7.67 | 10.34 | | | | |
| | | Juice | 17.12 | 8.01 | -5.31 | 7.63 | | | | |

### 2.4.2.1 Blood glucose

There was a significant main effect of treatment on blood glucose [F(2,68)=8.89, p<0.001]. Pairwise comparisons showed significantly higher blood glucose levels following supplementation of the juice treatment when compared to control (p=0.002). See figure 2.4a. There were no significant effects following supplementation of Delcyan™.

#### 2.4.2.2 Platelet MAO-B

There was a significant main effect of treatment on blood platelet MAO-B activity [$F(2,16)=15.2$ $p<0.001$]. Pairwise comparisons showed a decrease in platelet MAO-B activity after supplementation with the juice treatment when compared to control ($p<0.001$). See figure 2.4b. There were no significant differences between active treatment groups. There was no effect of the Delcyan$^{TM}$ treatment on blood platelet MAO-B.

#### 2.4.2.3 Monoamines

The repeated measures ANOVA revealed a significant main effect of treatment [$F(2,32)=12.18$ $p<0.001$] on plasma levels of normetanephrine. Pairwise comparisons showed levels of normetanephrine were significantly higher after supplementation of the juice treatment when compared to the control ($p<0.001$) and Delcyan$^{TM}$ ($p<0.001$). There were no effects of Delcyan$^{TM}$. See figure 2.4c.

The repeated measures ANOVA revealed a significant main effect of treatment [$F(2,32)=21.296$ $p<0.001$] on plasma levels of DHPG. Pairwise comparisons revealed levels of DHPG were significantly higher after supplementation of the juice treatment when compared to the control ($p<0.001$) and Delcyan$^{TM}$ ($p<0.001$). There were no effects of Delcyan$^{TM}$. See figure 2.4d.

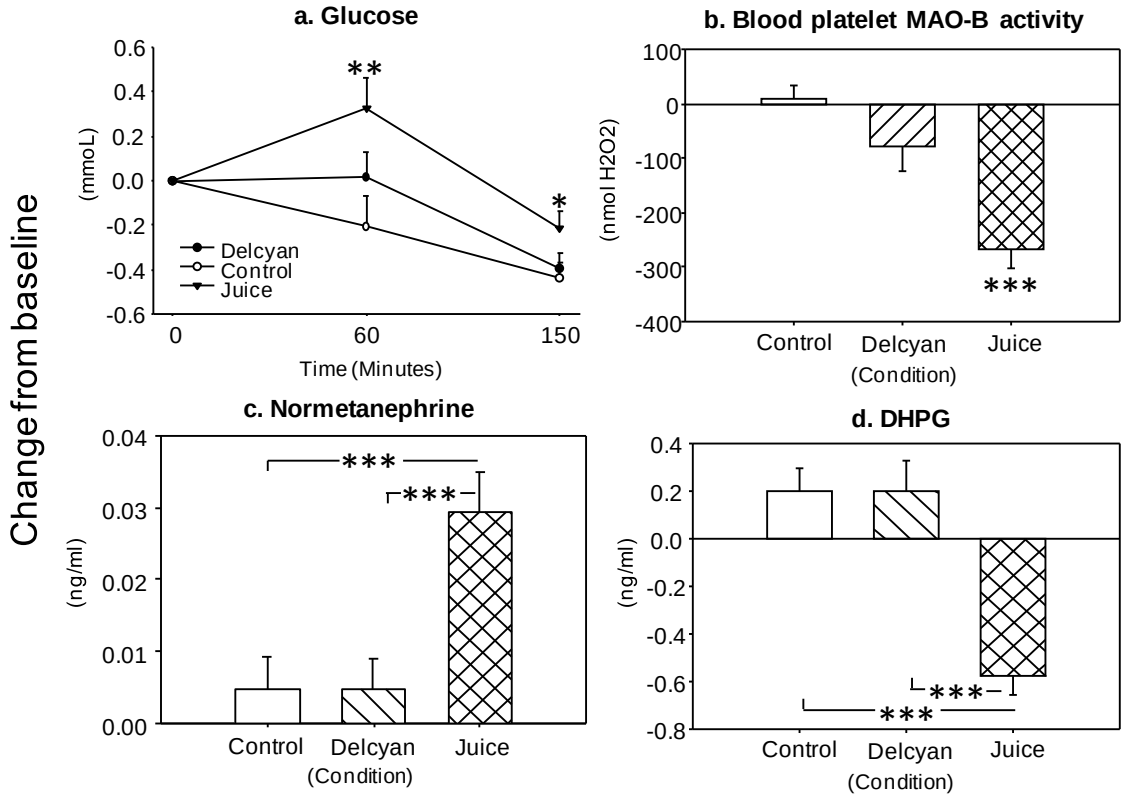



*Figure 2.4 Mean change from baseline scores following control, Delcyan™ and juice treatments. Significant differences compared to control (a,b) are indicated and compared to control and Delcyan™ (c,d) (*p<0.05 **p<0.01 ***p<0.001).*

### 2.4.2.4 Blood plasma anthocyanin levels

The repeated measures ANOVA revealed a significant effect of treatment on plasma levels of C3G, C3R, D3G and D3R [F(2,34)=27.5 (p<0.001)], [F(2,34)=33.7 (p<0.001)], [F(2,34)=112.51 p<0.001), [F(1.45,25.25)=96.26 p<0.001) respectively.

Plasma C3G, C3R, D3G and D3R levels were significantly higher after supplementation with the Delcyan™ treatment when compared to control p<0.001, p=0.001, p<0.001, p<0.001 and the Blackadder juice treatment p<0.001, p<0.001, p<0.003, p<0.001 respectively.

Plasma C3G, C3R, D3G and D3R levels were also significantly higher after supplementation of the Blackadder juice treatment when compared to control (p<0.001), (p<0.001), (p=0.003) and (p<0.001) respectively. A graphical representation of anthocyanin levels in blood plasma can be seen in figure 2.5a.

Following supplementation, the repeated measures ANOVA revealed a significant effect of the treatment on combined levels of C3G, C3R, D3G and D3R in blood plasma (combined anthocyanin level is the total amount of anthocyanins measured in blood plasma after supplementation of the study treatment) after consumption of the study treatments, [F (2,32)=105.34, p<0.0001]. Pairwise comparisons revealed that combined anthocyanin levels were significantly higher after consumption of the Delcyan$^{TM}$ (p<0.001) and juice (p<0.001) treatment when compared to control. Levels were also significantly after supplementation of the Delcyan$^{TM}$ extract when compared to the Blackadder juice extract (p<0.001). A graphical representation of combined anthocyanin levels in blood plasma can be seen in figure 2.5b.

*Table 2.8 Means and SD for the amount of measured anthocyanins given to the participants and the amount found in blood plasma (change from baseline) 150 minutes post supplementation of the Delcyan$^{TM}$ and Blackadder juice treatments*

| Treatment | Anthocyanin | Amount supplemented mg/kilo body weight | Average amount in plasma (nM) |
|---|---|---|---|
| Delcyan$^{TM}$ | Cyanidin glucoside | 0.80 | 0.41 ± 0.31 |
| | Delphinidin glucoside | 1.23 | 1.31 ± 0.91 |
| | Cyanidin rutinoside | 4.12 | 8.57 ± 2.89 |
| | Delphinidin rutinoside | 2.97 | 11.74 ± 4.51 |
| Blackadder juice | Cyanidin glucoside | 0.32 | 0.19 ± 0.15 |
| | Delphinidin glucoside | 0.57 | 0.77 ± 0.61 |
| | Cyanidin rutinoside | 3.57 | 6.36 ± 2.26 |
| | Delphinidin rutinoside | 2.74 | 7.85 ± 2.50 |
| Control | Cyanidin glucoside | 0 | -0.02 ± 0.12 |
| | Delphinidin glucoside | 0 | 0.00 ± 0.012 |
| | Cyanidin rutinoside | 0 | -0.03 ± 0.09 |
| | Delphinidin rutinoside | 0 | 0.03 ± 0.19 |

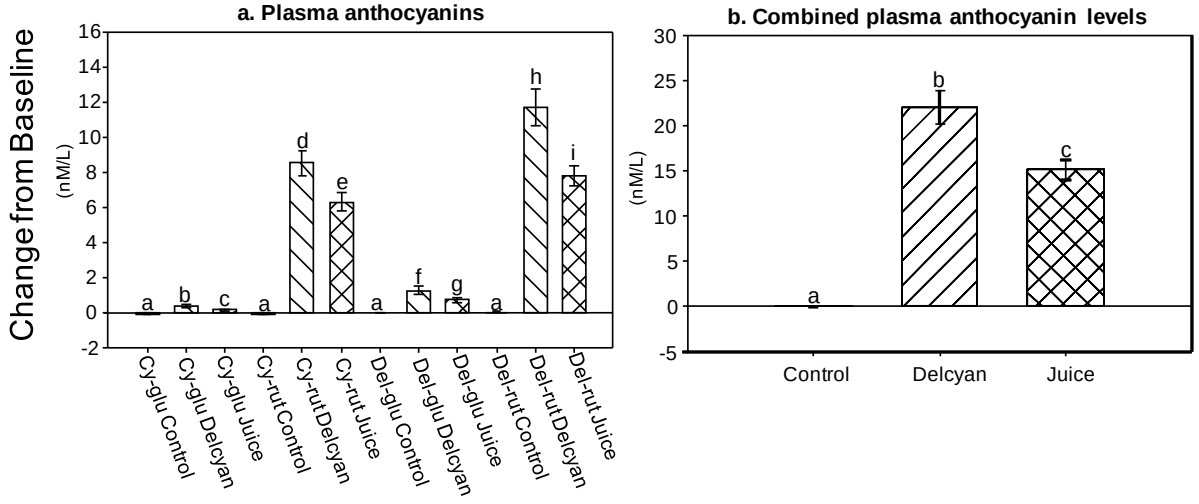



*Figure 2.5 Grouped mean blood plasma anthocyanins for each anthocyanin (a) and combined plasma anthocyanins (b) (combined anthocyanin is the sum of measured anthocyanins for each condition). Means with a different letter are significantly different to each other.*

## 2.5 Discussion and conclusion

The current study has outlined evidence of positive modulation of behaviour following administration of two blackcurrant extracts when compared to control, with no negative effects of either active treatment found. Improvements in RVIP accuracy were found after supplementation of the Delcyan$^{TM}$ extract and improvements in reaction time on the digit vigilance task were found after supplementation of the Blackadder juice extract. Although there were no other significant effects on behaviour, the juice treatment demonstrated a number of physiological effects not present following Delcyan$^{TM}$. These comprised of an extremely powerful inhibition of platelet MAO-B activity (96%) and a significant reduction in plasma normetanephrine (60%) and DHPG (~35.5%) when measured 2.5 hours after supplementation. The blackcurrant juice treatment also showed a significantly sustained (over both time points) increase in blood glucose when compared to control despite being sugar matched.

An increase in accuracy was shown during the RVIP task after supplementation with the Delcyan$^{TM}$ treatment, irrespective of task repetition, with no evidence of slowed reaction times. In regards to the juice treatment, there was evidence of an attenuation of the increase of digit vigilance reaction times seen with repeated testing, with no evidence of decreased accuracy. This improvement was seen during repetitions one, four and seven (70, 100 and 140 minutes post-supplementation, respectively). Further evidence for a modulation of behaviour following the blackcurrant extracts comes from non-significant trends observed on the treatment*repetition ANOVAs for Bond-Lader alertness ratings and mental fatigue visual analogue scales showed. These indicated a pattern of attenuation in decreased self-reported alertness and increased ratings of fatigue following supplementation of the Delcyan$^{TM}$ treatment but only reached significance after the final repetition of the 70 minute attentionally demanding cognitive battery. A graphical representation can be seen in appendix IV.a and IV.b.

There is evidence of direct cellular and molecular interactions of flavonoids on rodent brains (Spencer *et al.*, 2009) and changes in central (Francis *et al.*, 2006) and peripheral (Matsumoto *et al.*, 2005b) vascular function in humans after consumption of flavonoid-rich fruits. However, definitive mechanisms driving the behavioural effects in the present study are currently unknown, especially after supplementation of the Delcyan$^{TM}$ treatment, which had no significant effect upon any of the physiological measures.

As expected, blood plasma anthocyanins D3G, D3R, C3G and C3R were significantly increased 2.5 hours after supplementation of both blackcurrant treatments when compared to control. Measured blood plasma anthocyanins were also greater after supplementation of the Delcyan$^{TM}$ treatment when compared to the juice treatment. When all four measured anthocyanins were combined there was a 30% increase in plasma concentration following Delcyan$^{TM}$ when compared to the juice treatment. However, this extract contained 20% more of the measured anthocyanins than the juice drink. Although there was a significant difference in blood plasma anthocyanins between the two blackcurrant treatments, in line with past published research (Mazza *et al.*, 2002; Nielsen *et al.*, 2003; Matsumoto *et al.*, 2005b), anthocyanin quantities found in blood plasma were less than one percent of that ingested. It must be noted that vitamins and minerals, other than L-ascorbic acid, were not quantified in any of the study treatments. However, to my knowledge there are no reported cognitive or behavioural effects of acute vitamin or mineral supplementation. Given that the major phenolic constituents of each treatment were anthocyanins and both extracts affected attention based tasks, this may indicate that the effects of blackcurrant upon attention processing are directly related to their anthocyanin content; an acute effect which has previously been shown in children aged 7-9 years (Whyte & Williams, 2012). The specific demands of the two attention tasks are, however, not equal, with a higher demand both in processing, and duration of the RVIP task when compared to the digit

vigilance task. The RVIP contains a higher working memory element than the digit vigilance task, potentially indicating changes in numeric working memory processing as well attention, a cognitive outcome which has previously been shown to be sensitive to flavonoid-rich cocoa (Scholey *et al.*, 2010) and ginkgo biloba (Rigney *et al.*, 1999). However, until replication of the behavioural effects presented in the current has been achieved, it is difficult to elaborate further at this point

In terms of MAO-B activity, this is the first demonstration of a clinically significant inhibition of platelet MAO-B following blackcurrant supplementation. Central MAO-B inhibitors have been used for several decades for the treatment of depressive disorders and neurodegenerative diseases (Youdim & Bakhle, 2006) and have also been shown to improve cognitive processing when given to non-demented Parkinson patients (Hanagasi *et al.*, 2011). Most importantly MAO-B inhibitors have the potential to attenuate the breakdown of endogenous neurotransmitters, reducing levels of $H_2O_2$ associated with deamination of dopamine (Pizzinat *et al.*, 1999). Although the current study only measured MAO-B inhibition in peripheral tissue, if the inhibition can be shown to be centrally active, the clinical applications of a MAO inhibitor from a commonly consumed fruit could be vast. Potential applications include attenuating cognitive decline associated with natural ageing, as well as in clinical populations, including those suffering from early stage Parkinson's disease, whom are known to respond favourably to MAO inhibitors (Hanagasi *et al.*, 2011). DHPG, a metabolite largely determined by MAO-A dependent metabolism of noradrenalin (Scheinin *et al.*, 1991), which is a marker for reduced MAO-A activity after administration of pharmacological MAO-A inhibitors (Zimmer, 1990), was also found to be reduced after consumption of the Blackadder blackcurrant juice extract in the current study. This effect was not seen after consumption of the Delcyan$^{TM}$ extract, highlighting that, in addition to MAO-B inhibition, the Blackadder juice treatment possesses MAO-A inhibitory properties.

The observed reduction in DHPG is approximately two thirds of the reduction found after administration of 100mg of the selective MAO-A inhibitor drug moclobemide (Koulu *et al.*, 1989). These changes in DHPG did not coincide with an accumulation of adrenalin or noradrenalin in the current study, which is in line with previous research investigating acute supplementation of MAO-A inhibitors in humans (Illi *et al.*, 1996). In addition to decreased levels of DHPG, indicating MAO-A inhibition, we also see an increase in normetanephrine, a metabolite of noradrenalin via catechol-O-methyl transferase (COMT). This increase is potentially indicative of increased noradrenalin breakdown through COMT as a result of inhibition of the MAO-A enzyme. The ability of a MAO inhibitor to exert mood altering properties relies on its ability to inhibit MAO in the central nervous system, modulating the breakdown of dopamine, noradrenalin and serotonin in the brain. Serotonin is predominantly deaminated by the MAO-A isoform and is related to the aetiology of depression, for this reason, reversible MAO-A inhibitors yield the best therapeutic results in depressive patients (Gareri et al., 2000). Although MAO-B inhibitors do not possess anti-depressive properties on their own, MAO-B inhibition is useful for alleviating early symptoms associated with Parkinsonism, elevating dopamine and phenylethylamine in the brain (Riederer & Youdim, 1986). As MAO-B expression in the brain increases with age (Fowler *et al.*, 1997), and as a consequence of neurological diseases, the effectiveness of a MAO-B inhibitor in the brain where the ratio of MAO-B much outweighs that of MAO-A is increased in conjunction with supplementation of the dopamine precursor L-DOPA, at least in early stages of disease (Cools, 2006). Therefore, supplementation of a dopamine pre-curser with the MAO-B inhibiting Blackadder juice extract could yield greater results. Also related to this MAO inhibitory effect is a non-significant modulation of plasma prolactin observed in the current study where post-dose prolactin is lower after consumption of the Blackadder juice extract when compared to control. Although gamma-aminobutyric acid (GABA), serotonin, adrenalin and noradrenalin are slight rate limiting factors of prolactin secretion, dopamine is the most important hypothalamic prolactin inhibiting

factor, (Fitzgerald & Dinan, 2008) indicating that general and potentially central dopaminergic tone could have been affected by supplementation of the Blackadder juice drink. Although these prolactin findings are hindered by a small sample size and between subjects design, they illuminate the need for further research.

The blackcurrant juice treatment also showed a significantly sustained (over both time points) increase in blood glucose when compared to control, despite being sugar matched; an effect not seen with supplementation of the Delcyan$^{TM}$ treatment. Blood glucose was elevated by 0.53mmo/L 60 minutes and 0.23mmol/L 150 minutes post supplementation of the juice treatment. Although these results must be interpreted with caution as there were only two post-dose blood glucose measurements, this result shows a clear effect of the juice treatment on blood glucose. Therefore, a more thorough investigation needs to be completed to ascertain a full post supplementation blood glucose profile. Based upon the current findings, the effect on glucose appears to resemble the pattern after supplementation of apple juice where the post-prandial peak in blood glucose level is reduced resulting in a higher blood glucose reading one hour after supplementation (Johnston *et al.,* 2002). The main difference between the study treatments was phenolic acids at 0mg in the Delcyan$^{TM}$ treatment and 61mg in the juice treatment per 60kg of bodyweight, which could provide further evidence of the slowing of glucose transport from the gut via direct inhibition of intestinal epithelial glucose transporters by phenolic acids as described by Manzano and Williamson (2010). The study also showed no effect of acute blackcurrant supplementation on blood pressure or heart rate, which is consistent with the findings by Jin *et al.,* (2011).

The findings of the present study demonstrate, for the first time, a positive modulation of behaviour in a “young and healthy” adult cohort after supplementation of a blackcurrant extract. This is also the first evidence of a clinically significant reduction in MAO activity following ingestion of a commonly consumed fruit. The results suggest

that the MAO-B inhibition found in this study cannot be wholly responsible for the behavioural effects observed as both active conditions positively influenced attention based cognitive tasks, whereas only the juice treatment inhibited MAO-A and MAO-B. The finding of more robust effects on attention following Delcyan$^{TM}$, containing higher levels of anthocyanins, may indicate that these effects are attributable to the anthocyanin content and that any effects on MAO are independent of these. The possibility that a MAO-A and MAO-B inhibiting blackcurrant drink will exert favourable effects on cognitive modulation of non-clinical populations deserves further investigation. More exploration therefore needs to be undertaken to ascertain if other cognitive paradigms, especially those which have previously been shown to be sensitive to flavonoid-rich nutritional interventions in rats and humans, specifically memory tasks and paradigms sensitive to changes in levels of dopamine, are modulated after supplementation of a MAO inhibiting blackcurrant juice.

# CHAPTER 3. DOSE-RESPONSE EFFECTS OF THE BLACKADDER BLACKCURRANT JUICE EXTRACT ON MOOD, COGNITION AND NEUROENDOCRINOLOGY IN HEALTHY ADULTS

## 3.1 Introduction

Chapter two of this thesis presented data in support of patent findings (Bormann & Schatton, 1996) showing improvements to attention following acute supplementation of a blackcurrant extract to young healthy participants. The results from the previous chapter also suggested that different post-harvest preparation techniques applied to blackcurrant berries can yield different physiological responses. Previous findings, both in animal models (Joseph *et al.*, 1999; Casadesus *et al.*, 2004; Ramirez *et al.*, 2005; Wang *et al.*, 2006; Van Praag *et al.*, 2007; Williams *et al.*, 2008) and in the limited published human studies (Krikorian *et al.*, 2010a; Krikorian *et al.*, 2010b; Krikorian *et al.*, 2012), show positive effects of berries and their constituents upon several aspects of memory; a cognitive process which was not investigated in the first intervention study of this thesis. The nature of this published research is the attenuation of natural cognitive decline in aged animal models after the ingestion of flavonoid-rich foods. There is a similar focus in human intervention studies and, as discussed in chapter one and two of this thesis, positive effects upon spatial and verbal memory after 12 weeks supplementation of cranberry juice (Krikorian *et al.*, 2010a) or blueberry juice (Krikorian *et al.*, 2010b) have been reported in elderly adults with age related memory decline.

Although the above literature focuses upon cognitive performance in aged adults after consumption of flavonoid-rich berries, supplementation with other flavonoid-rich foods has been shown to elicit improvements to memory in younger participants. This research has highlighted that dose selection and time of post-dose cognitive assessments have an impact on the findings in flavonoid based intervention studies (Kennedy *et al.*, 2000; Scholey *et al.*, 2010). It must also be noted that Shukitt-Hale *et*

*al.,* (2006) have demonstrated differential neurocognitive responses at different doses within the same time frame in animal models after supplementation with grape juice. Further to this, Willis *et al.,* (2009) demonstrated dose-related effects in terms of motor and cognitive improvements in rats after supplementation with low and moderate doses of walnut, but impairment of both motor function and cognition with higher doses. Different findings shown at different time points and different doses (Kennedy *et al.*, 2000) may be due to the biphasic nature of flavonoid pharmacokinetics, where metabolites of flavonoid compounds re-emerge in the blood stream at a later time point (Jin *et al.*, 2011). In terms of intervention studies assessing the effect of berry supplementation on behaviour in a healthy young cohort, the only published study to date reported no significant positive or negative effects of acute supplementation of 10ml/kg of body weight (21mg/kg polyphenols) with 100% concord grape juice upon implicit memory or mood, as measured by a modified version of the word fragment completion task (Hendrickson & Mattes, 1998). The lack of effects in this study may be indicative of the implicit memory measure used, or other specifics of the study, such as, measuring effects post-prandially. The above evidence and data presented in chapter two, however, suggest that further exploration of acute effects of berries on memory performance in young adults is warranted.

Although *in vitro* data demonstrates the MAO inhibitory effects of anthocyanins (Dreiseitel *et al.*, 2009b), data from chapter two of this thesis highlighted that they are unlikely to be the sole compound driving the inhibition of MAO-A and MAO-B. The anthocyanin enriched Delcyan$^{TM}$ treatment elicited no significant effect on either MAO isoform, whereas, the Blackadder juice treatment with the full blackcurrant profile of polyphenols (standardised at 500mg of polyphenols per 60kg of body weight) inhibited both MAO subtypes. In addition to MAO-inhibition, there was also indication of modulation of prolactin following the Blackadder juice extract in the previous chapter. Interpretation of these findings was hindered by poor sample size, however, the effects

warrant further investigation as the hormone prolactin may be regulated by central dopaminergic tone. The relevance of this to behaviour is demonstrated by a plethora of scientific papers and review articles exploring the effects of dopamine on behaviour. For example, the lesioning of dopaminergic terminals or neurons (Viallet *et al.*, 1983; Amalric & Koob, 1987) and administration of $D_2$ dopaminergic receptor antagonists (Amalric *et al.*, 1993; Marrow *et al.*, 1993) decrease movement time and increase reaction times in animal models. The opposite is true after administration of dopamine receptor agonists, with decreased reaction times in animal models (Smith & Kieval, 2000). Reductions in CNS dopamine activity are purported to be associated with deficits in attention in clinical populations, such as, sufferers of attention deficit disorders (Lahoste *et al.*, 1996) and executive dysfunction (Levy *et al.*, 2002). In addition, reductions in motor control (Cooper *et al.*, 1991) and increases in impulsiveness (O'sullivan *et al.*, 2011) in sufferers of Parkinson's disease have been observed.

Oxidative stress occurs when levels of reactive oxygen species are greater than the capacity of the antioxidant defence system (Halliwell, 2008). Studies have shown that maintaining this equilibrium in the brain is of particular importance during ageing (see Floyd & Hensley, 2002 for a full review). Along with oxidative stress, increases in hydrogen peroxide ($H_2O_2$) occur in the brain during ageing, playing a significant role in cell death (Cavazzoni *et al.*, 1999). Although anthocyanins have been shown to be powerful antioxidants *in vitro*, the levels found in plasma are not great enough to cause an antioxidative effect *in vivo*, which could explain null effects found by Jin *et al.,* (2011). It would, therefore, be beneficial to quantify plasma ferric reducing/antioxidant power (FRAP) activity after consumption of the Blackadder blackcurrant extracts. This would not only assess the extracts direct potential to protect against reactive oxygen species and reduce oxidative stress, but additionally, assess whether the reduction in MAO-B activity, which is associated with deamination of amines producing $H_2O_2$

(Pizzinat *et al.*, 1999), could potentially cause an indirect effect upon FRAP. The FRAP assay is a direct measure of the total antioxidant potential of a sample (Benzie & Strain, 1996). FRAP results are only based on the antioxidant reducing potential of the ferric ion, and not the antioxidant preventative effect. In a recent study comparing the antioxidant potential of a guava extract (Thaipong *et al.*, 2006), FRAP analysis was shown to best correlate to polyphenol and ascorbic acid levels when compared to other measures such as 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) antioxidant assays.

In light of the research outlined above, and the effects described in chapter two, which saw the Blackadder juice extract impact attention processes and MAO; the aim of the current study is to ascertain if the positive behavioural and physiological effects of the Blackadder juice extract gathered can be replicated, and if they are dose and time dependent. A dose of the Blackadder juice extract standardised at 500mg of total polyphenols, which was shown to be effective at modulating both psychological and physiological parameters in chapter two, will again be used. Sequentially, reduced doses of the extract standardised at 250mg of total polyphenols and 125mg of total polyphenols will also be assessed. The first cognitive assessment will begin 60 minutes post-dose, the same time point as chapter one. A second cognitive assessment will then be implemented 150 minutes post-dose. One hundred and fifty minutes is a time point where MAO was shown to be inhibited by 96% in chapter two, and a time point where a secondary peak of potentially bioactive metabolites of flavonoid compounds re-emerge in the blood stream (Jin *et al.*, 2011). Secondly, the results from study one will be expanded by exploring memory paradigms shown to be sensitive to flavonoid supplementation in animal models and aged human trials as discussed in this section. Specifically these will include verbal, spatial and working memory, memory span and psychomotor performance. A final aim is to ascertain if supplementation of levels of

flavonoids from a blackcurrant extract, with levels of anthocyanins high enough to be quantified in plasma, can have an effect upon blood plasma FRAP.

## 3.2 Materials and methods

### 3.2.2 Design

The project investigated the acute effects on cognitive function, neuroendocrinology and mood of a single consumption of three blackcurrant drinks with varying levels of berry polyphenols versus a sugar, flavour, volume and appearance matched control. The study followed a double-blind, counterbalanced, placebo controlled, repeated measures design. Participants were randomly allocated to treatment orders as selected through a Williams Latin Square (Williams, 1949).

### 3.2.3 Participants

Forty healthy adults were recruited for the study, of these, 32 completed all study days (14 male, 18 female). Demographical information for the 32 participants who completed all study days can be found in table 3.1

*Table 3.1 Mean participant characteristics*

| Measure | Average measurement | SD | Range |
|---|---|---|---|
| Age (Years) | 21 | 3.61 | 18-35 |
| Height (m) | 1.7 | 0.09 | 1.53-1.89 |
| Mass (kg) | 67.37 | 9.72 | 50.4-90 |
| BMI ($m^2$) | 23.05 | 2.37 | 18-35 |

Participants were recruited using opportunity sampling from Northumbria University. Participants received £80 to recompense them for any expense they may have incurred during participation in the trial. Before potential participants were enrolled in the study, they attended a 90 minute training session. During this training session, participants gave their signed consent to participate in the study and were screened for any contraindications to the study, with the use of an exclusion questionnaire (see appendix I). In brief, all participants reported themselves to be healthy, not pregnant, non-tobacco users. Participants were not using dietary supplements or over the counter or recreational drugs (excluding the contrac eptive pill), did not have any sensitivities to any of the study treatments and had a body mass index below 35kg/$m^2$. Participants then completed three repetitions of the study day tasks to ensure they met

the required minimum standards on cognitive tasks to participate in the study and to reduce the likelihood of practice effects.

The study received ethical approval from the Northumbria University School of Life Sciences Ethics Committee and was conducted according to the Declaration of Helsinki (1964).

### 3.2.4 Treatments

The Blackadder juice which was given to participants in chapter two was again used in this present study. This juice was processed from the same cultivar of berry, but from a different year (one year later) (Blackadder cultivar, cultivated and processed in 2011 by Plant and Food Research Ltd, New Zealand). The crude Blackadder juice extract is a cold pressed crude juiced extract yielding approximately 100ml of juice containing ~500mg of polyphenols per 135g of fresh fruit. The Blackadder cultivar was juiced at Massey University, Palmerston North, New Zealand by the food solution team at PFR. The juice extract was then sent to Assure Quality Ltd, Auckland, New Zealand to be microbiologically tested. The juice extract was declared fit for human consumption.

Participants received four drinks with a one week washout period in between treatments. These drinks contained either; 0mg of polyphenols (control), 125mg, 250mg or 500mg of total polyphenols in the form of a cold pressed blackcurrant juice drink. In each case, drinks were comprised of 3.44g of glucose, 4.63g of fructose, 0.8g of sucrose, 6g of Splenda® and 50ml of an inert synthetic blackcurrant flavouring (blackcurrant flavour cordial, Schweppes, UK). The total volume of the drink was made up to 200ml with water. All quantities discussed are based on a 60kg person, drink quantities were calculated per kilo of body weight. The phytochemical breakdown of study drinks can be found in tables 3.2 and 3.3.

*Table 3.2 Anthocyanins and other phenolic compounds in each of the treatment conditions (mg per 60kg of bodyweight)*

| Treatment | Anthocyanins mg/60kg b.w | Other phenolics mg/60kg b.w | Total phenolics mg/60kg b.w |
|---|---|---|---|
| Control | 0 | 0 | 0 |
| 125mg | 92.96 | 32.03 | 125 |
| 250mg | 184.04 | 65.95 | 250 |
| 500mg | 372.80 | 127.19 | 500 |

*Table 3.3 Anthocyanins and other phenolic compounds in each of the treatment conditions.(mg per kilo of body weight, average dose given (mg) and dose range (mg))*

| **Treatment** | **Anthocyanins (mg/kg)** | **Anthocyanin average dose (mg)** | **Dose Range (mg)** | **Other polyphenols (mg/kg)** | **Other polyphenols average dose (mg)** | **Dose range (mg)** | **total polyphenols (mg/kg)** | **Total polyphenols average dose (mg)** | **Dose range (mg)** |
|---|---|---|---|---|---|---|---|---|---|
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 125mg | 1.54 | 103.74 | 77-138 | 0.53 | 35.71 | 26-47 | 2.08 | 140.1 | 105-187 |
| 250mg | 3.06 | 206.15 | 154-275 | 1.09 | 73.43 | 54-98 | 4.16 | 280.2 | 209-374 |
| 500mg | 6.21 | 418.36 | 312-558 | 2.11 | 142.1 | 106-189 | 8.33 | 561.1 | 419-749 |

### 3.2.5 Cognitive tasks

All cognitive measures and mood scales were delivered using the Computerised Mental Performance Assessment System (COMPASS) described in chapter two. Task stimuli were responded to using a peripheral response box (Cedrus RB-530) as shown below in figure 3.1. In order to assess the robustness of the effects of the Blackadder juice extract upon digit vigilance as outlined in chapter two, the digit vigilance task was combined with several cognitive tasks and repeated in a fashion similar to that of chapter two. Differences in the speed of the stimuli in the digit vigilance task were also investigated to assess whether increases in cognitive demand affect task performance. Other cognitive tasks focused upon paradigms shown to be sensitive to flavonoid supplementation in animal models and aged human trials discussed in section 3.1. Specifically, verbal, spatial and working memory, memory span and psychomotor performance.

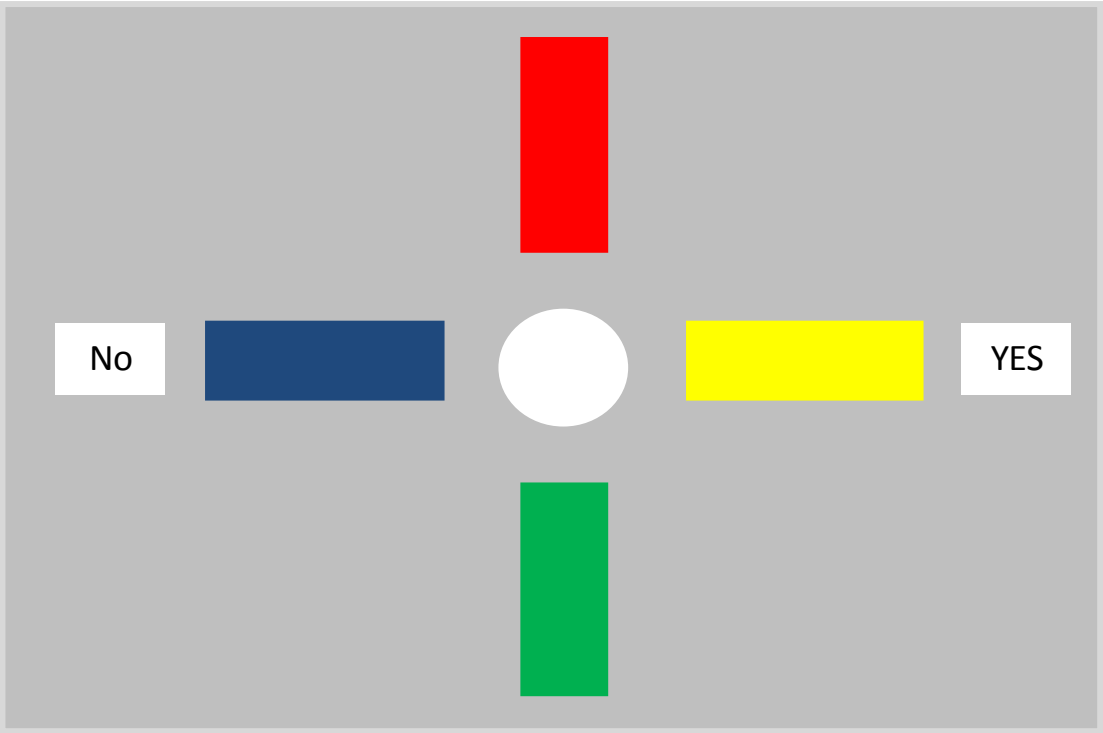



*Figure 3.1 Peripheral response box configuration used by participants to respond to stimuli during computerised cognitive tasks.*

**3.2.5.1 Dual task:** A dual task was used to incite a performance deficit in individuals who otherwise may be performing at levels near to their peak. This creates the opportunity for any facilitation by the intervention drinks to become apparent. Motor movement and verbal learning memory were assessed with the use of the dual task. Participants were played 20 words through a set of headphones whilst tracking an * around the computer screen with an optical mouse. Participants were asked to memorise as many of the words as possible whist keeping the mouse cursor as close to the tracking stimulus as they could. The task lasted for 60 seconds. Participants were advised to complete both tasks to the best of their ability with no advice given to prioritise one task over the other.

**3.2.5.2 Immediate word recall:** The participant was given 60 seconds to write down as many of the words presented during the dual task as possible. The number of words correctly recalled was recorded as a percentage of the total possible.

**3.2.5.3 Digit vigilance slow:** A target digit was randomly selected and constantly displayed to the right of the screen. A series of digits were presented in the left of the screen at the rate of 80/ per minute and the participant was required to press the centre button on the peripheral response box as quickly as possible every time the digit in the series matched the target digit. The task lasted two minutes and there were 30 stimulus-target matches. Task measures were accuracy (%), reaction time (msec) and number of incorrect responses (False Alarms).

**3.2.5.4 Digit vigilance fast:** The task is the same as digit vigilance slow task outlined above and used in chapter two, however, digits were presented in the left of the screen at the rate of 150/ per minute. This increase in the speed of the stimulus increases the cognitive demand of the task, therefore, placing a greater demand upon the attention of the participant. Task measures were accuracy (%), reaction time (msec) and number of incorrect responses.

**3.2.5.5 Corsi blocks:** A computerised adaptation of the Corsi blocks (Miner, 1971) task was used to assess visuo-spatial working memory. Nine blue squares were presented on a black background screen. The blue squares then changed to red and back to blue in a random sequence. Participants were asked to follow the sequence and respond via a mouse click. The task began at level four with four of the nine blue squares lighting up in sequence, and was repeated five times at each level. Task difficulty increased in increments with the number of blue squares lighting up as the levels increased. The task ended if the participant did not correctly recall 3 or more sequences on a level. The task was scored as span score derived from the average of the last 3 correctly completed trials.

**3.2.5.6 Telephone number task:** The telephone number task assesses working memory using an array of digits. The participant was shown a random 9 digit number

on the computer screen for 10 seconds. This number then disappeared from the screen for 5 seconds before a key pad appeared on the screen for the participant to type in the number which was previously presented, using the mouse. There were 10 presentations of numbers. The task was scored as average time to complete a sequence and the number of correct nine digit number entries presented as a percentage of the total possible correct recalls.

**3.2.5.7 Three back:** The N-Back task is one of the most popular working memory tasked used to invoke brain activation in fMRI studies (Owen *et al.*, 2005). The N-back task is a useful tool for assessing inter-individual differences in working memory (Jaeggi *et al.*, 2010). Participants were presented with a series of letters appearing on the computer screen one at a time. Participants were asked to respond using the “yes” or “no” button on the response pad if the letter on the screen was presented 3 letters previously. There were 45 letter presentations with 15 target stimuli. The task was scored as a percentage number of correct responses and overall reaction time (msec).

**3.2.5.8 Simple reaction time*:*** The participant was instructed to press the response button as quickly as possible every time an arrow was presented on the screen. Fifty stimuli were presented with an inter-stimulus interval that varied randomly between 1 and 3.5 seconds. Reaction times were recorded in msec.

**3.2.5.9 Delayed word recall:** The participant was given 60 seconds to write down as many of the words presented during the dual task as possible. The number of words correctly recalled was recorded as a percentage of the total possible.

**3.2.5.10 Word recognition:** The 20 words which were presented during the dual task and 20 unique distracter words were presented on the computer monitor. Words were presented individually and randomly. The participant was asked to respond using the

"yes" button on the computer keyboard if the word had been presented during the dual task and the "no" button if it had not. The word remained on the screen until the participant responded. The number of words correctly recalled was recorded as a percentage of the total possible and incorrect responses were recorded as the error. Overall reaction time was also recorded.

### 3.2.6 Mood

**3.2.6.1 Bond-Lader visual analogue scales**: The scales comprise of a total of sixteen 100mm lines anchored at either end by antonyms (e.g. alert-drowsy, calm-excited, etc.) on which participants mark their current subjective position. Scores from the 16 Bond-Lader visual analogue scales were combined as recommended by the authors to form three mood factors: 'alert', 'calm' and 'content' (Bond & Lader. 1974).

**3.2.6.2 Visual analogue scales**: Participants were asked to subjectively rate how difficult they perceived the tasks and how mentally energised, physically energised and motivated they felt. Visual analogue scales were displayed electronically at the end of every set of tasks. The scale was anchored "Not at all" on the left of the scale and "extremely" on the right.

**3.2.6.3 State anxiety questionnaire** The State-Trait Anxiety Inventory (STAI) is a commonly used measure of trait and state anxiety (Spielberger Cd, 1970). It is a 40 item scale which indicates the intensity of feelings of anxiety, distinguishing between state anxiety and trait anxiety. In the case of the present study, only the state (Y1) section of the inventory was used to assess changes in mood state.

The order of tasks in the baseline and post-dose cognitive assessments can be seen in figure 3.2a and 3.2b.

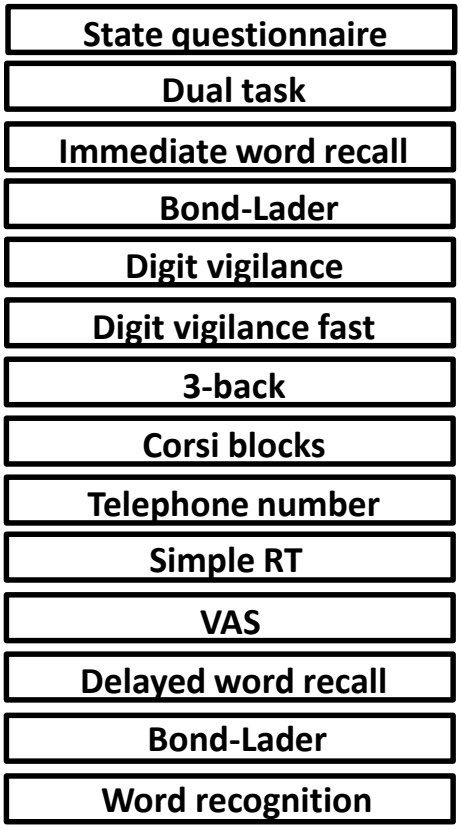



*Figure 3.2a*

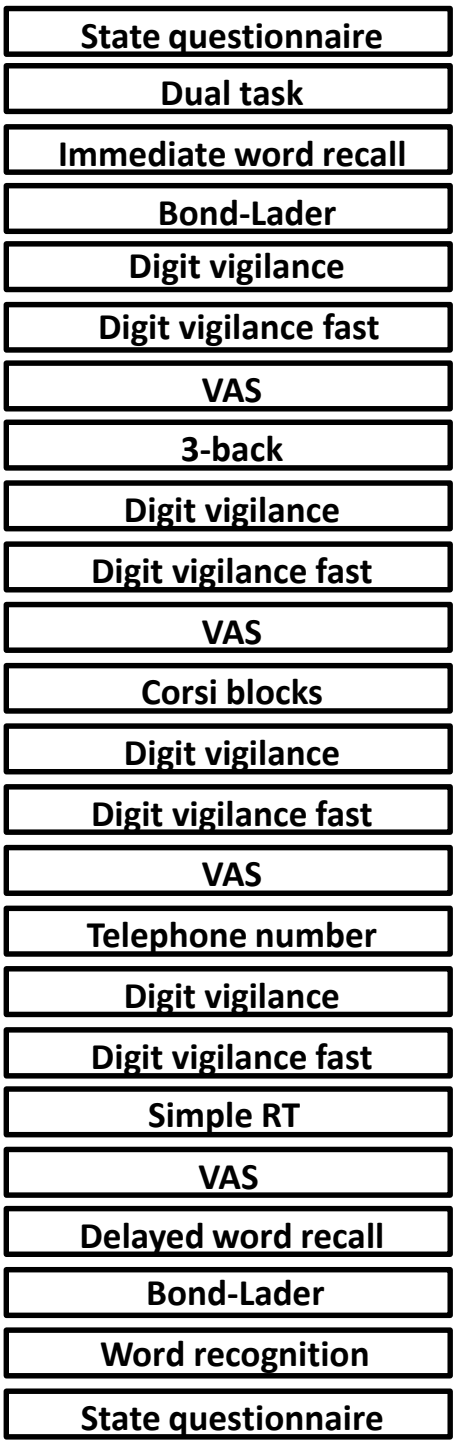



Figure 3.2b

*Figure 3.2 The order of tasks in the baseline cognitive assessment (3.2a) and the post-dose cognitive assessment (3.2b). VAS=Visual analogue scale*

### 3.2.6 Blood analysis

Venous blood samples (2x5ml) were collected at baseline and 120 minutes after supplementation of treatments. Samples were collected in 5ml BD vacutainers© (Becton, Dickinson and company). Both receptacles were treated with anticoagulants, one with lithium heparin (LH) and one with ethylenediaminetetraacetic acid (EDTA).

Whole blood samples treated with LH and EDTA were prepared for storage using the methods described in section 2.2.5 Prepared samples were stored at -80ºC until analysis was performed.

#### 3.2.6.1 Monoamine oxidase-B activity

A subsection of eight participants were analysed for MAO-B activity. Isolated platelet pellets were tested for MAO-B activity using the same methods as described in section 2.2.5.1.

#### 3.2.6.2 Prolactin analysis

A subsection of nine participants were analysed for prolactin analysis. Analysis was conducted as described in section 2.2.5.4.

#### 3.2.6.3 Ferric reducing antioxidant power assay

The ferric reducing/antioxidant power (FRAP) assay is a direct measure of the total antioxidant potential of a sample (Benzie & Strain, 1996). At low pH, ferric iron ($Fe^{3+}$) is converted to its ferrous form ($Fe^{2+}$); this chemical reaction results in the formation of a blue colour to the solution. (Benzie & Strain, 1996). The intensity of this blue colour is proportional to the antioxidant capacity of the sample. The method measures absorbance at 593nm. FRAP results are only based on the antioxidant reducing potential of the ferric ion, and not the antioxidant preventative effect. Full methods can be seen in appendix VI.

### 3.2.7 Procedures

Each participant was required to attend a total of four study days conducted at least 7 days apart, to ensure a sufficient wash out between conditions. Cognitive testing took place in a laboratory with participants visually and auditorally isolated from each other. On arrival at their first session, participants were randomly allocated to a treatment regime using a Latin square design that counterbalanced the order of treatments across the three active days of the study. On all 4 testing days participants arrived at the lab in the morning (8:00am), after a 12 hour overnight fast, and firstly completed one repetition of the baseline COMPASS computerised cognitive assessment (figure 3.2a). Participants then gave a 10mL baseline venous blood sample taken by a trained phlebotomist. They were then orally supplemented with treatment in the form of a single serve juice drink (200ml per 60kg weight) and asked to sit and watch TV or read quietly in a designated waiting room during a 60 minute resting absorption period. Sixty minutes after supplementation, the first post-dose state anxiety questionnaire was completed followed by the first post-dose COMPASS assessment (figure 3.2b). After completion of the assessment (120 minutes post-dose), participants immediately gave a second 10 ml venous blood sample. The blood sample was taken 120 minutes post-dose and not 150 minutes post-dose as in chapter two of this thesis to limit any impact of venepuncture upon the second cognitive assessment. Participants were then given a 30 minute break before completing a final post-dose state anxiety questionnaire and the second post-dose COMPASS assessment. Participants were then free to leave. A diagram of the study visit running order can be seen in figure 3.3.

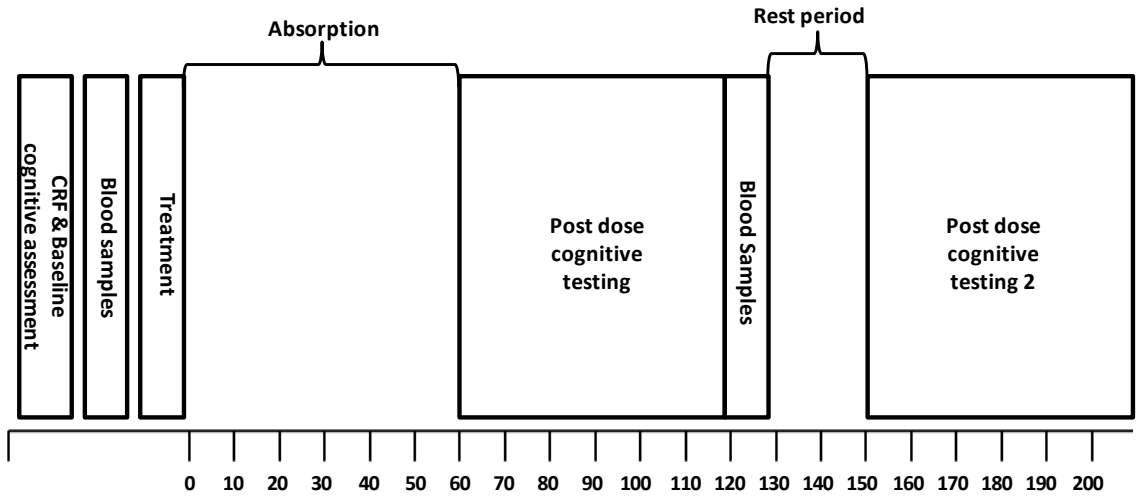



*Figure 3.3 Study day running order (minutes). CRF=Case report form.*

## 3.4 Statistical analysis

Mood, cognitive scores and the physiological measures were analysed as 'change from baseline' using the SPSS statistics package. The null hypothesis was rejected with a p value <0.05. Baseline differences were calculated for all measures using a one way (treatment) ANOVA.

Repeated measures ANOVAs (General linear model) (Treatment [control, 125mg, 250mg and 500mg] × assessment [1 to 2] × completion [1 to 4] for digit vigilance and VAS OR treatment [control, 125mg, 250mg and 500mg] × assessment [1 to 2] for Bond-Lader and all other cognitive assessments conducted). Plasma FRAP levels, platelet MAO-B and prolactin were analysed by one-way (treatment) repeated measures ANOVA. Mauchly`s test of sphericity was used to assess equality of the variances of the differences between factors. Where sphericity had been violated, Huynh-Feldt corrections for non-sphericity were implemented. Post hoc comparisons (pairwise) were used where the initial ANOVA revealed a p value <0.05 to ascertain any differences between treatments for the whole session, at each assessment and at each task repetition. Partial Bonferroni corrections were used to protect for error against multiple comparisons and, therefore, the p value was multiplied by the number

of treatments being compared to control (5 for MAO, PRL and FRAP, 3 for all other outcomes). Adjusted p values are reported.

## 3.5 Results

Prior to analysis of change from baseline data, mean pre-dose scores for all four treatments (control, 125mg, 250mg and 500mg) for each outcome were subjected to a one way repeated measures ANOVA. There were no significant differences found between any variables at baseline.

### 3.5.1 Cognitive performance

Mean pre-dose baseline and change from baseline scores for each behavioural condition and all ANOVA outcomes are presented in tables 3.4a, 34b, 3.5a, 3.6a and 3.6b. Significant differences are presented in figure 3.3. Unchanged “raw” data tables can be found in appendix X. Only ANOVA results for behavioural measures which generated significant effects are reported below.

*Table 3.4a Mean pre-dose baseline and change from baseline scores, standard deviations for the digit vigilance physiological parameter over the two measured assessments*

| Measure | N | Treatment | Baseline | | Session 1 (60 minutes) | | | | | | | | Session 2 (150 minutes) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Repetition 1 | | Repetition 2 | | Repetition 3 | | Repetition 4 | | Repetition 1 | | Repetition 2 | | Repetition 3 | | Repetition 3 | |
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Digit vigilance accuracy (%) | 28 | Control | 97.48 | 2.78 | -0.24 | 3.05 | -0.16 | 5.09 | -3.89 | 8.37 | -4.45 | 8.99 | -4.29 | 11.91 | -2.55 | 8.36 | -1.59 | 4.23 | -2.54 | 5.53 |
| | | 125mg | 97.11 | 3.46 | -0.08 | 4.55 | -2.06 | 4.23 | -4.52 | 7.25 | -3.10 | 6.91 | -2.70 | 6.12 | -5.24 | 8.93 | -5.40 | 7.24 | -6.67 | 10.37 |
| | | 250mg | 97.63 | 2.40 | -1.27 | 4.12 | -3.25 | 6.44 | -2.70 | 5.07 | -5.00 | 7.97 | -1.91 | 4.94 | -3.25 | 6.15 | -4.76 | 8.74 | -3.57 | 7.87 |
| | | 500mg | 96.37 | 5.99 | -0.24 | 7.01 | -2.78 | 7.83 | -5.08 | 7.95 | -2.78 | 5.53 | 0.00 | 7.11 | -1.19 | 9.04 | -3.73 | 8.55 | -2.38 | 7.13 |
| Digit vigilance false alarms (Number) | 28 | Control | 0.03 | 0.18 | 0.00 | 0.27 | -0.04 | 0.19 | -0.04 | 0.19 | -0.04 | 0.19 | -0.04 | 0.19 | -0.04 | 0.19 | -0.04 | 0.19 | 0.07 | 0.38 |
| | | 125mg | 0.00 | 0.00 | 0.04 | 0.19 | 0.07 | 0.38 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 | 0.19 | 0.04 | 0.19 | 0.00 | 0.00 | 0.04 | 0.19 |
| | | 250mg | 0.03 | 0.18 | -0.04 | 0.19 | 0.00 | 0.27 | -0.04 | 0.19 | 0.00 | 0.27 | 0.00 | 0.27 | 0.00 | 0.27 | -0.04 | 0.19 | 0.00 | 0.27 |
| | | 500mg | 0.00 | 0.00 | 0.04 | 0.19 | 0.04 | 0.19 | 0.04 | 0.19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.07 | 0.38 | 0.14 | 0.45 | 0.04 | 0.19 |
| Digit vigilance reaction time (msec) | 28 | Control | 397.2 | 33.65 | 7.67 | 22.01 | 26.52 | 31.20 | 30.16 | 25.66 | 35.97 | 33.23 | 15.18 | 28.17 | 24.07 | 34.77 | 29.13 | 31.86 | 24.87 | 31.05 |
| | | 125mg | 404.9 | 33.11 | 3.23 | 32.94 | 25.05 | 32.13 | 33.94 | 33.17 | 35.55 | 36.84 | 9.25 | 32.37 | 18.91 | 33.29 | 32.43 | 31.99 | 39.32 | 42.77 |
| | | 250mg | 397.2 | 29.80 | 8.04 | 22.46 | 21.39 | 27.26 | 26.51 | 31.35 | 31.83 | 32.85 | 14.58 | 27.93 | 22.08 | 29.42 | 29.62 | 33.61 | 31.03 | 38.21 |
| | | 500mg | 405. 7 | 38.13 | 13.84 | 21.44 | 26.67 | 24.40 | 29.64 | 30.64 | 40.39 | 36.12 | 21.39 | 28.42 | 29.07 | 32.01 | 34.88 | 26.37 | 35.10 | 31.32 |
| Digit vigilance fast accuracy (%) | 29 | Control | 93.19 | 7.14 | 0.23 | 7.26 | -2.22 | 7.70 | -4.14 | 8.81 | -4.21 | 8.59 | 0.31 | 7.02 | 0.54 | 6.99 | -2.99 | 11.75 | -2.68 | 10.05 |
| | | 125mg | 92.69 | 6.72 | -1.53 | 4.57 | -3.30 | 7.02 | -8.05 | 12.03 | -8.97 | 12.76 | -2.68 | 5.89 | -4.06 | 5.82 | -3.99 | 7.69 | -5.52 | 8.20 |
| | | 250mg | 93.84 | 6.51 | -3.14 | 10.47 | -5.90 | 6.27 | -7.66 | 10.83 | -7.66 | 10.93 | -4.67 | 5.61 | -4.67 | 6.99 | -6.36 | 11.54 | -5.75 | 8.56 |
| | | 500mg | 91.83 | 7.96 | -1.61 | 9.14 | -2.99 | 10.59 | -5.36 | 11.13 | -4.67 | 14.17 | -0.31 | 7.10 | -1.99 | 8.64 | -4.60 | 9.12 | -2.45 | 10.17 |
| Digit vigilance fast false alarms (Number) | 29 | Control | 1.71 | 1.99 | 0.00 | 1.73 | 0.07 | 2.45 | 0.41 | 2.13 | 0.62 | 2.23 | 0.07 | 2.39 | 0.17 | 1.83 | 1.10 | 2.64 | 0.86 | 2.50 |
| | | 125mg | 3.19 | 8.78 | 0.00 | 1.79 | -0.24 | 1.77 | 1.10 | 2.35 | 0.34 | 2.06 | 0.62 | 2.19 | 0.45 | 2.47 | -0.03 | 1.92 | 0.10 | 1.97 |
| | | 250mg | 1.84 | 1.93 | -0.03 | 2.35 | 0.17 | 2.21 | 0.62 | 2.44 | 0.34 | 2.04 | -0.17 | 2.04 | 0.31 | 1.81 | 0.69 | 2.65 | 0.48 | 2.59 |
| | | 500mg | 1.71 | 1.60 | -0.28 | 1.85 | 0.41 | 2.16 | 0.69 | 2.12 | 0.28 | 1.94 | -0.24 | 2.15 | 0.28 | 1.94 | 0.69 | 2.42 | 1.07 | 1.98 |
| Digit vigilance fast reaction time (msec) | 29 | Control | 427.6 | 33.61 | 1.77 | 26.36 | 15.36 | 29.37 | 22.84 | 33.88 | 30.47 | 36.78 | -0.68 | 31.53 | 9.77 | 32.53 | 15.87 | 33.48 | 13.55 | 29.45 |
| | | 125mg | 428.2 | 34.52 | 5.10 | 25.34 | 22.90 | 41.06 | 28.09 | 40.88 | 33.31 | 40.21 | 11.16 | 34.57 | 12.38 | 39.16 | 25.66 | 41.63 | 28.99 | 35.26 |
| | | 250mg | 422.7 | 31.95 | 10.16 | 29.80 | 30.37 | 34.64 | 28.03 | 28.01 | 32.08 | 38.91 | 15.09 | 28.57 | 22.09 | 31.35 | 22.95 | 32.13 | 22.46 | 31.78 |
| | | 500mg | 425.0 | 33.97 | 11.61 | 35.98 | 28.02 | 32.33 | 37.27 | 37.97 | 35.82 | 39.01 | 16.80 | 26.23 | 29.44 | 32.72 | 28.75 | 26.26 | 31.72 | 33.88 |

*Table 3.4b Main ANOVA and ANOVA interaction outcomes for the digit vigilance physiological parameter over the two measured assessments*

| Measure | Effect of treatment | Treatment*repetition interaction | Treatment*session interaction |
|---|---|---|---|
| Digit vigilance accuracy (%) | F=1.05 p>0.1 | F=0.40 p>0.1 | F=2.40 p=0.09 |
| Digit vigilance false alarms (Number) | F=1.04 p>0.1 | F=1.84 p>0.1 | F=0.77 p>0.1 |
| Digit vigilance reaction time (msec) | F=0.52 p>0.1 | F=0.67 p>0.1 | F=0.83 p>0.1 |
| Digit vigilance fast accuracy (%) | F=2.84 p=0.043 | F=0.51 p>0.1 | F=0.80 p>0.1 |
| Digit vigilance fast false alarms (Number) | F=0.03 p>0.1 | F=1.08 p>0.1 | F=1.70 p>0.1 |
| Digit vigilance fast reaction time (msec) | F=1.62 p>0.1 | F=1.04 p>0.1 | F=0.75 p>0.1 |

*Table 3.5 Mean pre-dose baseline and change from baseline scores, standard deviations and ANOVA outcomes for cognitive paradigms over both measured epochs*

| Measure | N | Treatment | Baseline | | Session 1 (60 minutes) | | Session 2 (150 minutes) | | Effect of Treatment | Treatment * session interaction |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | | |
| Simple RT | 30 | Control | 293.5 | 36.69 | 21.17 | 58.19 | 30.09 | 113.7 | F=0.90 p>0.1 | F=1.27p>0.1 |
| | | 125mg | 301.8 | 52.58 | 46.15 | 154.0 | 1.77 | 58.18 | | |
| | | 250mg | 282.9 | 32.40 | 50.15 | 122.9 | 61.12 | 147.7 | | |
| | | 500mg | 297.2 | 50.67 | 58.25 | 167.4 | 16.92 | 40.98 | | |
| Corsi blocks span | 30 | Control | 6.61 | 0.61 | -0.19 | 0.66 | -0.18 | 0.74 | F=0.50 p>0.1 | F=0.79 p>0.1 |
| | | 125mg | 6.37 | 0.76 | -0.04 | 0.88 | 0.01 | 0.91 | | |
| | | 250mg | 6.38 | 0.70 | -0.29 | 0.94 | -0.24 | 0.85 | | |
| | | 500mg | 6.29 | 0.76 | -0.07 | 0.99 | -0.21 | 0.92 | | |
| 3-back % correct | 30 | Control | 89.93 | 7.69 | -1.41 | 6.92 | 2.07 | 8.13 | F=0.25 p>0.1 | F=0.92 p>0.1 |
| | | 125mg | 89.19 | 7.78 | -0.22 | 6.61 | 0.52 | 5.91 | | |
| | | 250mg | 90.22 | 7.84 | -0.96 | 7.71 | -0.52 | 7.69 | | |
| | | 500mg | 89.71 | 6.64 | -1.71 | 8.33 | 0.07 | 7.14 | | |
| 3-back missed sequences | 30 | Control | 0.43 | 0.94 | 0.13 | 0.57 | -0.03 | 0.56 | F=0.67 p>0.1 | F=0.40 p>0.1 |
| | | 125mg | 0.70 | 1.58 | -0.23 | 1.17 | -0.20 | 1.42 | | |
| | | 250mg | 0.50 | 1.04 | 0.10 | 0.66 | 0.00 | 0.53 | | |
| | | 500mg | 0.53 | 1.20 | 0.30 | 2.42 | -0.03 | 1.13 | | |
| 3-back reaction time | 30 | Control | 652.7 | 189.1 | -31.12 | 119.9 | -57.41 | 126.8 | F=0.40 p>0.1 | F=0.28 p>0.1 |
| | | 125mg | 612.7 | 195.7 | -16.07 | 82.90 | -30.29 | 98.08 | | |
| | | 250mg | 642.7 | 193.3 | -56.07 | 90.78 | -29.14 | 92.78 | | |
| | | 500mg | 630.3 | 177.7 | -35.42 | 111.9 | -39.49 | 92.81 | | |
| Telephone % correct | 31 | Control | 38.33 | 26.25 | 6.67 | 26.82 | 12.50 | 22.50 | F2.09 p>0.1 | F=0.21 p>0.1 |
| | | 125mg | 42.92 | 27.01 | 2.08 | 24.80 | 4.17 | 22.58 | | |
| | | 250mg | 42.50 | 27.97 | 0.00 | 28.99 | 0.00 | 27.27 | | |
| | | 500mg | 42.92 | 23.37 | -0.42 | 24.45 | -6.67 | 19.35 | | |
| Telephone reaction time | 32 | Control | 6397 | 1087 | -74.99 | 1061 | -394.4 | 998.6 | F=1.30 p>0.1 | F=0.04 p>0.1 |
| | | 125mg | 6471 | 1484 | -183.5 | 1046 | -504.4 | 1133 | | |
| | | 250mg | 6434 | 1177 | -52.84 | 1194 | -419.2 | 698.1 | | |
| | | 500mg | 6776 | 1165 | -451.2 | 882.1 | -728.3 | 674.7 | | |
| Tracking accuracy (twips) | 30 | Control | 4110 | 408.2 | -412.6 | 210.7 | -31.43 | 269.7 | F=1.43 p>0.1 | F=0.46 p>0.1 |
| | | 125mg | 4218 | 421.1 | -304.4 | 340.1 | -9.38 | 223.5 | | |
| | | 250mg | 4169 | 425.1 | -351.0 | 216.3 | -62.57 | 216.7 | | |
| | | 500mg | 4209 | 448.2 | -337.9 | 335.1 | 5.05 | 245.4 | | |
| Word recognition % correct | 30 | Control | 74.41 | 11.17 | -7.25 | 11.95 | -10.17 | 11.39 | F=0.65 p>0.1 | F=1.17 p>0.1 |
| | | 125mg | 74.42 | 8.35 | -4.08 | 11.27 | -6.17 | 9.49 | | |
| | | 250mg | 72.92 | 9.89 | -4.25 | 15.24 | -3.83 | 16.48 | | |
| | | 500mg | 73.58 | 8.63 | -3.67 | 9.23 | -9.50 | 10.31 | | |
| Word recognition reaction time | 30 | Control | 1.31 | 0.205 | 0.00 | 0.18 | -2.57 | 0.28 | F=0.83 p>0.1 | F=1.06 p>0.1 |
| | | 125mg | 1.30 | 0.14 | 0.01 | 0.22 | -2.57 | 0.25 | | |
| | | 250mg | 1.29 | 0.16 | 0.05 | 0.27 | -2.52 | 0.33 | | |
| | | 500mg | 1.35 | 0.19 | -0.03 | 0.14 | -2.60 | 0.24 | | |
| Immediate word recall % correct | 32 | Control | 37.73 | 14.10 | -4.30 | 12.89 | -3.13 | 13.91 | F=0.68 p>0.1 | F=1.89 p>0.1 |
| | | 125mg | 40.78 | 12.69 | -1.33 | 11.36 | -8.20 | 11.66 | | |
| | | 250mg | 36.41 | 9.69 | -2.97 | 9.01 | -5.23 | 12.29 | | |
| | | 500mg | 35.47 | 12.02 | -0.23 | 11.51 | -2.73 | 12.37 | | |
| Immediate word recall error | 32 | Control | 0.69 | 0.93 | 0.20 | 1.20 | 0.28 | 1.44 | F=1.36 p>0.1 | F=1.06 p>0.1 |
| | | 125mg | 0.56 | 0.716 | 0.28 | 0.92 | 0.25 | 1.19 | | |
| | | 250mg | 0.53 | 0.718 | 0.16 | 0.92 | 0.59 | 1.74 | | |
| | | 500mg | 0.91 | 1.03 | -0.34 | 0.97 | 0.03 | 1.38 | | |
| Delayed word recall % correct | 32 | Control | 28.05 | 14.71 | -11.17 | 12.31 | -11.33 | 14.23 | F=1.22 p>0.1 | F=1.07 p>0.1 |
| | | 125mg | 28.82 | 12.08 | -6.56 | 12.73 | -11.88 | 9.88 | | |
| | | 250mg | 28.05 | 9.22 | -11.17 | 9.22 | -14.06 | 14.41 | | |
| | | 500mg | 26.56 | 12.45 | -7.42 | 12.39 | -8.52 | 13.81 | | |
| Delayed word recall error | 32 | Control | 0.687 | 0.78 | 1.05 | 1.50 | 0.88 | 1.26 | F=1.83 p>0.1 | F=1.12 p>0.1 |
| | | 125mg | 0.875 | 1.40 | 0.63 | 1.76 | 0.47 | 1.39 | | |
| | | 250mg | 0.625 | 0.71 | 0.72 | 1.08 | 1.44 | 1.72 | | |
| | | 500mg | 1.00 | 1.41 | 0.31 | 1.71 | 0.50 | 1.65 | | |

*Table 3.6a Mean pre-dose baseline and change from baseline scores, standard deviations for each mood parameter over both measured assessments*

| Measure | N | Treatment | Baseline | | Session 1 (60 minutes) | | | | | | | | Session 2 (150 minutes) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Repetition 1 | | Repetition 2 | | Repetition 3 | | Repetition 4 | | Repetition 1 | | Repetition 2 | | Repetition 3 | | Repetition 4 | |
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| VAS mentally energised | 32 | Control | 45.06 | 11.93 | 0.03 | 12.52 | -3.84 | 12.06 | -6.47 | 12.86 | -6.34 | 12.75 | 2.75 | 10.36 | -3.28 | 13.11 | -3.09 | 13.81 | -4.69 | 12.13 |
| | | 125mg | 46.91 | 11.47 | 1.84 | 13.42 | -5.31 | 17.29 | -3.75 | 17.12 | -7.63 | 16.48 | 2.44 | 11.32 | -1.09 | 13.65 | 0.09 | 14.10 | -1.03 | 15.47 |
| | | 250mg | 46.13 | 15.55 | 4.75 | 13.04 | -0.84 | 14.65 | -1.03 | 11.72 | -1.59 | 11.66 | 2.47 | 12.31 | -2.25 | 13.51 | -1.97 | 12.42 | -2.72 | 10.29 |
| | | 500mg | 44.63 | 12.47 | -1.44 | 13.84 | -6.81 | 14.81 | -7.25 | 16.07 | -7.88 | 15.68 | 0.47 | 12.09 | -1.34 | 15.09 | -1.47 | 13.57 | -2.16 | 16.75 |
| VAS motivation | 32 | Control | 47.88 | 14.66 | -0.72 | 16.17 | -4.63 | 13.47 | -6.72 | 16.59 | -9.03 | 15.85 | -0.16 | 14.64 | -3.75 | 16.51 | -7.13 | 14.76 | -4.94 | 17.44 |
| | | 125mg | 49.78 | 10.25 | 0.87 | 17.40 | -4.00 | 13.28 | -6.34 | 19.04 | -6.03 | 18.85 | 1.53 | 15.91 | -4.19 | 18.81 | -3.75 | 18.60 | -2.16 | 20.08 |
| | | 250mg | 47.56 | 15.96 | 1.69 | 12.45 | -3.13 | 13.44 | -6.88 | 11.02 | -8.22 | 11.68 | -2.47 | 14.49 | -6.81 | 15.00 | -9.47 | 13.53 | -8.41 | 14.47 |
| | | 500mg | 49.50 | 12.56 | -1.88 | 12.40 | -5.44 | 12.44 | -5.28 | 11.51 | -7.22 | 12.68 | 0.16 | 12.11 | -0.47 | 13.04 | -1.63 | 14.22 | -1.81 | 14.25 |
| VAS physically energised | 32 | Control | 48.97 | 13.05 | -0.59 | 11.44 | -3.78 | 12.70 | -7.19 | 13.24 | -7.13 | 13.34 | 1.03 | 11.10 | -2.94 | 12.50 | -3.69 | 12.29 | -4.13 | 13.41 |
| | | 125mg | 49.31 | 11.03 | 1.25 | 11.39 | -3.66 | 12.61 | -3.09 | 15.33 | -5.56 | 13.77 | 1.41 | 11.72 | -1.56 | 11.77 | 0.16 | 15.20 | 1.34 | 16.89 |
| | | 250mg | 45.47 | 13.35 | 1.44 | 14.97 | -0.38 | 12.36 | -1.16 | 15.38 | -4.22 | 14.14 | -0.34 | 11.24 | -2.09 | 12.83 | -2.63 | 14.52 | -5.00 | 12.70 |
| | | 500mg | 46.81 | 11.80 | -1.59 | 8.84 | -6.72 | 12.61 | -5.75 | 12.35 | -7.69 | 13.13 | -1.44 | 9.98 | -1.00 | 10.90 | -0.66 | 13.29 | -3.69 | 11.87 |
| STA-I | 32 | Control | 31.31 | 1.32 | -0.13 | 0.47 | 2.09 | 0.85 | 0.06 | 0.72 | 2.09 | 1.00 | | | | | | | | |
| | | 125mg | 32.10 | 1.23 | -0.10 | 0.77 | 1.52 | 0.70 | -0.07 | 0.84 | 0.80 | 0.93 | | | | | | | | |
| | | 250mg | 31.53 | 1.13 | -0.53 | 0.74 | 1.66 | 0.90 | 0.00 | 0.97 | 1.09 | 1.15 | | | | | | | | |
| | | 500mg | 31.31 | 1.32 | -1.27 | 0.59 | 1.97 | 0.87 | -0.55 | 0.73 | .900 | 1.06 | | | | | | | | |
| Bond-Lader calm | 32 | Control | 62.84 | 11.71 | -4.95 | 9.26 | -3.91 | 9.08 | -2.86 | 10.24 | -4.25 | 13.70 | | | | | | | | |
| | | 125mg | 62.97 | 11.44 | -1.47 | 8.73 | -0.55 | 8.73 | -1.48 | 11.08 | -3.39 | 7.98 | | | | | | | | |
| | | 250mg | 64.23 | 10.80 | -5.89 | 7.45 | -2.89 | 9.16 | -4.75 | 10.55 | -2.89 | 9.76 | | | | | | | | |
| | | 500mg | 63.34 | 11.69 | -1.39 | 9.47 | -0.67 | 9.01 | -1.61 | 11.04 | -3.02 | 9.41 | | | | | | | | |
| Bond-Lader content | 32 | Control | 61.99 | 13.51 | -0.73 | 6.37 | -3.86 | 8.75 | -0.03 | 7.72 | -0.84 | 7.72 | | | | | | | | |
| | | 125mg | 62.41 | 12.73 | 0.20 | 7.36 | -1.75 | 8.42 | 1.08 | 10.44 | 1.11 | 9.68 | | | | | | | | |
| | | 250mg | 63.54 | 12.73 | -1.99 | 6.16 | -3.04 | 5.96 | -1.84 | 7.24 | -2.37 | 7.47 | | | | | | | | |
| | | 500mg | 63.15 | 11.37 | 1.09 | 7.45 | -1.48 | 7.67 | 0.82 | 6.73 | -0.78 | 7.97 | | | | | | | | |
| Bond-Lader alert | 32 | Control | 52.89 | 13.57 | 1.73 | 8.98 | -6.92 | 13.61 | 0.49 | 9.81 | -4.53 | 10.36 | | | | | | | | |
| | | 125mg | 55.73 | 12.32 | 1.63 | 9.82 | -7.94 | 14.07 | -0.06 | 12.60 | -3.16 | 13.81 | | | | | | | | |
| | | 250mg | 53.53 | 14.71 | 1.11 | 9.21 | -6.96 | 9.43 | -1.29 | 8.76 | -8.62 | 11.02 | | | | | | | | |
| | | 500mg | 51.63 | 11.24 | 1.03 | 9.10 | -6.93 | 12.65 | 0.85 | 8.67 | -1.57 | 12.98 | | | | | | | | |

*Table 3.6b Main ANOVA and ANOVA interaction outcomes for each mood parameter over both measured treatments*

| Measure | Effect of treatment | Treatment*repetition interaction | Treatment*session interaction |
|---|---|---|---|
| VAS mentally energised | F=0.61 p>0.1 | F=1.43 p>0.1 | F=1.57 p>0.05 |
| VAS motivation | F=0.51 p>0.1 | F=1.34 p>0.1 | F=0.29 p>0.05 |
| VAS physically energised | F=0.69 p>0.1 | F=1.29 p>0.1 | F=1.573 p>0.05 |
| Bond-Lader calm | F=1.24 p>0.1 | F=1.56 p>0.1 | F=0.92 p>0.05 |
| Bond-Lader content | F=1.10 p>0.1 | F=0.87 p>0.1 | F=0.81 p>0.05 |
| Bond-Lader alert | F=0.50 p>0.1 | F=0.80 p>0.1 | F=1.38 p>0.05 |
| STAI | F=0.252 p>0.1 | | |

### 3.5.1.1 Digit vigilance fast

The repeated measures ANOVA revealed an overall treatment effect on digit vigilance accuracy [F (3,28)=2.843, p=0.04]. Post hoc comparisons revealed a significant reduction (p=0.04) in accuracy after supplementation of the 250mg treatment when compared to control. A graphical representation can be seen in figure 3.4.

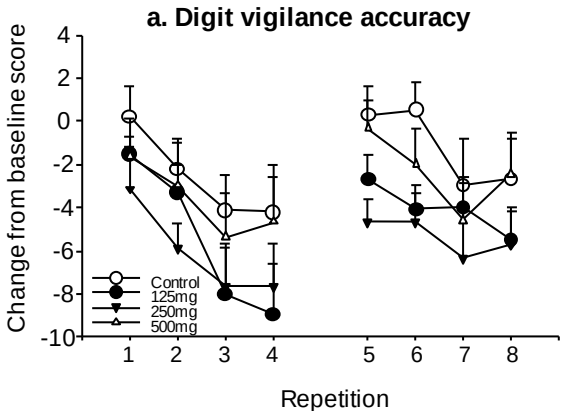



*Figure 3.4. Change from baseline scores for digit vigilance accuracy during cognitive testing sessions 1 (1-4) and 2 (5-8). There was a main effect of treatment, therefore no individual repetitions are highlighted.*

### 3.5.2 Physiological parameters

Mean pre-dose baseline and change from baseline scores for each physiological condition are presented in table 3.5 and significant differences are presented in figure 3.4. Unchanged "raw" data tables can be found in appendix X. Only ANOVA results for physiological measures which generated significant effects, are reported below.

*Table 3.5 Mean pre-dose baseline and change from baseline scores, standard deviations and main ANOVA outcomes for each physiological parameter over both measured epochs*

| Measure | N | Treatment | Baseline | | Post Dose | | Effect of treatment |
|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | |
| Platelet MAO-B | 8 | Control | 2517 | 1556 | -532.1 | 856.1 | F=5.63 p=0.005 |
| | | 125mg | 2391 | 1600 | -1323 | 1219 | |
| | | 250mg | 2366 | 1897 | -1502 | 905.4 | |
| | | 500mg | 2276 | 1319 | -1369 | 1053 | |
| FRAP | 10 | Control | 859.9 | 110.7 | 72.98 | 73.78 | F=0.91 p>0.1 |
| | | 125mg | 917.7 | 113.1 | -13.17 | 26.45 | |
| | | 250mg | 843.5 | 124.9 | -20.84 | 23.50 | |
| | | 500mg | 873.2 | 105.7 | 3.48 | 18.68 | |
| Prolactin | 9 | Control | 340.4 | 187.1 | -123.7 | 161.5 | F=7.93 p=0.003 |
| | | 125mg | 367.4 | 158.7 | -179.7 | 108.3 | |
| | | 250mg | 325.9 | 124.2 | -147.9 | 95.82 | |
| | | 500mg | 357.1 | 116.3 | -197.8 | 91.91 | |

### 3.5.2.1 MAO-B

There was a significant main effect of treatment on blood platelet MAO-B activity [F(1.3,10.8)=8.93, p=0.009]. Post hoc comparisons showed significantly reduced MAO-B activity after supplementation of the 250mg (p=0.008) and 500mg (p=0.02) study treatments and a trend reduction after supplementation of the 125mg (p=0.094) study treatment compared to control. Blackcurrant treatments were not significantly different to each other. See figure 3.4a.

### 3.5.2.2 Prolactin

The repeated measures ANOVA revealed an overall treatment effect [F (2.52,17.64)=7.29, p=0.003]. After corrections for multiple comparisons, post hoc comparisons revealed a significant reduction in blood plasma prolactin levels after supplementation of the 500mg (p=0.006) and the 125mg treatment (p=0.03) when compared to control. See figure 3.5b.

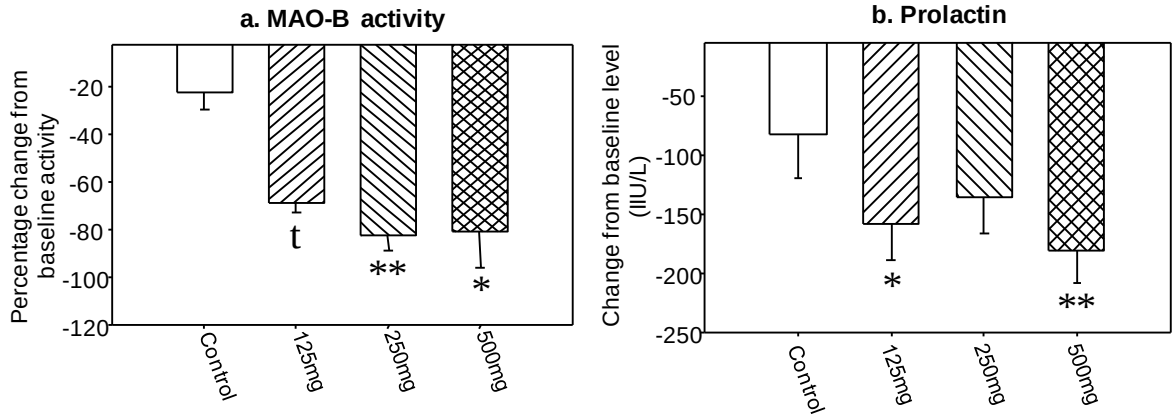



*Figure 3.5 Change from baseline scores for platelet MAO-B activity (a) and plasma prolactin levels (b) 120 minutes post supplementation.*

### 3.6 Discussion and conclusion.

The current study assessed the effect of the three Blackadder treatment interventions upon several different aspects of memory, which have been shown to be sensitive to nutritional interventions in both human and animal models after supplementation of berry extracts. Retrieval memory, working memory, visuo-spatial working memory and memory span, as well as verbal learning memory were assessed 1 hour and 2.5 hours post-supplementation. None of these were significantly affected by any intervention dose at either post-dose cognitive assessment. From the array of cognitive tasks utilised in the present study, spatial and retrieval memory have previously been shown to be impacted in humans by flavonoid-rich extracts, albeit not in blackcurrant form (Pipingas *et al.*, 2008; Ryan *et al.*, 2008; Krikorian *et al.*, 2010a; 2010b). Other memory outcomes assessed had previously proven sensitive to interventions in animal models (Casadesus *et al.*, 2004; Ramirez *et al.*, 2005; Wang *et al.*, 2006; Van Praag *et al.*, 2007; Williams *et al.*, 2008). This literature, both in humans and animal models, surrounding the modulation of memory after supplementation of berry constituents is, however, focused upon aged animal models and humans; with all results pertaining to modulations of cognitive behaviour after chronic supplementation. It is, therefore, hypothesised that the lack of behavioural effects could be associated with the age of the cohort used in the present study. It is probable that these healthy adult participants were already performing near to their peak (Salthouse, 2009). However, the improvement in digit vigilance response times found in chapter two, one hour post supplementation of the Blackadder juice, was not replicated in the present study. In fact, a reduction in accuracy was noted on the fast version of this task following 250mg. It is important to note that there are differences between the paradigms used in studies one and two of this thesis with different tasks preceding each repetition of the digit vigilance task; however, the lack of improvements in the current study suggests that the effects of blackcurrants in this population may be subtle or non-existent. It is possible that these effects are only seen under demanding conditions, outlining the

need for a longer and more demanding cognitive battery or an older study cohort before any positive modulations are exhibited.

The reductions in platelet MAO-B activity reported in chapter two were replicated in the present study. All blackcurrant drinks reduced MAO-B activity compared to placebo with 250mg and 500mg treatments inhibiting by ~ 80% and the 125mg treatment reducing activity by 68%, with the control showing a reduction of 22%. The lack of a clear dose response is most likely due to the small differences in doses used but there is potential that this dose response is affected by the type of inhibition. Although the measurement of inhibition type is out of the remit of the thesis, data outlined by (Dreiseitel *et al.*, 2009a) suggest that anthocyanins inhibit the MAO enzyme competitively, where the inhibiting compound acts as the enzyme substrate and binds directly to the site of action, allowing endogenous ligands to displace the active inhibitor competitively. If this was the case, it would be expected that the dose response in the present study would be greater. Instead, it is theorised that the inhibition is non-competitive, where, rather than binding to MAO enzyme at the site of action, the inhibiting compound instead changes the shape of the enzyme so it is only influenced by the concentration of the inhibiting compound and not concentrations of substrates. This is a factor which requires more attention to establish an $IC_{50}$ of the blackcurrant extract. In chapter two, the inhibition of platelet MAO-B was ˜96% at 150 minutes post supplementation, potentially outlining that the full effects of the treatment on MAO are not being seen at the 120 minute time point used in the current study.

To further investigate non-significant findings upon prolactin after consumption of the Blackadder blackcurrant extract in study one, plasma prolactin was again measured, this time in a larger cohort utilising a within subjects design. Prolactin was reduced after supplementation of all of the blackcurrant drinks, but only reaching statistical significance after the 125mg (48%) and 500mg (55.5%) blackcurrant drinks. The

reason for the inability of the 250mg treatment to significantly reduce prolactin is unknown; nevertheless, there was a non-significant (45.2%) reduction from baseline blood plasma level following 250mg, a reduction of 10% when compared to control. The 55.5% reduction following the 500mg drink is a 20% reduction when compared to placebo. As the predominant control of prolactin secretion into plasma is via hypothalamic inhibition of lactotroph activity (Ben-Jonathan & Hnasko, 2001) and the most important hypothalamic prolactin inhibiting factor is dopamine, this reduction in prolactin possibly indicates an increase in central dopaminergic tone after the supplementation of the blackcurrant drinks. Therefore, potentially outlining a therapeutic impact upon symptoms of neuro-degenerative disease. In order to indirectly measure the dose effects of the Blackadder juice extract upon MAO-A, it would have been beneficial to assess the effects of different doses of the study treatments upon plasma DHPG which was shown to be increased in chapter two of this thesis. This was, however, out of the remit of the current study.

Results from the current study showed that there were no significant reductions in plasma FRAP after any dose of the Blackadder blackcurrant extract 120 minutes post supplementation. The purpose of measuring the plasma FRAP activity in the present study was not only to assess the juice's direct potential to protect against reactive oxygen species and reduce oxidative stress, but to assess if the reductions in MAO-B activity, which are associated with deamination of amines producing $H_2O_2$ (Pizzinat *et al.*, 1999), could cause an indirect effect upon FRAP. Results reported by Jin *et al*., (2011) showed no effects of a 20% blackcurrant upon blood plasma FRAP. However, they used a much lower dose of blackcurrant and found no major berry constituents in blood plasma, whereas, anthocyanins were shown to be absorbed and measurable in plasma after consumption of the juice treatment in chapter two of this thesis. No effect upon FRAP has also been reported after consumption of a 500ml blueberry extract (Pedersen *et al.*, 2000).

In conclusion, the findings of the present study do not suggest that supplementation of the blackcurrant juice acutely modulates measured memory or attention paradigms 1 or 2.5 hours post-supplementation. Platelet MAO-B was, however, strongly inhibited in a dose responsive manner along with significant reductions in peripheral prolactin levels. The findings outline the need for a full time course of MAO inhibition to be established. Further to this, in line with human and animal data published in the literature, the impact of the blackcurrant extracts on an older population and outcomes pertaining to the attenuation of natural cognitive decline should be investigated.

# CHAPTER 4. THE EFFECT OF THE BLACKADDER BLACKCURRANT JUICE EXTRACT UPON PRE-FRONTAL CORTICAL HAEMODYNAMICS IN HEALTHY ADULTS

## 4.1 Introduction

During natural ageing cerebral blood flow reduces by up to 0.5% per year (Leenders *et al.*, 1990). Preservation of blood flow, including the maintenance of endothelial function and large artery elasticity, are inversely associated with the incidence of cardiovascular disease (CVD) (Targonski *et al.*, 2003) and vascular dementia (Dede *et al.*, 2007). Furthermore, epidemiological observations demonstrate that diets high in phytochemicals such as anthocyanins can significantly reduce incidence of CVD risk factors, dementia and stroke (Curin *et al.*, 2006; He *et al.*, 2006). There is a growing body of human nutritional intervention studies demonstrating the ability of fruit polyphenols to modulate haemodynamics in the brain (Francis *et al.*, 2006; Kennedy *et al.*, 2010; Krikorian *et al.*, 2012; Wightman *et al.*, 2012) and in the periphery (Matsumoto *et al.*, 2005a; Matsumoto *et al.*, 2005b). Regulation of blood flow, especially within the human brain, is complex, with multiple overlapping regulatory paradigms and key structural components, such as, cerebral pressure auto-regulation, flow metabolism coupling and neurogenic regulation (Peterson *et al.*, 2011). Recent human intervention studies focusing upon cerebral blood flow and cognitive activities postulate increases or decreases in blood flow to levels of nitric oxide (Kennedy *et al.*, 2010; Wightman *et al.*, 2012). As discussed in section 1.4.2, berry constituents have been shown to up-regulate eNOS (Andriambeloson *et al.*, 1998) and inhibit iNOS (Chen *et al.*, 2001), however, there is no data published detailing their effects upon neuronal nitric oxide synthase (nNOS), manipulation of which, is essential if changes in cerebral flow post-intervention are to been seen (Santizo *et al.*, 2000; Kitaura *et al.*, 2007). All published human intervention studies pertaining to blackcurrants and blood flow have focused upon peripheral blood flow modulation with varying outcomes being

reported. For example, significant increases in skin blood circulation have been observed after supplementing eight females with 140mg of blackcurrant polyphenols, containing 50mg of anthocyanins (Matsumoto *et al.*, 2005a). The same author also found that supplementing 17mg/kg of body weight of blackcurrant polyphenols increased peripheral blood flow and reduced shoulder stiffness during typing (Matsumoto *et al.*, 2005b). In contrast, no effect was found using flow mediated dilation on peripheral blood flow after supplementation of a 20% blackcurrant drink (Jin *et al.*, 2011).

Near Infrared Spectroscopy (NIRS) is a neuro-imaging tool which is used both clinically (Weber *et al.*, 2007) and in nutritional intervention studies (Kennedy *et al.*, 2010; Kennedy & Haskell, 2011) to assess the oxygen status of superficial cortical blood flow of human subjects at various stages of life. NIRS has been shown to be sensitive to age (Safonova *et al.*, 2004; Herrmann *et al.*, 2006) and pharmacological interventions (Bönöczk *et al.*, 2002; Brassard *et al.*, 2010; Watanabe *et al.*, 2011). In terms of intervention studies investigating the effects of phytochemicals on central blood flow using NIRS, Kennedy *et al.,* (2010), showed that supplementation of trans-resveratrol increased cerebral blood flow during cognitive demand in a dose-dependent manner. The intervention did not affect cognitive performance. A second intervention study assessed the impact of the tea polyphenol EGCG on cerebral haemodynamics in healthy young adults. The intervention caused a reduction in total cortical haemoglobin presence during cognitive tasks with no differences in hemispheric activity or effect of task cognitive difficulty. The authors attribute this to vasoconstriction properties potentially attributed to inhibition of nNOS. There were also no effects of the intervention on cognitive tasks. EGCG has also been associated with a significant overall increase in alpha, beta and theta activity, as measured by electroencephalogram, again, with no effect upon cognition. Reductions in self-related stress and increases in calmness have, however, been reported (Scholey *et al.*, 2012).

Although NIRS studies are limited, modulations of central blood flow after the administration of a nutritional intervention can be seen using other neuroimaging methods such as fMRI. Supplementation of 444ml of concord grape juice per day for 16 weeks has been shown to increase blood flow in the right anterior and posterior cortical regions in aged adults (65 and over) with mild cognitive decline during performance of the n-back working memory task (Krikorian *et al.*, 2012). Cocoa has also been shown to increase blood flow to grey matter two hours after the consumption of a flavan-3-ol rich cocoa drink containing 516mg of flavan-3-ols when compared to a control (Francis *et al.*, 2006). The same authors also reported that the short term chronic supplementation (5 days) of healthy adults with a cocoa drink, containing 172mg of flavan-3-ols, increased bold signal intensity during a cognitively demanding letter pair switching task, 90 minutes post consumption of the intervention drink, when compared to control (Francis *et al.*, 2006). Other nutritional interventions have highlighted that non-polyphenol compounds can also modulate haemodynamics. For example, Kennedy and Haskell (2011) showed that supplementation of 75mg of caffeine led to a decrease in total levels of haemoglobin and, therefore, a decrease in cerebral blood flow in the pre-frontal cortex. These effects are driven by interactions of caffeine with adenosine A2 receptors, which are widely distributed throughout the brain (Laurienti *et al.*, 2003). Watanabe *et al.,* (2002) demonstrated that supplementation of 8g of creatine for five days modulated task evoked increases in oxyhaemoglobin and decreases in deoxyhaemoglobin during cognitive tasks in healthy volunteers. Further to this, Jackson *et al.,* (2012) demonstrated a dose-related increase in total haemoglobin and oxyhaemoglobin during frontal cortex task performance after supplementing healthy adults with docosahexaenoic acid (DHA) rich omega 3 fish oils.

Supplementary oxygen (Moss *et al.*, 1998) and glucose (Kennedy & Scholey, 2000) have been shown to positively impact cognitive performance in healthy adults. Therefore, on the most basic level, increases in cerebral blood flow could potentially be

beneficial to acute cognitive performance via increasing the transport of blood borne metabolic substrates to the brain and increasing the transportation of waste products from the brain, which is necessary for natural brain function (Attwell *et al.*, 2010b). In practice, intervention studies suggest that manipulating cerebral haemodynamics, both increasing and decreasing, has a diminutive effect upon acute cognitive performance in a healthy young population over a wide range of cognitive paradigms (Kennedy *et al.*, 2010; Wightman *et al.*, 2012). However, chapter two of this thesis outlines a potential impact of blackcurrants upon cognitive performance in healthy young adults, particularly after sustained mental performance, a factor which has not been shown after supplementation with resveratrol or EGCG. Furthermore, neurotransmitter tone, which plays a key role in vasodilatation and the regulation of cerebral blood flow (Attwell *et al.*, 2010a), was seen to be affected after supplementation with the Blackadder juice in study one. A nutritional intervention, such as the 500mg dose of the Blackadder juice extract used in chapters two and three of this thesis, which is rich in flavonoids, but also has the potential to modulate monoaminergic tone could, therefore, impact cerebral blood flow on two levels, regulating endothelial-dependent nitric oxide and via a direct effect upon neurotransmitters.

In light of modulatory effects of nutritional interventions upon cerebral haemodynamics published in the literature (Kennedy *et al.*, 2010; Kennedy & Haskell, 2011; Wightman *et al.*, 2012), the current study will, for the first time, assess the impact of blackcurrant juice upon pre-frontal cortical haemodynamics using NIRS. The study will focus upon shallow pre-frontal cortical haemodynamics under cognitive demand, and at rest, utilising cognitive paradigms focusing upon central executive tasks and attention processing which have been shown to elicit an intervention-dependent cerebral blood flow response in the frontal cortex (Kennedy *et al.*, 2010; Kennedy & Haskell, 2011; Wightman *et al.*, 2012). As the timescale of any haemodynamic effects of blackcurrant consumption are not known, the study will observe haemodynamics during the resting

absorption period directly after consumption of the study intervention and throughout cognitive task performance, which will be completed at the same time point as in chapter two, 60 minutes post-dose, when anthocyanins are known to be present in blood plasma.

The primary aim of the study was, therefore, to assess if there was an impact of the Blackadder blackcurrant extract upon pre-frontal cortical haemodynamics in healthy young adults, during rest and during cognitive demand.

## 4.2 Materials and methods

### 4.2.1 Design

The project investigated the acute effects of a single serving of the Blackadder juice drink standardised to contain 500mg of polyphenols per 60kg of body weight versus a control upon measures of NIRS. Drinks were matched for volume, taste, appearance and sugars but differed in the amount of total polyphenols. The study followed a double-blind, counterbalanced, placebo controlled, repeated measures design. Participants were randomly allocated to treatment orders as selected through a Williams Latin Square (Williams, 1949).

### 4.2.2 Participants

Twenty two healthy adults participated in the study, of which 20 completed the study (6 male and 14 female). The mean age of the 20 participant's was 22.3±3.6 years, with a mean body mass index of 23.14±2.66 kg/m$^2$. Other participant characteristics can be found in table 4.1.

*Table 4.1 Mean participant characteristics*

| Measure | Average measurement | SD | Range |
|---|---|---|---|
| Age (years) | 22.3 | 3.6 | 18-30 |
| Height (m) | 1.69 | 0.07 | 1.58-1.85 |
| Mass (kg) | 66.84 | 12.14 | 47-95 |
| BMI (kg/m$^2$) | 23.14 | 2.66 | 18-29 |

Participants were recruited using opportunity sampling from Northumbria University, UK. Participants received £35 to recompense them for any expense they may have occurred to participate in the trial. Prior to acceptance onto the study potential participants attended a one hour training session. During this training session participants gave their signed consent to participate in the study and were screened for any contraindications to the study with the use of an exclusion questionnaire. A full list of exclusion criteria can be seen in appendix I. Participants then completed four repetitions of the study day tasks to ensure they met the required minimum standards to participate in the study.

The study received ethical approval from the Northumbria University School of Life Sciences Ethics Committee and was conducted according to the Declaration of Helsinki (1964).

### 4.2.3 Treatments

Participants received two intervention drinks with at least one week washout in between treatments. These drinks contained either 0mg of polyphenols (control) or 500mg polyphenols per 60kg of body weight in the form of a cold pressed blackcurrant juice (Blackadder cultivar, cultivated and processed in 2011 by Plant and Food Research Ltd, New Zealand (Blackadder Juice)). In each case, drinks comprised of 3.44g of glucose, 4.63g of fructose, 0.8g of sucrose, 6g of Splenda$^{TM}$ and 50ml of blackcurrant flavouring (Schweppes blackcurrant cordial). The total volume of the drink was made up to 200ml with water. All quantities discussed are based on a 60kg person, drink quantities were calculated per kilo of body weight.

*Table 4.2 Anthocyanins and other phenolic compounds in each of the study conditions (mg per 60kg of bodyweight)*

| Treatment | Anthocyanins mg/60kg | Other phenolics mg/60kg | Total phenolics (mg/60kg) |
|---|---|---|---|
| Control | 0 | 0 | 0 |
| Blackadder | 372.8 | 127.1 | 500 |

*Table 4.3 Anthocyanins and other phenolic compounds in each of the treatment conditions.(mg per kilo of body weight, average dose given (mg) and dose range (mg))*

| For Treatment | Anthocyanins (mg/kg) | Anthocyanin average dose (mg) | Dose Range (mg) | Other polyphenols (mg/kg) | Other polyphenols average dose (mg) | Dose range (mg) | total polyphenols (mg/kg) | Total polyphenols average dose (mg) | Dose range (mg) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Blackadder | 6.21 | 415 | 294-594 | 2.11 | 141 | 100-202 | 8.33 | 556 | 395-798 |

### 4.2.4 Cognitive and mood measures

All cognitive measures and mood scales were delivered using the Computerised Mental Performance Assessment System (COMPASS).

A modified version of the 60 minute cognitive demand battery (CDB) which has been shown to be sensitive to nutritional interventions (Scholey *et al.*, 2010) was used to assess NIRS outcomes during cognitive demand. A shortened version of the CDB has been shown to activate the pre-frontal cortex in brain imaging studies (Kennedy & Haskell, 2011). The modified cognitive demand battery lasted 30 minutes and involved three repetitions of the nine minute battery, consisting of serial 3 subtractions, serial 7 subtractions and rapid visual information processing followed by a mental fatigue visual analogue scale.

**4.2.4.1 Serial threes subtraction task:** Computerised versions of the serial subtraction tasks were implemented using tests of 2-minute duration. Participants were required to count backwards in threes from a given number as quickly and as accurately as possible using the number keys to enter each response. A random starting number between 800 and 999 was presented on the computer screen, which was cleared by the entry of the first response. The task was scored for number of responses, number of correct responses and number of errors. In the case of incorrect responses subsequent responses were scored as positive if they were scored as correct in relation to the new number.

**4.2.4.2 Serial 7 subtractions***:* This was identical to the Serial threes task except that it involved serial subtraction of sevens.

**4.2.4.3 Rapid visual information processing:** The participant was required to monitor a continuous series of digits for targets of three consecutive odd or three consecutive

even single digits. The single digits were presented at the rate of 100 per minute and the participant responded to the detection of a target string by pressing the space bar on the computer keyboard as quickly as possible. The task was continuous and lasted for 5 minutes, with 8 correct target strings being presented in each minute. The task was scored for percentage of target strings correctly detected, average reaction time for correct detections (msec), and number of incorrect responses (false alarms).

**4.2.4.4 Fatigue***:* Participants were asked to subjectively rate how mentally fatiguing they found the cognitive tasks. The visual analogue scales were displayed electronically at the end of every set of tasks. The scale was anchored “Not at all” and “extremely”, with higher scores representing more mental fatigue.

### 4.2.5 Near infrared spectroscopy

Functional near infrared spectroscopy (NIRS) utilises the optical absorption properties of oxygenated haemoglobin and deoxygenated haemoglobin after the introduction of near-infrared light through the intact skull for the purpose of functional neuroimaging (Kennedy *et al.*, 2010). When assessed by NIRS, the increase in blood flow in the surface layers of the cortex during localized neural activity is seen as an increase in the total concentration of oxyhaemoglobin and comparative decrease in deoxyhaemoglobin (Steinbrink *et al.*, 2006), with both variables corresponding strongly with the fMRI BOLD signal (Huppert *et al.*, 2006; Steinbrink *et al.*, 2006). Several peer reviewed intervention studies have utilised NIRS to assess the effects of nutritional interventions upon cerebral blood flow (Watanabe *et al.*, 2002; Jackson *et al.*, 2012). In the current study, cerebral blood flow was measured using the method reported by Kennedy *et al*., (2010), a method which has previously been shown to be sensitive to nutritional interventions with similar goals as the current study (Kennedy *et al.*, 2010; Kennedy & Haskell, 2011; Wightman *et al.*, 2012). Whereby, a 12-channel Oxymon system (Artinis Medical Systems BV, Zetten, Netherlands) was used to measure relative changes in absorption of near-infrared light at a time resolution of 10 Hz. The system emitted two

wavelengths of light (765 and 855 nm). The differential path length factor was adjusted according to the age of the participant. A simple 2-emitter/optode pair configuration was used in the current study with emitter/optode pairs positioned over the left and right frontal cortex by using a standard optode holder headband, which separated the emitter/optode pairs by 4 cm as seen in figure 4.1. Each pair therefore collected data from an area of prefrontal cortex that included the areas corresponding to the International 10–20 system Fp1 and Fp2 EEG positions. Proprietorial software was used to calculate relative concentration changes in oxyhaemoglobin, deoxyhaemoglobin, and total-haemoglobin by means of a modified Beer-Lambert law (Obrig & Villringer, 2003). The NIRS data output was time stamped at the start of each event segment (baseline, absorption and each cognitive task) to ensure that data corresponded to the relevant epoch. Events which could impact data (excessive head movements, head scratching, sneezing etc.) were also recorded for the purpose of data cleaning.

*Figure 4.1 NIRS headband (picture used with permission from the Brain Performance and Nutrition Research Centre)*

### 4.2.6 Procedures

Each participant was required to attend one screening session and two study days which were conducted at least seven days apart to ensure a sufficient wash out period between conditions. Cognitive testing took place in a laboratory where participants were individually tested in an environment isolated from noise and other physiological distractions. On arrival at their first session, participants were randomly allocated to a

treatment regime using a Latin square design that counterbalanced the order of treatments across the two active days of the study. On all testing days participants arrived in the laboratory at 8am (after a 12 hour overnight fast) and were firstly fitted with a NIRS headband as shown in figure 4.1. After a five minute seated resting period, NIRS recording began and continued throughout the entire session. Participants then completed one set of the cognitive demand battery which was used as the baseline cognitive measure. The participant then rested for 10 minutes, this was used as the pre-dose baseline measurement for NIRS paradigms. The participant was then supplemented with either the active treatment or matched control (depending on randomisation). After a one hour resting absorption period, during which the participant was required to sit and watch a DVD (Grand Designs, Channel4), the participant completed a modified version of the cognitive demand battery. The running order of the testing session can be seen in figure 4.2.

Originally, peripheral haemodynamics were also planned to be measured with the use of a photoplethysmography. However, observation of the data of the first two participants revealed that the placing of the photoplethysmography on the participants' finger impacted the primary NIRS outcome. The reason for this is not known, the measurement of peripheral haemodynamic measurement was, therefore, removed from the protocol and the data from the two participants in question was omitted from data analysis.

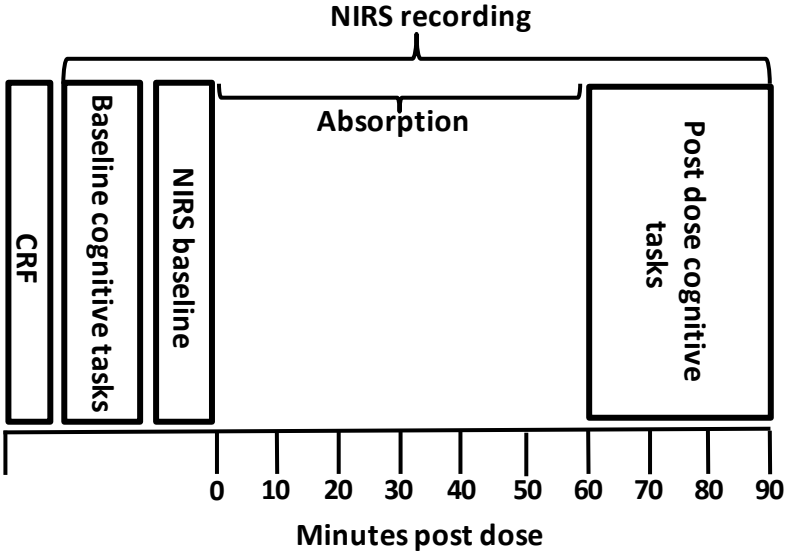



*Figure 4.2 Test session running order (minutes). CRF=case report form, NIRS=near infrared spectroscopy.*

## 4.3 Statistical analysis

Mood, cognitive scores and the NIRS measures were analysed as 'change from baseline' using the SPSS 18 statistics package. The null hypothesis was rejected with a p value <0.05. Baseline differences were calculated for all mood and cognitive measures using a one way (treatment) ANOVA.

Haemodynamics outcomes were assessed using 12, five minute, epochs during the 60 minute absorption and for the full duration of each cognitive task (2 minutes for serial subtraction tasks and five minutes for the RVIP task) during the cognitive demand battery (9 epochs). The baseline measure was calculated using the final two minutes of the 10 minute resting baseline period immediately prior to ingestion of the study drinks. Raw resting baseline measures can be found in appendix IX.

Cognitive measures and fatigue ratings were analysed with repeated measures ANOVAs (General linear model) by treatment [control and 500mg] and completion [1 to 3]). Repeated measures ANOVAs (General linear model) by treatment [control and 500mg], epoch [1 to 21] and hemisphere [left and right] were conducted on the entire

session for haemodynamic outcomes. Where no hemispheric differences were observed, data for the two hemispheres were combined and this factor (hemisphere) was omitted from the analyses. Mauchly`s test of sphericity was used to assess equality of the variances of the differences between factors. Where sphericity had been violated, Huynh-Feldt corrections for non-sphericity were implemented.

## 4.4 Results

Prior to analysis of change from baseline data, mean pre-dose scores for control and juice treatments for each cognitive and mood outcome were subjected to a one way repeated measures ANOVA. There were no significant differences found between treatments on any measures.

### 4.4.1 Cognitive performance and mood

Mean pre-dose baseline and change from baseline scores for each behavioural measure are presented in table 4.4. Unchanged “raw” data tables can be found in appendix X.

*Table 4.4 Mean pre-dose baseline and change from baseline scores, standard deviations and main ANOVA and ANOVA interaction outcomes for all behavioural outcomes*

| Measure | N | Treatment | Baseline | | Repetition 1 | | Repetition 2 | | Repetition 3 | | Effect of treatment | Treatment*repetition interaction |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | | |
| Serial 3 incorrect responses | 20 | Control | 1.95 | 12.27 | 0.30 | 10.77 | 0.75 | 8.32 | 1.45 | 13.95 | F=0.46 p>0.1 | F=0.40 p>0.1 |
| | | Juice | 2.80 | 17.39 | -0.75 | 11.13 | 0.75 | 14.43 | 1.30 | 20.83 | | |
| Serial 3 correct responses | 20 | Control | 44.20 | 51.11 | 0.45 | 18.83 | 1.30 | 30.58 | 0.70 | 35.16 | F=0.97 p>0.1 | F=0.72 p>0.1 |
| | | Juice | 42.85 | 76.74 | 1.20 | 31.04 | 0.90 | 25.00 | -0.80 | 36.98 | | |
| Serial 3 number of responses | 20 | Control | 46.15 | 54.74 | 0.75 | 15.42 | 2.05 | 28.38 | 2.15 | 30.17 | F=0.47p>0.1 | F=0.73 p>0.1 |
| | | Juice | 45.65 | 69.13 | 0.45 | 29.50 | 1.65 | 18.54 | 0.50 | 23.82 | | |
| Serial 7 incorrect responses | 20 | Control | 2.95 | 12.09 | 0.00 | 13.30 | 0.10 | 10.25 | 0.90 | 11.42 | F=0.16 p>0.1 | F=2.1 0p>0.1 |
| | | Juice | 2.80 | 12.78 | -0.05 | 9.34 | 0.50 | 12.35 | -0.05 | 14.76 | | |
| Serial 7 correct responses | 20 | Control | 24.25 | 49.01 | 2.80 | 30.53 | 2.80 | 28.75 | 2.55 | 22.45 | F=0.07 p>0.1 | F=.20 p>0.1 |
| | | Juice | 25.25 | 54.02 | 2.75 | 18.86 | 3.05 | 22.03 | 2.75 | 28.80 | | |
| Serial 7 number of responses | 20 | Control | 27.20 | 45.06 | 2.80 | 23.11 | 2.90 | 22.14 | 3.45 | 19.71 | F=0.01 p>0.1 | F=0.71 p>0.1 |
| | | Juice | 28.05 | 48.87 | 2.70 | 16.93 | 3.55 | 22.31 | 2.70 | 27.34 | | |
| RVIP correct responses | 20 | Control | 53.50 | 62.59 | -0.25 | 36.44 | -1.50 | 33.72 | 0.13 | 51.87 | F=0.45 p>0.1 | F=0.99 p>0.1 |
| | | Juice | 55.79 | 57.76 | -0.63 | 42.13 | -5.00 | 53.20 | -4.38 | 42.28 | | |
| RVIP false alarms | 20 | Control | 0.85 | 26.59 | -0.15 | 4.65 | 0.30 | 6.66 | 0.15 | 7.15 | F=0.03 p>0.1 | F=2.79 p>0.1 |
| | | Juice | 0.84 | 35.04 | 0.30 | 7.83 | -0.15 | 4.17 | 0.55 | 5.12 | | |
| RVIP reaction time | 20 | Control | 482.88 | 54.45 | 0.36 | 193.37 | -4.74 | 179.69 | -5.81 | 218.41 | F=0.09 p>0.1 | F=0.39 p>0. 1 |
| | | Juice | 482.53 | 55.75 | -1.44 | 162.57 | 17.32 | 269.09 | -34.83 | 524.55 | | |
| Fatigue | 20 | Control | 28.60 | 77.90 | 16.00 | 94.66 | 22.10 | 114.89 | 25.80 | 126.20 | F=1.63 p>0.1 | F=0.56 p>0.1 |
| | | Juice | 23.75 | *90.30* | 11.15 | 50.14 | 19.25 | 71.48 | 23.35 | 93.59 | | |

### 4.4.2 Haemodynamics

Mean change from baseline scores for each haemodynamic epoch are presented in table 4.3 and 4.4. A graphical representation is presented in figure 4.2. Resting baseline data was omitted from tables as it is an arbitrary figure. This data can be found in appendix IX. Only ANOVA results for physiological measures which generated significant effects are reported below.

*Table 4.5 Mean change from baseline scores (mmol/L), standard deviations (SD) and ANOVA outcomes for left hemisphere (LH), right hemisphere (RH) and hemispheres combined (combined) for NIRS outcomes total-HB Oxy-HB and deoxy-HB throughout the whole testing procedure*

| Measure | N | Treatment | 1-5 minute epoch | | 6-10 minute epoch | | 11-15 minute epoch | | 16-20 minute epoch | | 21-25 minute epoch | | 26-30 minute epoch | | 31-35 minute epoch | | 36-40 minute epoch | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Combined OxyHb | 20 | Control | -1.33 | 1.33 | -1.65 | 1.22 | -1.06 | 1.65 | -0.64 | 1.73 | -0.41 | 1.97 | -0.31 | 1.83 | 0.05 | 2.12 | 0.1 | 2.85 |
| | | Juice | -0.57 | 1.24 | -1 | 1.41 | -0.72 | 1.42 | -0.31 | 1.59 | 0.05 | 1.9 | 0.18 | 2.14 | 0.47 | 2.03 | 0.36 | 2.02 |
| OxyHb LH | 20 | Control | -1.75 | 1.8 | -2.1 | 1.52 | -1.45 | 1.96 | -0.95 | 1.85 | -0.85 | 2.18 | -0.7 | 2.08 | -0.2 | 2.38 | -0.4 | 3.05 |
| | | Juice | -0.65 | 1.31 | -1.1 | 1.37 | -0.95 | 1.57 | -0.7 | 1.92 | -0.3 | 2.15 | -0.2 | 2.24 | 0.25 | 2.2 | 0.05 | 2.44 |
| OxyHb RH | 20 | Control | -1 | 1.3 | -1.3 | 1.34 | -0.6 | 1.7 | -0.35 | 1.84 | 0 | 2.34 | 0.05 | 1.93 | 0.3 | 2.08 | 0.6 | 2.89 |
| | | Juice | -0.25 | -3.58 | -0.45 | 0.45 | 0.5 | 2.24 | 0.65 | 3.35 | 1.25 | 7.16 | 1.95 | 9.84 | 1.95 | 10.73 | 2.15 | 9.84 |
| Combined DeoxyHb | 20 | Control | 0.34 | 0.49 | 0.57 | 0.44 | 0.37 | 0.44 | 0.2 | 0.45 | 0.01 | 0.46 | -0.02 | 0.48 | -0.16 | 0.54 | -0.1 | 0.86 |
| | | Juice | 0.02 | 0.41 | 0.4 | 0.59 | 0.25 | 0.72 | 0.06 | 0.75 | -0.03 | 0.84 | -0.15 | 0.77 | -0.33 | 0.66 | -0.42 | 0.79 |
| DeoxyHb LH | 20 | Control | 0.34 | 0.48 | 0.6 | 0.43 | 0.39 | 0.56 | 0.26 | 0.55 | 0.02 | 0.51 | -0.01 | 0.55 | -0.11 | 0.62 | 0.01 | 0.87 |
| | | Juice | 0.05 | 0.39 | 0.45 | 0.55 | 0.26 | 0.61 | 0.07 | 0.77 | -0.01 | 0.82 | -0.12 | 0.73 | -0.36 | 0.71 | -0.31 | 0.65 |
| DeoxyHb RH | 20 | Control | 0.35 | 0.57 | 0.54 | 0.56 | 0.35 | 0.49 | 0.13 | 0.52 | -0.01 | 0.58 | -0.03 | 0.65 | -0.22 | 0.58 | -0.2 | 0.98 |
| | | Juice | -0.01 | 0.51 | 0.34 | 0.7 | 0.25 | 0.9 | 0.05 | 0.8 | -0.06 | 0.96 | -0.18 | 0.92 | -0.29 | 0.72 | -0.52 | 1.2 |
| Combined TotalHb | 20 | Control | -0.99 | 1.26 | -1.08 | 1.26 | -0.68 | 1.54 | -0.44 | 1.77 | -0.41 | 1.99 | -0.33 | 1.89 | -0.12 | 2.29 | 0.01 | 3.36 |
| | | Juice | -0.55 | 1.24 | -0.6 | 1.33 | -0.47 | 1.33 | -0.25 | 1.7 | 0.02 | 2.07 | 0.03 | 2.34 | 0.15 | 2.01 | -0.06 | 2.36 |
| TotalHb LH | 20 | Control | -1.25 | 1.52 | -1.35 | 1.39 | -1.05 | 1.79 | -0.65 | 1.79 | -0.85 | 2.18 | -0.7 | 2.15 | -0.35 | 2.56 | -0.3 | 3.37 |
| | | Juice | -0.35 | 1.57 | -0.25 | 1.71 | -0.2 | 1.82 | 0.05 | 2.16 | 0.35 | 2.37 | 0.4 | 2.6 | 0.35 | 2.39 | 0.5 | 2.06 |
| TotalHb RH | 20 | Control | -0.85 | 1.53 | -0.75 | 1.62 | -0.4 | 1.85 | -0.1 | 2.1 | -0.05 | 2.5 | 0.2 | 2.33 | 0.05 | 2.48 | 0.35 | 3.72 |
| | | Juice | -0.75 | 1.29 | -0.8 | 1.47 | -0.75 | 1.86 | -0.8 | 2.04 | -0.3 | 2.41 | -0.3 | 2.7 | -0.1 | 2.27 | -0.45 | 3.28 |

| Measure | N | Treatment | 41-45 minute epoch | | 46-50 minute epoch | | 51-55 minute epoch | | 56-60 minute epoch | | Serial 3 repetition 1 | | Serial 7 repetition 1 | | RVIP repetition 1 | | Serial 3 repetition 2 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Combined OxyHb | 20 | Control | 0.48 | 3.51 | 0.44 | 3.88 | 0.13 | 3.69 | 0.35 | 3.66 | 1.87 | 4.25 | 1.19 | 4.16 | 1.64 | 3.93 | 1.95 | 4.66 |
| | | Juice | 0.75 | 2.22 | 1.05 | 1.9 | 1.48 | 2.05 | 1.71 | 1.95 | 3.08 | 2.86 | 2.83 | 2.16 | 2.49 | 2.23 | 3.18 | 2.5 |
| OxyHb LH | 20 | Control | 0 | 3.74 | 0 | 4.22 | -0.25 | 3.99 | 0.05 | 4.02 | 0.75 | 4.45 | 1.35 | 4.69 | 1.15 | 4.88 | 1.4 | 4.75 |
| | | Juice | 0.3 | 2.45 | 0.6 | 1.9 | 0.95 | 2.28 | 1.25 | 2.4 | 3.7 | 2.77 | 4.2 | 3.64 | 3.8 | 3.5 | 3.9 | 3.86 |
| OxyHb RH | 20 | Control | 0.95 | 3.68 | 0.9 | 3.82 | 0.6 | 3.93 | 0.6 | 3.62 | 1.6 | 4.31 | 2.15 | 3.88 | 2.2 | 4.3 | 2.3 | 4.18 |
| | | Juice | 2.4 | 9.84 | 1.85 | 9.17 | 2.15 | 1.41 | 0.42 | 1.99 | 0.45 | 2.21 | 0.55 | 2.37 | 0.44 | 2.55 | 0.53 | 2.3 |
| Combined DeoxyHb | 20 | Control | 0.18 | 1.3 | 0.35 | 1.25 | 0.39 | 1.28 | 0.27 | 1.27 | 0.02 | 0.99 | 0.13 | 1.18 | 0.08 | 1.21 | 0.05 | 1.09 |
| | | Juice | -0.47 | 0.88 | -0.4 | 0.85 | -0.38 | 0.86 | -0.56 | 0.79 | -0.64 | 0.93 | -0.55 | 0.86 | -0.61 | 0.88 | -0.5 | 0.68 |
| DeoxyHb LH | 20 | Control | 0.24 | 1.2 | 0.4 | 1.14 | 0.49 | 1.22 | 0.4 | 1.22 | 0.29 | 1.18 | 0.18 | 1.05 | 0.22 | 1.19 | 0.13 | 1.22 |
| | | Juice | -0.31 | 0.78 | -0.22 | 0.84 | -0.21 | 1.04 | -0.42 | 1.05 | -0.35 | 1.08 | -0.36 | 1.07 | -0.36 | 0.82 | -0.32 | 0.78 |
| DeoxyHb RH | 20 | Control | 0.12 | 1.5 | 0.29 | 1.44 | 0.29 | 1.5 | 0.14 | 1.44 | -0.03 | 1.31 | -0.13 | 1.05 | -0.07 | 1.32 | -0.05 | 1.24 |
| | | Juice | -0.63 | 1.4 | -0.59 | 1.26 | -0.55 | 1.04 | -0.7 | 0.92 | -0.75 | 0.97 | -0.37 | 0.78 | -0.86 | 1.35 | -0.65 | 0.69 |
| Combined TotalHb | 20 | Control | 0.66 | 4.42 | 0.78 | 4.82 | 0.52 | 4.66 | 0.62 | 4.63 | 1.9 | 4.85 | 1.32 | 5.03 | 1.72 | 4.74 | 2 | 5.34 |
| | | Juice | 0.28 | 2.49 | 0.65 | 2.09 | 1.1 | 2.11 | 1.15 | 1.98 | 2.44 | 2.93 | 2.28 | 2.32 | 1.88 | 2.53 | 2.68 | 2.45 |
| TotalHb LH | 20 | Control | 0.25 | 4.47 | 0.45 | 5.06 | 0.2 | 4.79 | 0.45 | 4.9 | 1.05 | 5.28 | 1.65 | 5.2 | 1.35 | 5.2 | 1.6 | 5.39 |
| | | Juice | 0.9 | 2.53 | 1.3 | 2.23 | 1.7 | 2.41 | 1.8 | 2.19 | 3.35 | 3.27 | 3.55 | 4.2 | 2.8 | 3.21 | 3.9 | 3.52 |
| TotalHb | 20 | Control | 1.05 | 4.66 | 1.1 | 4.9 | 0.9 | 4.96 | 0.8 | 4.75 | 1.5 | 5.12 | 2.1 | 4.85 | 2.05 | 4.76 | 2.15 | 4.8 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RH | | Juice | -0.3 | 3.28 | 0 | 2.64 | 0.25 | 2.51 | 0.45 | 2.63 | 1.3 | 2.54 | 1.25 | 2.65 | 0.8 | 2.63 | 1.85 | 2.43 |

| Measure | N | Treatment | Serial 7 repetition 2 | | RVIP repetition 2 | | Serial 3 repetition 3 | | Serial 7 repetition 3 | | RVIP repetition 3 | | Effect of treatment | Treatment*epoch interaction | Treatment* hemisphere interaction | Treatment* hemisphere *epoch interaction |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | | | | |
| Combined OxyHb | 20 | Control | 1.83 | 4.15 | 1.57 | 4.05 | 1.82 | 4.66 | 1.67 | 4.44 | 1.72 | 3.95 | F=1.89 p>0.1 | F=1.52 p>0.1 | F=7.26 p=0.014 | F=5.30 p=0.006 |
| | | Juice | 3.27 | 2.54 | 2.6 | 2.29 | 3.25 | 2.71 | 2.91 | 2.65 | 2.97 | 2.61 | | | | |
| OxyHb LH | 20 | Control | 1.7 | 5.07 | 1.3 | 5.01 | 1.05 | 4.35 | 1.05 | 4.47 | 1.3 | 4.16 | | | | |
| | | Juice | 3.8 | 3.74 | 4.4 | 4.08 | 3.15 | 2.89 | 3.15 | 3.05 | 3.7 | 3.51 | | | | |
| OxyHb RH | 20 | Control | 2.3 | 4.62 | 2.4 | 4.48 | 2.15 | 3.95 | 2.15 | 3.88 | 2.2 | 3.86 | | | | |
| | | Juice | 0.47 | 2.24 | 0.54 | 2.6 | 0.58 | 2.23 | 0.46 | 2.01 | 0.5 | 2.3 | | | | |
| Combined DeoxyHb | 20 | Control | 0.04 | 1.17 | 0.15 | 1.28 | 0.07 | 1.13 | 0.17 | 1.26 | 0.11 | 1.18 | F=5.7 p=0.02 | F=2.73 p=0.43 | F=2.73 p=0.046 | F=1.50 p>0.1 |
| | | Juice | -0.48 | 0.67 | -0.4 | 0.63 | -0.34 | 0.92 | -0.4 | 0.73 | -0.38 | 0.76 | | | | |
| DeoxyHb LH | 20 | Control | 0.15 | 1.23 | 0.24 | 1.33 | 0.25 | 1.41 | 0.14 | 1.29 | 0.18 | 1.24 | | | | |
| | | Juice | -0.02 | 1.06 | -0.26 | 0.72 | -0.13 | 0.84 | -0.62 | 0.73 | -0.2 | 0.89 | | | | |
| DeoxyHb RH | 20 | Control | -0.06 | 1.14 | 0.07 | 1.34 | 0.09 | 1.2 | 0 | 1.08 | 0.04 | 1.19 | | | | |
| | | Juice | -0.93 | 1.24 | -0.55 | 0.68 | -0.67 | 1.02 | -0.66 | 1.08 | -0.56 | 0.77 | | | | |
| Combined TotalHb | 20 | Control | 1.87 | 4.98 | 1.72 | 4.86 | 1.88 | 5.27 | 1.84 | 5.17 | 1.84 | 4.69 | F=0.32 p>0.1 | F=0.73 p>0.1 | F=7.06 p=0.01 | F=2.64 p=0.02 |
| | | Juice | 2.79 | 2.46 | 2.2 | 2.34 | 2.91 | 2.75 | 2.52 | 2.63 | 2.6 | 2.64 | | | | |
| TotalHb LH | 20 | Control | 1.85 | 5.82 | 1.25 | 5.2 | 1.4 | 5.68 | 1.45 | 5.69 | 1.5 | 4.98 | | | | |
| | | Juice | 3.5 | 3.87 | 2.9 | 3.26 | 3.65 | 3.72 | 4.3 | 4.39 | 3.55 | 3.76 | | | | |
| TotalHb RH | 20 | Control | 2.2 | 5.2 | 2.15 | 4.74 | 2.1 | 5.12 | 2.3 | 5.24 | 2.35 | 4.79 | | | | |
| | | Juice | 1.95 | 1.88 | 1.6 | 2.01 | 1.35 | 2.56 | 1.5 | 2.16 | 1.6 | 2.23 | | | | |

#### 4.4.2.1 Oxyhaemoglobin

Analysis of the entire session revealed a significant treatment*hemisphere interaction for oxyhaemoglobin [F(1,19)=7.265, p=0.01]. Pairwise comparisons showed an increase in oxyhaemoglobin in the left hemisphere following consumption of the Blackadder juice when compared to control (p=0.038), with no effect of treatment on the right hemisphere.

There was also a significant treatment*hemisphere*epoch interaction for the entire session [F(2.33,44.33)=5.30, p=0.006]. Pairwise comparisons revealed an increase in oxyhaemoglobin in the left hemisphere after consumption of the Blackadder juice drink when compared to control at the following epochs; 5 minutes (p=0.003), 10 minutes (p=0.048), serial 3s repetition 1 (p=0.002), serial 7s repetition 1 (p=0.003), RVIP repetition 1 (p=0.004), serial 3s repetition 2 (p=0.004), serial 7s repetition 2 (p=0.021), RVIP repetition 2 (p=0.001), serial 3s repetition 3 (p=0.030), serial 7s repetition 3 (p=0.031) and RVIP repetition 3 (p=0.003) with no interpretable effect of treatment on the right hemisphere. A graphical representation of all NIRS oxyhaemoglobin outcomes can be seen in figure 4.3.

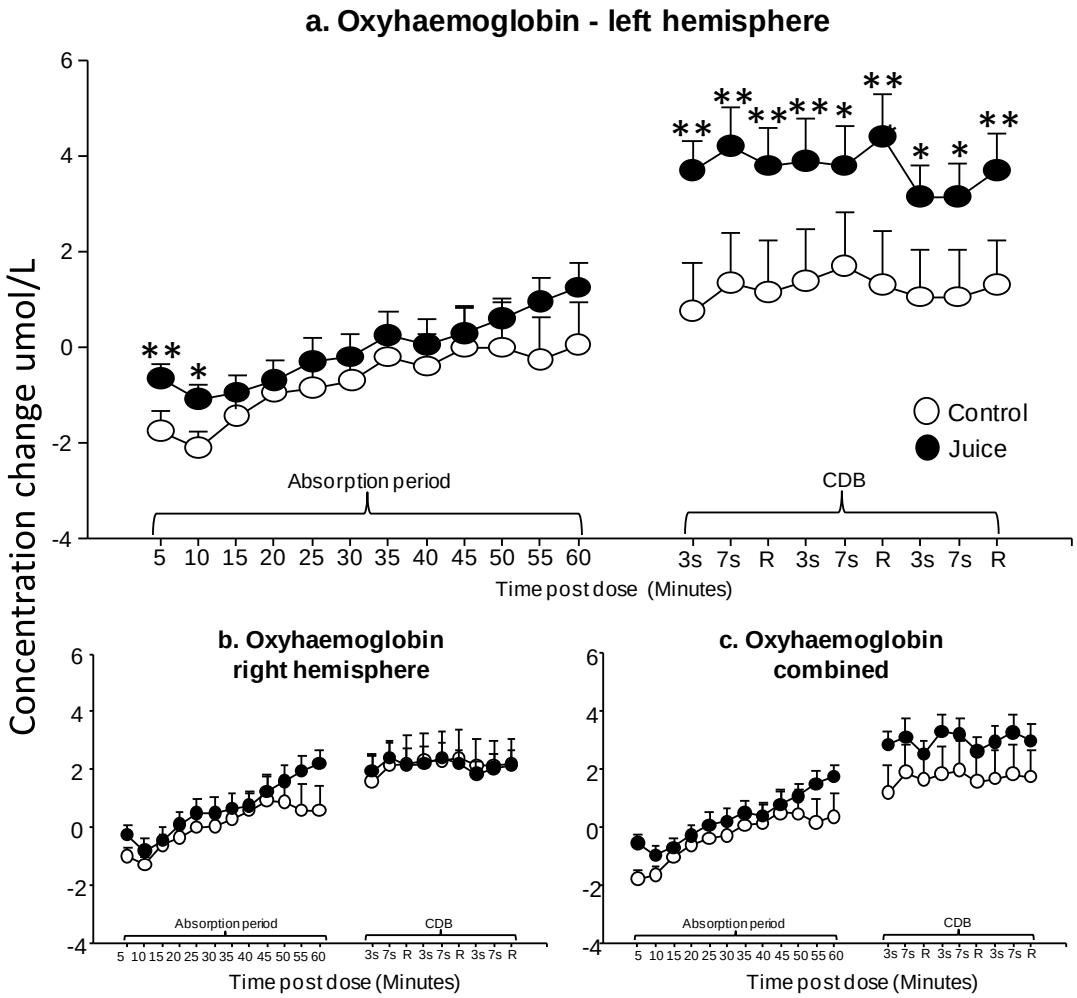



*Figure 4.3 Change from baseline oxyhaemoglobin throughout the 60 minute absorption period and cognitive demand battery (CDB) (a) the left hemisphere, (b) the right hemisphere and (c) left and right hemispheres combined. Significant differences are indicated on the graph (*p<0.05, **p<0.01).*

### 4.4.2.2 Deoxyhaemoglobin

The ANOVA of the entire session revealed a significant effect of treatment on deoxyhaemoglobin [F(1,19)=5.70, p=0.021]. This was due to a decrease in deoxyhaemoglobin after consumption of the Blackadder juice when compared to control.

The ANOVA also revealed a significant treatment*epoch interaction for the entire session [F(3.31,63.05)=2.73, p=0.046]. Pairwise analysis revealed a decrease in deoxyhaemoglobin after the Blackadder juice treatment when compared to control at the 5 minute (p=0.027), 50 minute (p=0.018), 55 minute (p=0.010), 60 minute (p=0.009), serial 3s repetition 1 (p=0.013), serial 7s repetition 3 (p=0.017) epochs.

Trends towards the same pattern of modulation were seen at the 45 minute (p=0.06), serial 7s repetition 1 (p=0.088), RVIP repetition 1 (p=0.05), serial 3s repetition 2 (p=0.068), serial 7s repetition 2 (p=0.069), RVIP repetition 2 (p=0.076) and serial 3s repetition 3 (p=0.053) epochs. A graphical representation of all NIRS deoxyhaemoglobin outcomes can be seen in figure 4.4

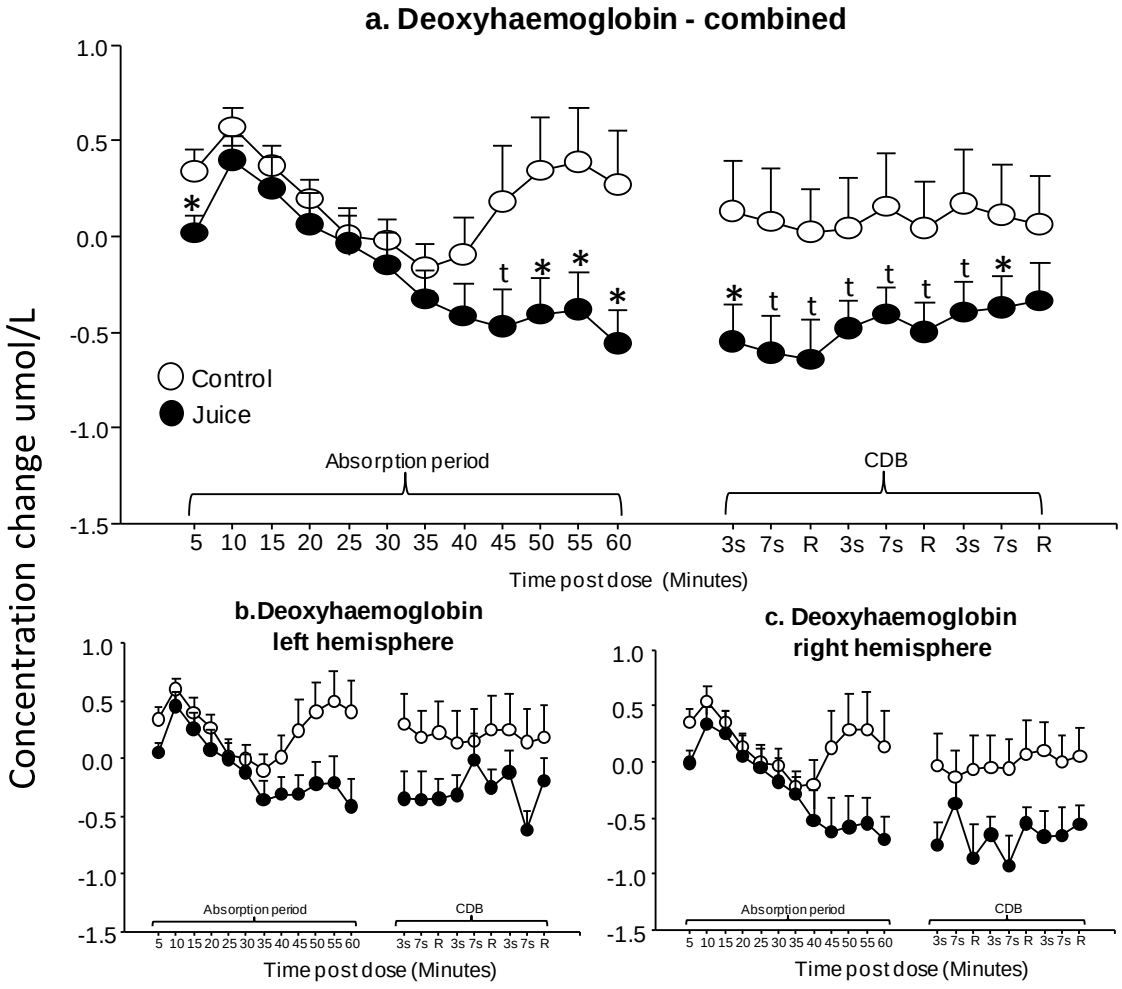



*Figure 4.4 Change from baseline deoxyhaemoglobin throughout the 60 minute absorption period and cognitive demand battery (CDB) for (a) combined left and right hemisphere, (b) the left hemisphere and (c) the right hemisphere. Significant differences are indicated on the graph (*p<0.05, t p<0.1).*

### 4.4.2.3 Total haemoglobin

There was a significant treatment*hemisphere interaction for total haemoglobin measured over the entire session [F(1,19)=7.06, p=0.016]. Pairwise comparisons revealed a trend towards an increase in total haemoglobin in the left hemisphere after consumption of the Blackadder juice extract when compared to control (p=0.09).

The ANOVA also revealed a significant treatment*hemisphere*epoch interaction for total haemoglobin over the entire session [F(5.93,112.68)=2.64, p=0.020]. Pairwise comparisons revealed an increase in total haemoglobin in the left hemisphere when compared to control at the; 10 minute (p=0.032), serial 3s repetition 1 (p=0.031), serial 7s repetition 1 (p=0.040), serial 3s repetition 2 (p=0.026), serial 3s repetition 3 (p=0.042), serial 7s repetition 3 (p=0.014) and RVIP repetition 3 (p=0.047) epochs.

A trend towards the same pattern of modulation was seen at the 5 minute epoch (p=0.058). A graphical representation of all NIRS total haemoglobin outcomes can be seen in figure 4.5

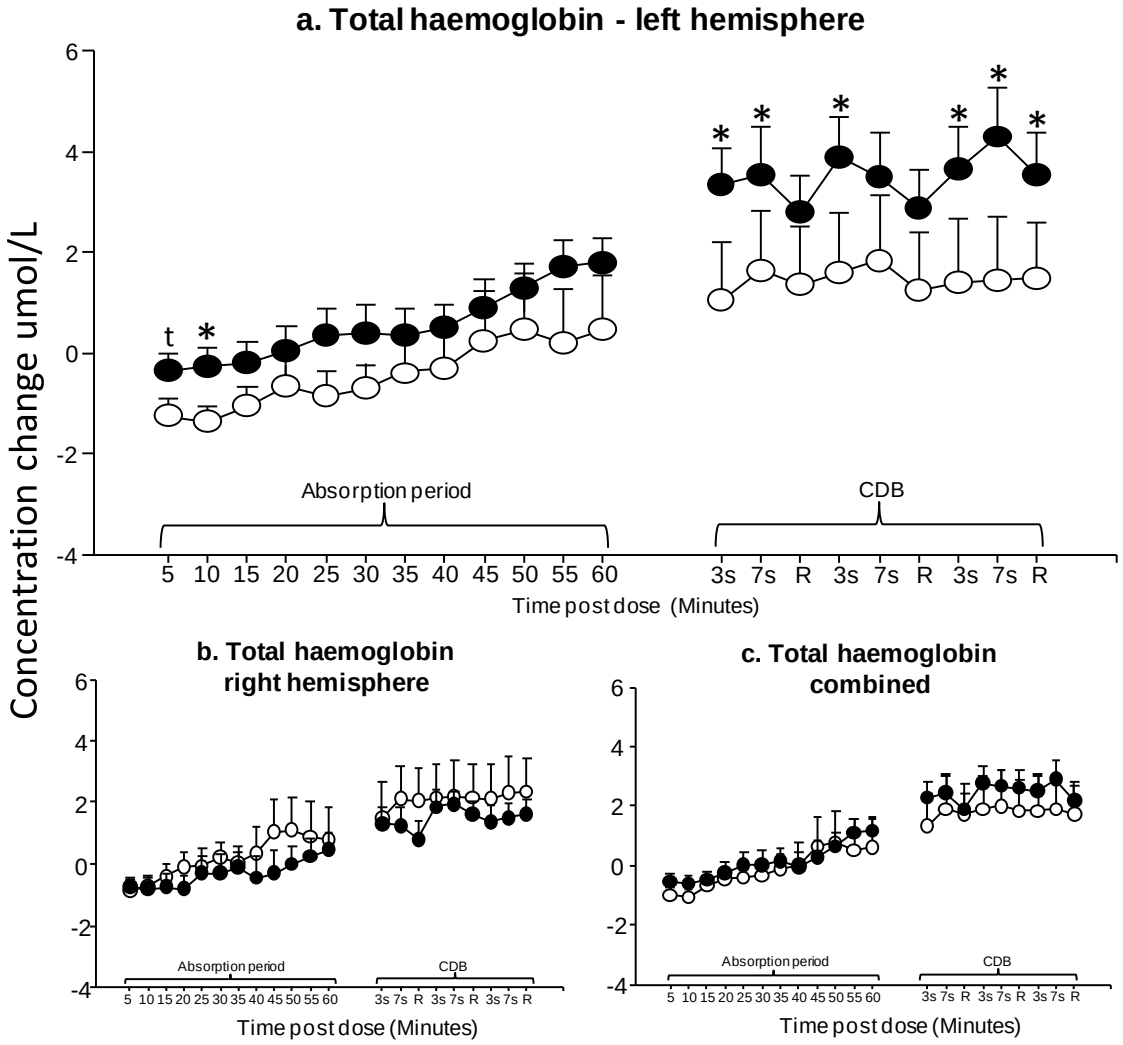



*Figure 4.5 Change from baseline total haemoglobin throughout the 60 minute absorption period and cognitive demand battery (CDB) for (a) the left hemisphere, (b) the right hemisphere and (c) left and right hemisphere combined. Significant differences are indicated on the graph (tp<0.1,*p<0.05, **p<0.01).*

## 4.5 Discussion and conclusion

The aim of the current study was to assess the impact of the MAO inhibiting Blackadder blackcurrant juice extract upon shallow pre-frontal cortical haemodynamics. The study focused upon two aspects, pre-frontal cortical haemodynamics under cognitive demand and pre-frontal cortical haemodynamics at rest. The overall pattern of task-related changes in local haemoglobin concentrations independent of intervention was as expected (Kennedy *et al.*, 2010; Kennedy & Haskell, 2011; Wightman *et al.*, 2012), with increases in oxyhaemoglobin and decreases in deoxyhaemoglobin during cognitive task performance and, therefore, increases in regional cerebral blood flow.

When compared to placebo, the consumption of the Blackadder juice extract resulted in significant modulations of pre-frontal cortex haemoglobin concentrations during resting absorption and cognitive task performance, with apparent hemispheric differences. An increase in oxyhaemoglobin and total haemoglobin in the left hemisphere, but not the right, was seen after consumption of the blackcurrant drink when compared to control, with significant increases being found throughout the cognitive demand period with no effect of task type. In relation to concentration changes in deoxyhaemoglobin, supplementation of the Blackadder blackcurrant extract caused a significant or near to significant trend reduction in overall deoxyhaemoglobin (left and right hemispheres combined), beginning during the resting absorption period, 45 minutes post supplementation of the blackcurrant juice. This reduction continued throughout the remaining absorption period and throughout the cognitive testing period, again with no effect of task type.

With regards to intervention-related modifications in concentrations of total and oxygenated haemoglobin, increases have been observed during cognitive demand after oral supplementation of resveratrol (Kennedy *et al.,* 2010), anti-depressants

(Kohmura *et al.*, 2013) and vinpocetine (Bönöczk *et al.*, 2002); however, with no hemispheric differences seen. As increased oxyhaemoglobin and total haemoglobin have been linked to increased neural activity during cognitive demand in healthy young adults when measured by NIRS (Hoshi & Tamura, 1993), the rise in total haemoglobin in response to post-dose tasks in the current study could demonstrate neurovascular coupling, which is further up-regulated in the left hemisphere after consumption of the blackcurrant juice. As the tasks in the present study have been shown to increase activity in the pre-frontal cortex (Coull *et al.*, 1996) and the pre-frontal left hemisphere (Burbaud *et al.*, 1999), the hemispheric differences found in the present study could be an effect of the study intervention amplifying a natural difference in hemispheric activity in response to cognitive performance. However, it must be noted that there is no observable difference in hemispheric activity shown in the current study after consumption of the control intervention. When assessed via PET without a nutritional intervention, the RVIP task has been shown to elicit increases in regional cerebral blood flow bilaterally in the inferior frontal gyri, parietal cortex and fusiform gyrus, as well as lateralised right-sided activation in superior frontal gyrus (Coull *et al.*, 1996). When RVIP was compared to a simplified version of the task with the working memory element removed, the right frontal activations were no longer apparent. The authors consider these findings consistent with the existence of a right fronto-parietal network for sustained attention, and a left fronto-parietal network for working memory. Therefore, the increase in left hemisphere regional cerebral blood flow following blackcurrant supplementation in the current study, could be indicative of increased neuronal activation in brain areas associated with working memory.

Given the increase in oxygenated haemoglobin following supplementation of the Blackadder extract, the relative decrease in deoxygenated haemoglobin is as expected. This is due to the rate of utilisation of oxygen being slower than the rate at which it is provided. However, the effects on deoxygenated haemoglobin do not show

the lateralisation observed with oxygenated haemoglobin, which, coupled with the time of onset of effects suggests that another mechanism is involved in the effects on deoxyhaemoglobin. The significant effects upon deoxyhaemoglobin seem to be brought about by a divergence in effects at around 35 minutes post-consumption. Prior to this, both treatments have followed a similar pattern of increase at 10 minutes post-consumption followed by a decline up until 35 minutes. At this point, deoxyhaemoglobin levels begin to rise back up in the control group until they reach a level similar to that observed at baseline. Conversely, values in the Blackadder juice group continue to fall before levelling off for the remainder of the session. This decrease in deoxyhaemoglobin may outline neuronal efficiency. The reason for this pattern of modulation is unknown. As both the active and control drink followed the same pattern of modulation until 35 minutes post-dose, it would suggest that the effects are caused by a common denominator. Drinks are matched for sugars, a tentative explanation could be that more glucose is available for utilisation and is therefore the ratio of lactate and glucose in neuronal tissue changes in favour of glucose. As less oxygen is needed to use glucose as a fuel when compared to lactate (Larrabee, 1995), less oxygen is needed to maintain cognitive activities. It must also be noted that a significant increase in regional cerebral blood flow, as indexed by total haemoglobin, was seen at the ten minute epoch, post-consumption of the blackcurrant intervention as compared to control. It seems unlikely that this early effect is attributable to modulations of biological mechanisms by phenolic compounds within the study drinks, with a more plausible explanation being that it is related to subtle differences between sensory properties of the Blackadder juice extract and the control drink. fMRI clearly shows that a number of factors related to the sensory properties of a food or drink can directly modulate frontal cortex activity, including differing tastes (Smits *et al.*, 2007) and flavours (Marciani *et al.*, 2006).

Substantiated evidence of underlying mechanisms driving changes in haemodynamics after administration of a nutritional intervention are yet to be fully understood. Although anthocyanins have been found in the brains of rats after oral ingestion (Andres-Lacueva *et al.*, 2005), bioavailability data from chapter two, and data in the literature (Matsumoto *et al.*, 2001; Jin *et al.*, 2011) show the bioavailability of anthocyanin compounds to be extremely low and, therefore, their direct effect upon the site of action is doubtful. However, given that nitric oxide synthesis plays a major role in the modulation of localised blood flow in the neural tissue of animal models (Kitaura *et al.*, 2007), the pattern of increased oxyhaemoglobin and total haemoglobin in the left hemisphere during cognitive tasks only could indicate intervention-dependent, nitric oxide synthesis driven neurovascular coupling in the frontal cortex. This neurovascular coupling involves the coordinated interaction of neurons, glia, and vascular cells and is, therefore, a key regulator in the maintenance of normal blood flow in the cerebral cortex during brain activity. For this reason, nitric oxide synthesis is a suggested therapeutic target for improving brain function in pathologies associated with cerebrovascular dysfunction (Girouard & Iadecola, 2006). MAO inhibitors act upon neurotransmitters which affect regional cerebral blood flow (Mckim, 2003), and are mediators of nitric oxide in animal models of hypertension (Sturza *et al.*, 2013), therefore, the impact of MAO inhibition by the Blackadder blackcurrant extract cannot be overlooked as a possible mediator of the haemodynamic changes seen in the current study. Dopamine, which is deaminated by MAO-B, has now been established as a modulator of neuronal blood flow (Krimer *et al.*, 1998) and is theorised to be implicated in diminished blood flow in pathological diseases such as Parkinson's disease (Leenders *et al.*, 1985).Therefore, increased CNS levels of dopamine modulated by inhibition of central MAO-B could potentially drive increases in neuronal activity and, therefore, modulated haemodynamics in the current study. Reductions in levels of deoxyhaemoglobin coincide with the appearance of berry anthocyanins in blood plasma which reach maximal concentrations at ˜1 hour post consumption

(Matsumoto *et al.*, 2001). Pharmacokinetics of anthocyanins pre-one hour post oral ingestion have not been published; however, it seems reasonable to assume that anthocyanins would be present in blood plasma 45 minutes post ingestion.

The study treatment showed no effect upon cognitive task performance. Although previously shown to be sensitive to cocoa flavonoids (Scholey *et al.*, 2010), the tasks employed here were chosen on the basis that they have previously been shown to activate the prefrontal cortex (Drummond *et al.*, 1999; Kazui *et al.*, 2000) and generate a haemodynamic response in the frontal cortex (Kennedy & Haskell, 2011; Wightman *et al.*, 2012), rather than because they have been shown to be sensitive to polyphenol supplementation.

Results from the current study outline a heightened NIRS response in brain regions responsible for task performance after supplementation with the Blackadder blackcurrant extract when compared to control. This is not coupled with an increase in cognitive performance in measured paradigms. To attempt to assess if blood flow changes are general, or solely an artefact of an amplification of neurovascular coupling in a specific brain region following the blackcurrant intervention, a suggestion for future research would be to assess cognitive tasks which are not dependent on frontal cortex activity alongside tasks which are. Furthermore, the cohort used in the present study had no apparent issues pertaining to cerebral blood flow. The tight regulation of blood flow in the brain could mean that sufficient blood flow already exists for maximal cognitive performance and, therefore, increasing blood flow above this threshold does not have any acute benefits upon cognitive performance. The use of an aged cohort, a cohort in which cerebral blood flow has potentially reduced (Leenders *et al.*, 1990), could highlight differential cognitive results. Indeed, recent unpublished “pilot” findings from the Brain, Performance and Nutrition Research Centre indicated that supplementing five healthy middle aged participants with either 500mg of resveratrol or

an inert placebo increased regional cerebral blood flow but also increased serial three correct responses, a finding which was not shown in a younger cohort (unpublished observation, private communication). To assess if changes in blood flow are also evident in the periphery, it would have been beneficial to measure peripheral and cerebral blood flow together. However, as discussed in the methods section of this chapter, data from the initial two participants in this study illuminated that attempts to measure peripheral blood flow with a photoplethysmography (digital volume pulse) compromised the primary NIRS outcome and was, therefore, omitted from the study to be used in chapter five of this study.

Reductions in cerebral blood flow are associated with natural ageing (Leenders *et al.*, 1990) and neurological disease (Dede *et al.*, 2007). Results from this thesis outline the ability of the Blackadder blackcurrant juice extract to increase cerebral blood flow. Therefore, the effects of the Blackadder juice extract in addition to other types of blackcurrant extracts upon brain function deserves further investigation.

# CHAPTER 5. A PHARMACODYNAMIC ASSESSMENT OF THE EFFECTS OF THE BLACKADDER BLACKCURRANT JUICE EXTRACT UPON PLATELET MAO-B, PLASMA PROLACTIN, BLOOD GLUCOSE AND PERIPHERAL BLOOD FLOW

## 5.1 Introduction

As detailed in chapter one, the phytochemical constituents in blackcurrant extracts have the potential to impact *in vitro* physiological parameters closely related to the regulation of human behaviour and cognitive performance. The *in vivo* efficacy of some of these physiological effects have been presented in the previous three intervention chapters; in particular, inhibition of peripheral MAO activity, modulation of shallow pre-frontal cortical haemodynamics, interactions with hormone secretion and regulation of blood glucose.

In relation to the inhibition of MAO activity, it is important to distinguish between the two isoforms of MAO which exist, MAO-A and MAO-B. Each MAO enzyme has a substrate preference, inhibitor specificity, and tissue distribution. For example the percentage of total MAO-B in neuronal tissue is 75% whereas only 25% is MAO-A (Saura Marti *et al.*, 1990). In terms of pharmacological inhibitors of the MAO enzyme, two types are currently used clinically. These are irreversible and reversible inhibitors. These two groups of MAO inhibitors contain two subgroups; selective inhibitors, which inhibit only one MAO enzyme isoform and non-selective inhibitors which inhibit both MAO isoforms. Irreversible inhibitors are used to “knock out” the MAO enzyme fully (Yamada & Yasuhara, 2004). This means that MAO enzyme activity only resumes once the enzyme has regenerated, consequently, the inhibition of MAO activity is dependent on both the pharmacokinetics of the inhibitor and the ability of the body to regenerate the enzyme, which can take up to three weeks (Pfizer.Inc., 2007). Unlike irreversible inhibitors, the bond between the reversible MAO inhibiting drug and the MAO enzyme

is reversible and, therefore, the inhibition relies solely on the pharmacokinetics and affinity to the enzyme of the inhibiting drug. For example, phenelzine, which is one of the last remaining non-selective, irreversible MAO inhibitors still prescribed for anxiety and depressive illness to patients who are non-responsive to more modern serotonin re-uptake inhibitors, has an elimination half-life of 11.6 hours and the inhibition of MAO enzyme lasts up to three weeks (Pfizer.Inc., 2007). In contrast, Toloxatone, a reversible selective inhibitor of MAO-A, inhibits MAO-A for only six hours before activity returns to baseline values (Berlin *et al.*, 1990). Previous chapters have outlined the efficacy of the Blackadder juice drink in the inhibition of both MAO isoforms in the periphery and potentially for MAO-B centrally. Chapter three of this thesis also outlined a significant reduction in blood prolactin after consumption of the Blackadder blackcurrant juice drink, which can be representative of central dopaminergic tone, with reductions of ~55% two hours post-supplementation. These findings are also consistent with inhibition of prolactin secretion by the central dopamine receptor D2 agonist Bromocriptine, 12mg of which reduces peripheral prolactin by ~60% two hours post-dose (Luciana *et al.*, 1998). Bromocriptine is prescribed to treat neurodegenerative diseases such as parkinsonism (Korczyn *et al.*, 1999) and neuroendocrine issues such as hyperprolactinemia (Crosignani, 2006) via antagonism of dopamine D2 receptors on normal and tumour lactotrophs (Tansey & Schlechte, 2001)

The active compound or compounds contained in the Blackadder juice drink responsible for the inhibition of MAO and the reduction in prolactin levels in previous chapters is not yet elucidated and the biphasic nature of blackcurrant phytochemical absorption (Jin *et al.*, 2011) indicate that there could be time related effects of MAO inhibition with potential for more than one maximal inhibition time point. The pharmacodynamics of the inhibition are of great interest, to assess the maximal levels of MAO inhibition *(Cmax)*, the time to this maximum inhibition *(Tmax)* and the total duration of the MAO inhibition. This data is also invaluable with regards to the timing of

dosing, and safety. For example, the inhibition of both MAO isoforms inhibits the degradation of dietary amines in the digestive tract. It is know that if both isoforms are inhibited for prolonged periods tyramine can accumulate to dangerous levels potentiating a hypotensive crisis (tyramine effect). As data pertaining to the duration of the inhibition is essential, a measure will, therefore, be taken 24 hours post-supplementation.

Results from chapter two illustrate a modulation of blood glucose after consumption of the blackcurrant extracts. Glucose levels were greater at 60 and 150 minutes post-supplementation of the Blackadder juice. Although the major phenolic constituents of blackcurrants are anthocyanins, there are less abundant phenolic structures present in smaller quantities. There is evidence to show that when phenolic compounds are consumed orally with glucose tolerance test the rate and pattern of glucose uptake is modulated in rats. This pattern of modulation was not seen after intravenous administration, potentially outlining an attenuation of glucose absorption from the small intestine (Bassoli *et al.,* (2008). Further to this, Manzano & Williamson (2010) demonstrated an inhibition of sodium-glucose transport proteins and glucose transporter two in Caco2 cell monolayers after exposure to apple phenolic acids, supporting a reduction of glucose transport from the intestinal lumen after supplementation of phenolic acids. It is believed that data presented in chapter two could be a misrepresentation of the pattern of glucose absorption. Instead of increasing overall absorption, it is hypothesised that like apple juice (Johnston *et al.*, 2002), the blackcurrant drink reduces the post-prandial peak in blood glucose level resulting in a slower release of glucose from the digestive tract. Therefore, the higher blood glucose reading one hour post-supplementation of the Blackadder juice extract compared to control in chapter two may have been due to a slowing of glucose uptake rather than an overall increase in blood glucose. It is, therefore, important that the current study investigates more frequent time points to establish a pattern of glucose modulation

after consumption of the Blackadder juice extract. In addition, as blood lactate is a substrate of glucose metabolism and, therefore, rises with the presence of glucose in peripheral blood (Jackson *et al.*, 1973), blood lactate will be used as a measure to ensure that reduced glucose levels are due to a slowing of glucose absorption, rather than an increased utilisation.

There is an increasing body of data outlining the ability of nutritional interventions to modulate haemodynamics in humans. Chapter four of this thesis also highlighted a hemispheric-dependent modulation in cerebral haemodynamics after ingestion of the Blackadder juice. It is theorised that given that nitric oxide synthesis plays a major role in the modulation of localised blood flow in the neural tissue of animal models (Kitaura *et al.*, 2007), the pattern of increased oxyhaemoglobin and total haemoglobin in the left hemisphere during cognitive tasks, could indicate intervention-dependent, nitric oxide synthesis driven neurovascular coupling in the frontal cortex. Berry anthocyanins have been shown to induce endothelial dependent relaxation facilitated by up regulation of eNOS (Andriambeloson *et al.*, 1998) and to inhibit iNOS (Chen *et al.*, 2001) *in vitro*. Published *in vivo* human intervention studies, however, reveal contrasting results after supplementation of blackcurrant extracts. For example, Jin *et al,.* (2011) found no effects upon flow mediated dilation after acute supplementation of a 20% blackcurrant drink containing 52mg of polyphenols, the majority of which were phenolic acids. Whereas Matsumoto *et al.,* (2005a) showed increases in skin blood circulation after supplementing eight females with 140mg of blackcurrant polyphenols, containing 50mg of anthocyanins. A further study by Matsumoto *et al.,* (2005b) showed that supplementing 17kmg/kg of body weight of blackcurrant polyphenols increased peripheral muscle blood flow as measured by NIRS, and reduced shoulder stiffness during typing. Although there are methodological differences between these studies in relation to the haemodynamic methods, there are also major difference between phenolic profiles of the intervention drinks, most noticeably the amount and type of

anthocyanins and phenolics making a direct comparison between studies impossible. Although the specific literature outlining effects of blackcurrants upon peripheral haemodynamics seems to be ambiguous, endothelium dependent vasodilation and large artery stiffness have been shown to be positively affected by cocoa consumption in flow mediated dilation studies two hours post-dose (Heiss *et al.*, 2007; Faridi *et al.*, 2008a) using non-invasive methods, such as digital pulse amplitude tonometry in the index finger of healthy young (Fisher *et al.*, 2003) and healthy aged (Fisher & Hollenberg, 2006) adults. It is therefore reasonable to suggest that, as well as modulation of cerebral blood flow, a blackcurrant intervention with high levels of polyphenols, such as the Blackadder juice extract, could affect peripheral haemodynamics as measured via a non-invasive digital pulse amplitude tonometry method allowing several non-invasive measurements throughout a set time frame.

Given the evidence above, in order to create a sound platform for future behavioural research, it is necessary to further investigate the physiological findings from the previous investigational chapters of this thesis. In an attempt to establish a "therapeutic window", the current study will assess the $T_{max}$ and time course of the MAO-B inhibition outlined in chapters two and three of this thesis. The 500mg dose of the Blackadder juice extract will be used as this gave the most constant and highest inhibition of MAO-B. Further to this, a time scale of plasma prolactin levels, which have been shown to be reduced after supplementation of the Blackadder juice extract, will be investigated. Thirdly to extend the effects of the Blackadder juice extract upon blood glucose levels, a full post-prandial blood glucose profile will be taken following supplementation of the intervention drink. Finally, the effect of the Blackadder juice extract upon endothelium dependent vasodilation and large artery stiffness as measured by digital volume pulse will be investigated.

## 5.2 Materials and methods

### 5.2.1 Design

The project investigated the acute effects of a blackcurrant drink made from pressed blackcurrant berries (blackcurrant cultivar) standardised to contain 500mg of polyphenols and a sugar matched control drink on, blood platelet MAO-B activity, blood plasma prolactin, blood glucose and peripheral blood flow. Drinks were matched for volume, taste, appearance and sugars. The study followed a double-blind, counterbalanced, placebo controlled, repeated measures design.

Participants were randomly allocated to treatment orders as selected through a Williams Latin Square (Williams, 1949)..

### 5.2.2 Participants

Eight healthy male adults participated in the study. The mean age of the participants who completed the study was 25 years±1.7, with a mean body mass index of 24.99± 0.71 kg/$m^2$.

*Table 5.1 Mean participant characteristics*

| Measure | Average measurement | SD | Range |
|---|---|---|---|
| Age (years) | 25.3 | 4.7 | 20-35 |
| Height (m) | 1.81 | 0.07 | 1.7-1.95 |
| Mass (kg) | 82.31 | 4.73 | 75-89 |
| BMI (kg/$m^2$) | 24.99 | 2.01 | 21-27 |

Participants were recruited using opportunity sampling from Northumbria University, UK. Participants received £70 to recompense them for any expense they may have occurred to participate in the trial. Before participants were enrolled in the study they attended a 20 minute screening session. During this screening session participants gave their signed consent to participate in the study and were screened for any contraindications to the study with the use an exclusion questionnaire (an example can be seen in appendix I). Participants were also asked to confirm that they had never had issues giving blood.

The study received ethical approval from the Northumbria University School of Life Sciences Ethics Committee and was conducted according to the Declaration of Helsinki (1964). All participants gave their informed consent before their inclusion in the study.

### 5.2.3 Treatments

The Blackadder juice which was given to participants in chapter three and four was again used in this present study (Blackadder cultivar, cultivated and processed in 2011 by Plant and Food Research Ltd, New Zealand. As discussed in chapter three, the Blackadder juice extract is a crude juiced extract yielding approximately 70ml of juice containing ~500mg of polyphenols per 100g of fresh fruit, an amount of fruit easily eaten or drank in one sitting ensuring the ecological validity of the study. Participants received two treatment drinks with at least one week washout in between treatments. These drinks contained either 0mg of polyphenols (control) or 500mg polyphenols in the form of a cold pressed blackadder juice extract. Drinks were matched for sugars and taste as outlined in chapter four. The total volume of the drink was made up to 200ml with water. All quantities discussed are based on a 60kg person, drink quantities were calculated per kilo of body weight. A breakdown of anthocyanins and other phenolics in study drinks can be seen in tables 5.2 and 5.3.

*Table 5.2 Anthocyanins and other phenolic compounds in each of the treatment conditions (mg per 60kg of bodyweight)*

| Treatment | Anthocyanins mg/60kg | Other phenolics mg/60kg | Total phenolics (mg/60kg) |
|---|---|---|---|
| Control | 0 | 0 | 0 |
| Blackadder | 372.8 | 127.2 | 500 |

*Table 5.3 Anthocyanins and other phenolic compounds in each of the treatment conditions.(mg per kilo of body weight, average dose given (mg) and dose range (mg))*

| Treatment | Anthocyanins (mg/kg) | Anthocyanin average dose (mg) | Dose Range (mg) | Other polyphenols (mg/kg) | Other polyphenols average dose (mg) | Dose range (mg) | total polyphenols (mg/kg) | Total polyphenols average dose (mg) | Dose range (mg) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Blackadder | 6.21 | 511 | 465-555 | 2.11 | 173 | 158-188 | 8.33 | 685 | 624-744 |

### 5.2.4 Peripheral blood flow

Transmission of infrared light through the finger is proportional to blood volume, measurement of which gives a digital volume pulse (DVP). DVP is a non-invasive method of measuring reflective index and stiffness index via a photoplethysmography transducer placed on the figure that can been used as a sensitive index of nitrate bioavailability (Bass *et al.*, 1989). DVP has been shown to be sensitive to NO synthase manipulation (Klemsdal *et al.*, 1994), administration of vasoactive drugs and vascular ageing (Takazawa *et al.*, 1998). There are two factors pertaining to the DVP waveform. The first is formed as a result of the direct propagation of this pulse from the aortic root to the finger (figure 5.1a). As well as travelling down the arm, the direct pulse propagates along the aorta toward the lower body. At every change in diameter and at arterial bifurcations, part of the pulse is reflected back. All these reflections add up and can be considered as a single reflected wave arising from the lower body. This reflected wave travels back up the aorta and then down the arm to the finger to form the second, diastolic, part of the DVP waveform (figure 5.1b). The upper limb provides a common conduit for both the directly transmitted wave and the reflected wave and, therefore, has little influence on the contour of the DVP. Depending on the vascular tone and large artery stiffness, the characteristics of the DVP waveform may change. The PulseTrace PCA2 (Carefusion) was used to derive two indices from the contour analysis of the DVP: the reflection index (RI) and the stiffness index (SI). RI was used to assess endothelial function by measuring its change to potential endothelium dependent vasodilatory properties of the blackcurrant intervention and SI was used as a measure of large artery stiffness.

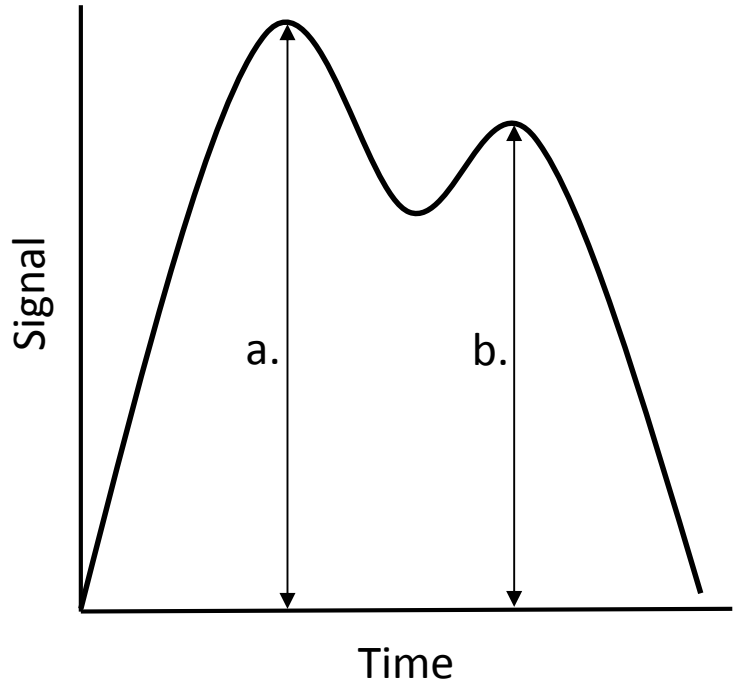



*Figure 5.1 An example DVP waveform. showing (a) pulse from the aortic root to the finger and (b) the reflected wave.*

### 5.2.5 Blood collection and storage

Blood was collected from an inlaying cannula in the left median cubital vein. Venous blood samples (15ml) were collected at baseline and 10 further time points post-supplementation of study treatments. Cannulae and connecting tubes were kept patent with saline solution containing 0.9% sodium chloride (Posiflush, BD Becton Dickinson Ltd, Oxford, UK). Samples were collected in either BD vacutainers© (Becton, Dickinson and company) or a with a 20µl end-to-end capillary (EKF Diagnostics). Vacutainer receptacles were treated with anticoagulants, one with lithium heparin (LH) and one with (EDTA). Blood samples were kept on ice and centrifuged within 30 minutes of collection. Whole blood samples treated with LH and EDTA were prepared for storage using the methods described in section 2.2.5. Prepared samples were stored at -80ºC until analysis was performed.

#### 5.2.5.1 MAO-B analysis

Isolated platelet pellets were tested for MAO-B activity using the same methods as described in section 2.2.5.1.

#### 5.2.5.2 Prolactin analysis

Prolactin analysis was measured by diagnostic Medlab, Auckland, New Zealand. Prolactin was analysed in 300µl of blood plasma collected in LH treated vacutainers and prepared for storage using the method outlined in section 2.2.5.4

#### 5.2.5.3 Glucose and lactate analysis

Twenty microlitres of whole blood was collected from the inlaying cannula in an end-to-end capillary (EKF Diagnostics) and immediately transferred into an EKF safe-lock cup prefilled with 1ml of haemolysis solution. The whole blood and haemolysis solution was then analysed using a Biosen C_line analyser (EKF diagnostics) for glucose (mmol/L) and lactate (mmol/L) within 30 minutes of collection. The manufacturer reports that the Biosen C_line analyser has a coefficient of variance (CV) of 1.5%.

### 5.2.6 Procedures

Participants were required to attend the laboratory a total of three times. The first was a screening visit to ensure eligibility, the second and third were study visits lasting a total of 4.5 hours. On all study day visits, participants arrived in the laboratory at 8am, and firstly gave a baseline DVP measurement after a five minute seated resting period. Participants were then cannulated in the left median cubital vein by a qualified phlebotomist. After a five minute seated rest, a DVP measurement was taken from the left index figure. Fifteen millilitres of blood was then drawn from the cannula using a 5ml vacutainer containing EDTA and a 10ml vacutainer containing LH. Dependent upon treatment allocation, the participant was then randomly supplemented with either the Blackadder juice drink or the matched control. A further 15ml of blood was then removed via the cannula; at; 15, 30, 45 60, 100, 120, 150, 180 and 240 minutes post-supplementation. DVP measurements were also taken in triplicate immediately prior to every cannulation blood sample. Participants were then free to leave. The participant was required to attend a 24 hour follow up visit where a further DVP measurement was

taken and 15ml of blood was removed via venepuncture. A graphical representation of the study running order can be seen in figure 5.2.

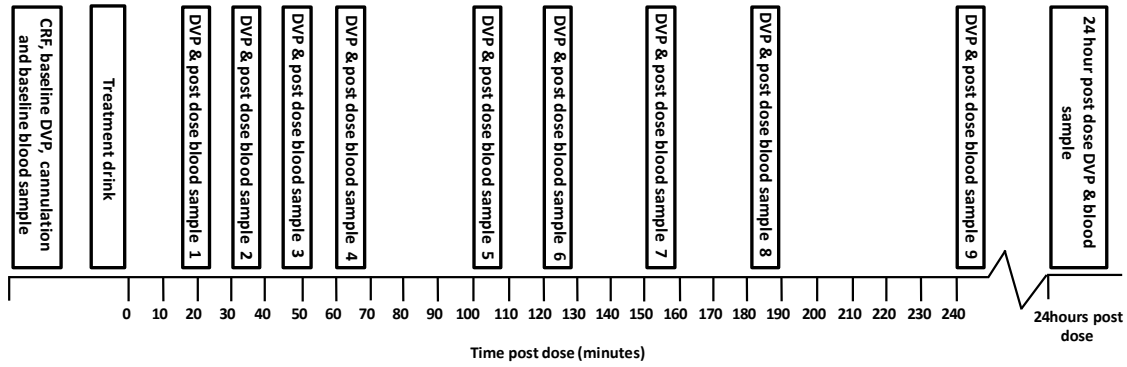



*Figure 5.2 Study day running order. Scale depicts minutes post supplementation of the study intervention. DVP=Digital volume pulse, CRF= case report form.*

### 5.2.7 Statistical analysis

For all outcomes, data was separated for the purpose of two analyses. These were, study day epochs, baseline through to 240 minutes post-supplementation for the purpose of assessing the $C_{max}$ and $T_{max}$ of the biological parameters up to four hours post-dose; and the 24 hour follow up visit to assess if values had returned to baseline 24 hours post-dose.

For blood parameters MAO-B activity, prolactin, glucose and lactate, an estimation of the area under the curve (AUC) was calculated using the incremental trapezoidal method. Data points below quantification (zero) were replaced with zero. AUC was calculated incrementally from time point; 0 to 15, 15 to 30, 30 to 45, 45 to 60, 60 to 100, 100 to 120, 120 to 150, 150 to 180 and 180 to 240 minutes using “unchanged” raw data (iAUC). Increments were then summed to give an area under the curve from time point zero, to the last observed study day concentration at 240 minutes post-supplementation ($AUC_{o\text{-}t}$). DVP parameters and 24 hour assesments were analysed as ‘change from baseline’. In addition to AUC, blood lactate and blood glucose data were also analysed using repeated measures ANOVA of change from baseline data (as used in chapter two of this thesis). This allowed comparisons to be made between

results from chapter two and the current data set so the hypothesis of a blunted blood glucose post-prandial peak could be tested. Both AUC and change from baseline analyses are therefore presented below in section 5.3 where any significant differences were found.

The null hypothesis was rejected with a p value <0.05. Baseline differences were calculated for all measures using a one way (treatment) repeated measures ANOVA. Statistical analysis was performed in the SPSS 18 statistics package using repeated measures ANOVAs (General linear model). Treatment [control and juice] × time point [1 to 9] for all outcomes and a one way repeated measures ANOVA (treatment [control and juice]) for $AUC_{o\text{-}t}$, 24 hour prolactin, 24 hour MAO-B, 24 hour DVP, 24 hour glucose and 24 hour lactate. Pairwise comparisons were conducted on all outcomes with a p value <0.5 from the initial ANOVA to ascertain any differences between treatments for the whole session and at specific epochs. Mauchly`s test of sphericity was used to assess equality of the variances of the differences between factors. Where sphericity had been violated, Huynh-Feldt corrections for non-sphericity were implemented. As only one treatment was being compared to control, no Bonferroni corrections were made.

## 5.3 Results

Prior to analysis of change from baseline data, mean pre-dose scores for control and juice treatments for each outcome were subjected to a one way repeated measures ANOVA. There were no significant differences found between treatments on any measures. Area under the curve data can be found in table 5.4. Mean pre-dose and post-dose change from baseline results for each haemodynamic and blood outcome are presented in table 5.5, 24 hour post-data presented in table 5.6. Unchanged "raw" data tables can be found in appendix X. Outcomes that elicited a significant effect in the initial ANOVA are outlined below and depicted in figure 5.3.

### 5.3.1 Blood glucose

There were no significant differences found for the AUC between treatments with regards blood glucose levels. The repeated measures ANOVA of change from baseline data revealed a significant treatment*epoch interaction [$F(3.65,25.60=3.19$, $p=0.033$]. Pairwise analysis revealed a significant reduction in blood glucose 15 minutes after supplementation of the juice treatment when compared to control ($p=0.027$). There were no significant effects of treatment at the 24 hour time point.

### 5.3.2 Prolactin

The one way treatment repeated measures ANOVA revealed a significant effect of treatment for the $AUC_{o-t}$ outcome prolactin [$F(1,7)=64.92$, $p<0.001$]. This was observed to be a significant reduction in prolactin after supplementation of the juice treatment when compared to control The treatment*increment ANOVA revealed a significant interaction [$F(2.7,18.88)=12.48$, $p<0.001$]. Pairwise analysis of the increments revealed significantly lower iAUC at 45-60 ($p=0.006$), 60-100 ($p=0.001$), 100-120 ($p<0.001$), 120-150 ($p=0.001$), 150-180 ($p=0.001$) and 180-240 minutes ($p=0.001$). There were no significant effects of treatment at the 24 hour time point.

### 5.3.3 Platelet MAO-B activity

The one way treatment repeated measures ANOVA revealed a significant effect of treatment for the $AUC_{o\text{-}t}$ [F(1,6)=61.1, p<0.001]. This was observed to be a significant reduction in AUC platelet MAO-B activity after supplementation of the juice treatment when compared to control The treatment*increment ANOVA revealed a significant treatment*increment interaction [F(2.04,12.28)=34.5, p<0.001]. Pairwise analysis of the increments revealed significantly lower iAUC at 0-15 (p=0.04), 15-30 (p<0.001), 30-45 (p<0.001), 45-60 (p<0.001), 60-100 (p<0.001), 100-120 (p<0.001), 120-150 (p<0.001), 150-180 (p=0.001) and 180-240 minutes (p=0.001). There were no significant effects of treatment at the 24 hour time point.

*Table 5.4 Incremental and total AUC data for the control and juice treatment for blood outcomes MAO-B prolactin, glucose and lactate and ANOVA outcomes*

| iAUC | MAO-B | | Prolactin | | Glucose | | Lactate | |
|---|---|---|---|---|---|---|---|---|
| | Control | Juice | Control | Juice | Control | Juice | Control | Juice |
| 0 to 15 minutes | 17305 | 12757 | 59983 | 46686 | 1.27 | 1.15 | 0.22 | 0.24 |
| 15 to 30 minutes | 16850 | 1375 | 61751 | 43898 | 1.45125 | 1.29 | 0.32 | 0.27 |
| 30 to 45 minutes | 14774 | 62.70 | 42613 | 35083 | 1.2275 | 1.25 | 0.36 | 0.35 |
| 45 to 60 minutes | 15052 | 0 | 32320 | 22589 | 0.96 | 1.07 | 0.33 | 0.37 |
| 60 to 100 minutes | 34648 | 0 | 68240 | 53746 | 2.38 | 2.47 | 0.67 | 0.80 |
| 100 to 120 minutes | 40410 | 0 | 34665 | 27514 | 1.2875 | 1.28 | 0.24 | 0.31 |
| 120 to 150 minutes | 43399 | 1195 | 34906 | 25365 | 1.9775 | 1.98 | 0.33 | 0.40 |
| 150 to 180 minutes | 44436 | 2338 | 61293 | 43778 | 2.03625 | 2.06 | 0.36 | 0.38 |
| 180 to 240 minutes | 91500 | 8833 | 59983 | 46686 | 4.16125 | 4.14 | 0.73 | 0.77 |
| Total AUC | 318379 | 26564 | 49471 | 37333 | 16.75 | 16.72 | 3.60 | 3.92 |
| Effect of treatment | F=61 p<0.001 | | F=64 p<0.001 | | F=0.12 p>0.1 | | F=1.55 p>0.1 | |
| Treatment*increment interaction | F=34 p<0.001 | | F=12 p<0.001 | | F=2.08 p=0.07 | | F=1.58 p>0.1 | |

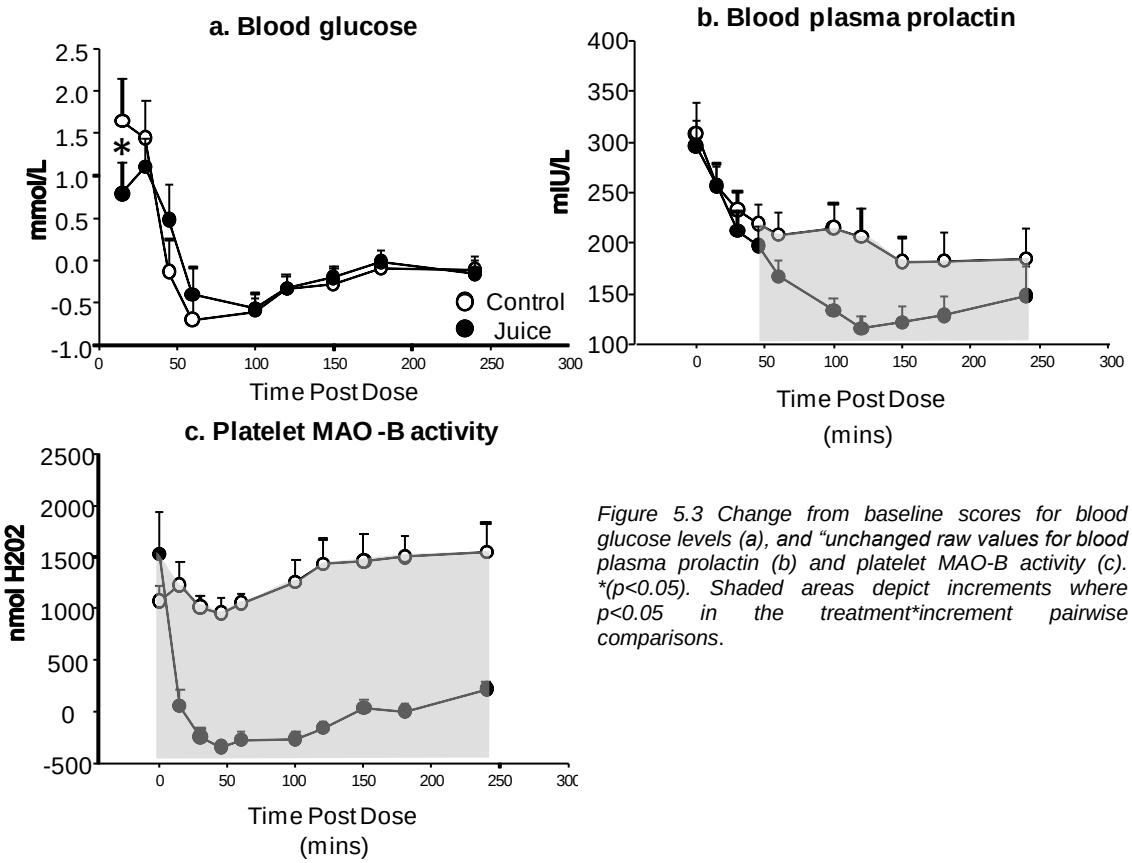



*Figure 5.3 Change from baseline scores for blood glucose levels (a), and "unchanged raw values for blood plasma prolactin (b) and platelet MAO-B activity (c). *(p<0.05). Shaded areas depict increments where p<0.05 in the treatment*increment pairwise comparisons*.

*Table 5.5 Mean pre-dose baseline and change from baseline scores, standard deviations and ANOVA outcomes for all haemodynamic parameters*

| Measure | Treatment | N | Baseline | | 15 minutes | | 30 minutes | | 45 minutes | | 1 hour | | 1.5 hours | | 2 hours | | 2.4 hours | | 3 hours | | 4 hours | | Effect of treatment | Treatment*epoch interaction |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | | |
| Heart rate (BPM) | Control | 8 | 60.5 | 11.22 | 0.88 | 4.55 | 4.75 | 11.8 | 5 | 3.42 | 5 | 4.54 | 2.38 | 6.86 | 0.5 | 4.34 | -4.5 | 4.87 | -2.38 | 5.5 | -1.88 | 5.11 | F=0.16 p>0.1 | F=1.60 p>0.1 |
| | Juice | | 63.63 | 8.52 | 1 | 6.19 | -3.13 | 3.68 | -1 | 6.39 | 0.63 | 4.17 | 1.75 | 2.25 | 0.13 | 6.22 | -5.75 | 4.71 | -4.38 | 2.62 | -7.5 | 3.38 | | |
| DVP - SI | Control | 8 | 5.71 | 1.08 | -0.12 | 0.72 | 0.73 | 2.9 | -0.18 | 0.52 | 0.06 | 0.65 | -0.39 | 0.71 | -0.25 | 0.48 | -0.3 | 0.88 | -0.31 | 0.89 | -0.52 | 0.77 | F=0.51 p>0.1 | F=1.62 p>0.1 |
| | Juice | | 6.63 | 0.97 | 0.34 | 0.45 | -0.08 | 0.45 | 0.48 | 1.27 | 0.12 | 0.97 | 0.1 | 0.88 | 0.07 | 0.74 | -0.22 | 0.73 | 0.01 | 0.38 | -0.29 | 0.6 | | |
| DVP - RI | Control | 8 | 61.88 | 16.34 | 7.13 | 12.15 | -4.88 | 7.02 | 3.75 | 15.97 | 5.75 | 7.23 | -5.88 | 10.91 | -0.75 | 5.85 | 5.13 | 13.57 | 5.5 | 17.39 | -1.75 | 21.47 | F=1.23 p>0.1 | F=1.29 p>0.1 |
| | Juice | | 69.13 | 12.98 | 7.25 | 9.59 | -1 | 11.34 | -7.38 | 13.88 | -4.38 | 12.77 | -4.88 | 15.49 | -5.88 | 17.33 | -2.88 | 12.47 | -1.63 | 11.31 | -4.5 | 10.24 | | |
| Glucose (mmol/L) | Control | 8 | 4.25 | 0.47 | 1.65 | 1.4 | 1.45 | 1.24 | -0.14 | 1.06 | -0.7 | 0.75 | -0.6 | 0.42 | -0.33 | 0.45 | -0.27 | 0.48 | -0.09 | 0.39 | -0.11 | 0.44 | F=0.028 p>0.1 | F=3.19 p=0.033 |
| | Juice | | 4.23 | 0.44 | 0.79 | 1.04 | 1.1 | 0.93 | 0.48 | 1.17 | -0.41 | 0.92 | -0.57 | 0.5 | -0.33 | 0.42 | -0.2 | 0.38 | -0.02 | 0.41 | -0.16 | 0.46 | | |
| Lactate (mmol/L) | Control | 8 | 0.72 | 0.24 | 0.33 | 0.63 | 0.68 | 0.55 | 0.6 | 0.49 | 0.47 | 0.33 | 0.07 | 0.23 | -0.05 | 0.16 | -0.04 | 0.13 | 0.03 | 0.14 | -0.02 | 0.16 | F=1.72 p>0.1 | F=2.27 p=0.069 |
| | Juice | | 0.93 | 0.37 | 0.06 | 0.24 | 0.33 | 0.28 | 0.6 | 0.39 | 0.53 | 0.38 | 0.05 | 0.23 | -0.05 | 0.26 | -0.17 | 0.37 | -0.14 | 0.28 | -0.18 | 0.41 | | |

*Table 5.6Mean change from baseline scores, standard deviations and ANOVA outcomes for MAO-B, prolactin glucose, lactate and DVP 24 hour parameters*

| Measure | Treatment | N | 24h | | Effect of treatment |
|---|---|---|---|---|---|
| | | | Mean | SD | |
| MAO-B (nmol $H^2O^2$) | Control | 6 | 665.59 | 596.15 | F=2.54 p>0.1 |
| | Juice | | -108.15 | 863.12 | |
| Prolactin (mIU/L) | Control | 6 | 26.16 | 137.91 | F=0.23 p>0.1 |
| | Juice | | 19.88 | 109.28 | |
| Glucose (mmol/L) | Control | 8 | 0.25 | 0.32 | F=0.20 P>0.1 |
| | Juice | | 0.11 | 0.75 | |
| Lactate (mmol/L) | Control | 8 | 0.21 | 0.54 | F=0.041 p>0.1 |
| | Juice | | -0.21 | 0.35 | |
| Heart Rate (BPM) | Control | 8 | 3 | 4.41 | F=2.95 p>0.1 |
| | Juice | | -6 | 8.93 | |
| DVP -SI | Control | 8 | -0.15 | 0.51 | F=0.14 p>0.1 |
| | juice | | -0.01 | 0.71 | |
| DVP -RI | Control | 8 | -6.13 | 14.25 | F=0.041 p>0.1 |
| | Juice | | -4.13 | 12.7 | |

## 5.4 Discussion

The current study details a fast and absolute inhibition of blood platelet MAO-B and a significant reduction in plasma prolactin after consumption of the Blackadder blackcurrant juice extract when compared to control. The data also demonstrate a blunted post-prandial blood glucose peak after consumption of the Blackadder blackcurrant juice extract when compared to control.

The current study illustrates a sustained significant reduction in platelet MAO-B of ~100% when compared to control. This reduction began 15 minutes post supplementation, the first post-dose epoch measured, and continued through to four hours post does, the last day one measurement. MAO-B activity had then returned close to the pre-dose baseline level 24 hours post-dose. These results show an increased inhibition compared to findings in chapters two and three, which revealed an inhibition closer to 85%. Unfortunately, because of the rapid inhibition of the MAO-B enzyme, it is not possible, from these data, to calculate a time to maximal inhibition. It would, therefore, be useful if future studies were conducted using several doses and shorter initial blood collection epochs. The active compound or compounds driving this inhibition are not known. Data in the literature outlines an inhibition of MAO-B by anthocyanins *in vitro* (Dreiseitel *et al.*, 2009a); however, this study used levels 1000 times higher than quantified in plasma after oral consumption. In addition maximal plasma concentrations do not occur until one to two hours post consumption (Mazza *et al.*, 2002), making it unlikely that they explain the effects seen at 15 minutes in the current study. Coupled with the inability of the Delcyan$^{TM}$ treatment (an anthocyanin-enriched extract) to inhibit MAO-B in chapter two, this outlines that at least alone, anthocyanins are not likely to be the compound driving MAO inhibition. No significant effects of the Blackadder blackcurrant juice extract were evident at the 24 hour post-dose follow up visit when compared to control. This result outlines a distinct reversible complete inhibition of peripheral MAO-B activity from the Blackadder juice extract.

When compared to pharmaceutical reversible MAO-B specific inhibitors such as Lazamabemide, the profile of MAO-B inhibition of Blackadder juice bears a remarkable similarity, with rapid inhibition of MAO-B in platelets of >90%, 30 minutes post-dose, maximal inhibition subsiding 16 hours post-dose and full restoration of enzyme activity returning 48 hours post-dose following a 100mg dose (Dingemanse *et al.*, 1997).

As anticipated, peripheral prolactin was significantly reduced after supplementation of the Blackadder juice drink when compared to placebo. Reductions were seen 30 minutes post-consumption, with significantly lower area under the curve increments beginning 45 minutes post-dose and continuing until the last measured concentration at 240 minutes post-dose. The maximal reduction appeared at the 120 minute epoch with a reduction of 61%. This reduction in prolactin coincides with that reported in chapter three, where a reduction of 55% was reported 120 minutes post-supplementation of the 500mg Blackadder juice drink. These findings are also consistent with inhibition of prolactin secretion by the central D2 receptor agonist Bromocriptine, 12mg of which reduces peripheral prolactin by ~ 60% two hours post-dose (Luciana *et al.*, 1998). These reductions in prolactin could have positive implications upon symptoms associated with hyperprolactinaemia, such as libido and sexual health (Buvat, 2003), but more importantly, as predominant control of prolactin is via hypothalamic inhibition of lactotroph activity and the most important hypothalamic prolactin inhibiting factor is dopamine (Ben-Jonathan & Hnasko, 2001), they indicate the possibility of a centrally active inhibition of MAO after ingestion of the Blackadder juice extract.

With regards to blood glucose modulation, it was hypothesised in chapter two that the time points used did not allow for a clear representation of the effect of the blackcurrant intervention upon post-prandial glucose profiles. As hypothesised, supplementation of the Blackadder juice significantly reduces the post-prandial peak of blood glucose, and

delays the peak by 15 minutes when compared to the control beverage, as shown in figure 5.3a. Although further time points were not significant a modulation of blood glucose occurs until 100 minutes post-consumption of the Blackadder extract with decreased levels until ~35 minutes post-dose then increased blood glucose levels until 100 minutes post-dose where glucose levels return to a level similar to control and remain that way until the last measured time point at 240 minutes post-dose. Blood glucose followed a similar pattern of modulation described by Törrönen *et al.,* (2012) and Wilson *et al.,* (2008), with reduced levels from the first post-dose measurement at 15 minutes until one hour post-dose. Peak plasma glucose levels published by Törrönen *et al.,* (2012) were, however, later than the findings in the current study with peaks at ~40 minutes post-consumption. However, Törrönen *et al.,* (2012) used sucrose meal, a disaccharide composed of the monomers fructose and glucose. Therefore, some digestion would need to take place for the glucose molecule to be cleaved from the sucrose compound before it could be transported to the blood stream, slowing absorption in both the active and control treatments. In the current study, glucose findings are coupled with a non-significant trend towards reduction in post-prandial lactate 15 minutes post-supplementation of the Blackadder blackcurrant juice extract when compared to control. As lactate is a by-product of glucose metabolism, this further supports the hypothesis that the pattern of glucose modulation was a result of slowed glucose absorption rather than an increase in metabolism (a graphical representation of this can be seen in appendix VII). Although samples in the current study were taken at regular time points via cannulation, it must be noted that the infrequent samples make the assumption that the modulation in blood glucose is linear between each sample. It would be beneficial to use such methods as interstitial continuous glucose monitoring as used by Dye *et al.,* (2010) to allow for a full “real time” profile of the effects of the blackcurrant extract upon blood glucose to be monitored.

In contrast with Matsumoto *et al.,* (2005a; 2005b), measures of endothelial dependent peripheral haemodynamics were not impacted by the Blackadder juice drink. However, Matsumoto *et al.,* (2005b) used a different technique, NIRS, and measured blood flow during typing. This measurement of blood flow could indicate an increase in haemodynamics due to an increased demand because of the typing task used. Therefore, like neuronal findings in chapter four this may suggest that a metabolic demand must be placed on the area, to increase blood flow, before a treatment effect can be seen. The results from the current study are in line with findings by Jin *et al*., (2011) who showed that measures of endothelial dependent peripheral haemodynamics were unchanged after consumption of a 20% blackcurrant drink. However, it must be noted that the dose of blackcurrant juice used by Jin *et al.,* (2011) contained majorly phenolic acids (80mg) whereas the extract in the current study contained a broad profile of polyphenols, including 372mg of anthocyanins. Therefore, direct comparison cannot be made. In light of results presented in chapter four of this thesis, it is, however clear that, more structured research needs to be conducted in relation to the effect of blackcurrants upon peripheral and cerebral blood flow, utilising standardised techniques, consistent methodologies and standardised extracts from varying post-harvest preparations at different doses.

In conclusion, along with a growing body of blackcurrant polyphenol pharmacokinetic data in the literature, these data provide invaluable pharmacodynamic information pertaining to the impact of the Blackadder juice upon blood glucose, platelet MAO-B and peripheral prolactin; therefore, creating a sound platform and an indication of a therapeutic window for future behavioural and physiological methodologies to be based.

# CHAPTER 6. THE SHORT TERM EFFECTS OF TWO BLACKCURRANT EXTRACTS ON MOOD AND COGNITION IN MIDDLE AGED ADULTS

## 6.1 Introduction

Chapter three of this thesis indicated no improvements upon any aspects of working memory, spatial memory, verbal memory, retrieval memory or motor processing outcomes in healthy young adults after acute supplementation of the Blackadder juice drink extract standardised at 125mg, 250mg or 500mg of total polyphenols. Data from the initial intervention study in this thesis, however, suggested that acute supplementation of blackcurrant extracts can attenuate reductions in cognitive behaviour associated with mental fatigue in healthy young adults, in particular, attention. It is hypothesised that the null effects of the Blackadder juice extract on memory in chapter three of this thesis could be associated with the age of the cohort used. These healthy adult participants were likely to be performing near to their cognitive peak (Salthouse, 2009), and unlike the initial intervention study, participants may not have been mentally fatigued to a high enough level to reveal subtle cognitive benefits. As discussed in previous chapters, epidemiological data suggest that diets high in fruit based phytochemicals, particularly flavonoids, can attenuate age related reductions in cognition (Letenneur *et al.*, 2007; Nurk *et al.*, 2009; Devore *et al.*, 2012; Kesse-Guyot *et al.*, 2012). Shorter, chronic, intervention studies in elderly humans have also demonstrated a very mild attenuation of age related cognitive decline, particularly with respect to episodic memory, recall memory, visual attention, visuo-spatial and verbal memory (Krikorian *et al.*, 2010a; Krikorian *et al.*, 2010b; Krikorian *et al.*, 2012). No such reports have been published in the literature in relation to acute studies or in a middle aged population.

Decrements in memory, spatial visualisation and reaction times have been shown by the age of 50 (Salthouse, 2009). These age related cognitive declines are coupled with age related changes in several neurobiological variables such as; reductions in

serotonin receptor binding (Sheline *et al.*, 2002), reductions in striatal dopamine binding (Volkow *et al.*, 2000), increases in brain MAO activity (Fowler *et al.*, 1997) and reductions in cortical blood flow (Leenders *et al.*, 1990); all of which are theorised to play a role in natural cognitive decline and could, therefore, be therapeutic targets in the battle against age related cognitive decline. Chapters two, three and five of this thesis have demonstrated a robust and powerful inhibition of peripheral MAO-A and -B and potentially central MAO-B following acute supplementation of the Blackadder juice extract. Coupled with reductions in levels of peripheral prolactin, at similar levels to that of D2 receptor agonists, it can be theorised that there is a potential inhibition of either or both MAO isoforms within the brain and, therefore, a potential increase in central nervous system levels of dopamine after consumption of the Blackadder juice drink; reductions of which, are thought to be a key factor in cognitive decline associated with natural ageing (Volkow *et al.*, 1998; Bãckman *et al.*, 2000). Unlike most central nervous system enzyme systems, MAO-B presence in neuronal tissue has been shown to increase with age. For example, in a positron emission tomography analysis of 21 participants aged between 23 and 86 years, Fowler *et al*., (1997) reported an increase in brain MAO-B activity of 7% per decade. These increases in MAO activity with age are linked with reduced levels of striatal dopamine and an increase in extracellular $H_2O_2$ (Kish *et al.*, 1992). Further to MAO inhibition, results from chapter four of this thesis highlight modulations of shallow pre-frontal cortical haemodynamics during cognitive demand after consumption of the Blackadder juice extract. As mentioned earlier, reductions in cortical blood flow of up to 0.5% per year are evident during natural ageing (Leenders *et al.*, 1990) and are associated with age related dementia (Dede *et al.*, 2007). Although acute increases in regional cerebral blood flow by a nutritional intervention has not been shown to influence acute cognitive performance in young adults, recent unpublished “pilot” findings from Northumbria University (The Brain, Performance and Nutrition Research Centre) indicated that supplementing five healthy middle aged participants with 500mg of resveratrol increased regional cerebral

blood flow. Additionally, serial three cognitive task correct responses increased, a response to the intervention which has not been seen in a young cohort (unpublished observation, private communication). Although other factors could have influenced the positive findings, this potentially indicates a beneficial effect of the modulation of cerebral haemodynamics upon cognitive performance in a middle aged cohort. Utilising data from previous studies and prior findings from this thesis, the current study will assess the time course of any behavioural effects of the intervention. These facets will be accessed via conducting multiple cognitive assessments throughout the day at time points when MAO activity is known to be inhibited, central blood flow is known to be modulated and time points when the “parent” compounds and biphasic metabolites of the blackcurrant extracts are known to be in the periphery.

There is some evidence suggesting that polyphenol rich fruits can attenuate the onset of natural ageing as outlined in animal models, and long term epidemiological observations indicate slower cognitive decline in cohorts with higher polyphenol intake. It, therefore, makes sense that cohorts who are suffering cognitive and psychomotor decline (which could be associated with reduced dopaminergic tone, reductions in cortical blood flow and increased MAO activity but not to an extent where any pathological effects can be seen), such as the middle aged (Leenders *et al.*, 1990; Fowler *et al.*, 1997; Volkow *et al.*, 1998; Salthouse, 2009), could respond positively to a flavonoid-rich, haemodynamic modulating and MAO inhibiting blackcurrant extract. Acute supplementation could heighten short term cognitive function whilst the long term consumption could delay the onset of age related cognitive decrements later in life. Although measurement of the effect of long term blackcurrant supplementation is not within the remit of this thesis, acute supplementation of the Blackadder juice drink in a middle aged cohort could positively affect dopamine related cognitive tasks that have been shown to be sensitive to natural ageing, such as finger tapping, working memory,

mental flexibility, attention related tasks and response inhibition (Salthouse, 1991; Volkow *et al.*, 1998; Salthouse, 2009).

Previous chapters in this thesis have shown a mild modulation of blood glucose after consumption of the Blackadder juice drink, more specifically, a reduction in immediate post-prandial blood glucose levels. These reductions are theorised to be brought about by the interaction of phenolic compounds with sodium-glucose transport proteins and glucose transporter 2, resulting in a slower uptake of glucose into the blood stream (Manzano & Williamson, 2010). These effects have been documented by other authors acutely after simultaneous consumption of sugars and phenolic rich cranberry juice (Wilson *et al.*, 2008b) and berry purée (Törrönen *et al.*, 2012). However, a later post-dose glucose challenge, without further phenolic supplementation, has not been documented. A secondary focus of this study will, therefore, assess whether a later 10g glucose load in the form of a drink will facilitate a similar pattern of glucose modulation as that seen with simultaneous glucose and blackcurrant polyphenol consumption in chapter five.

Given the above evidence, the current study aims to examine if acute supplementation of blackcurrant extracts in a middle aged population, whom will potentially be exhibiting early age related cognitive deficits, can augment memory, attention and executive function. The study will utilise two active treatments; the Delcyan$^{TM}$ powdered extract used in chapter two of this thesis, which is an anthocyanin-enriched extract and has been shown to have no effect upon central or peripheral MAO-B activity *in vivo;* and the Blackadder cultivar juice extract which contains the full blackcurrant phenolic profile and has been shown to inhibit both MAO isoforms and to modulate cortical haemodynamics *in vivo*. This will allow differentiation between the effects of a berry extract with a MAO-B inhibiting effect and an extract lacking this ability.

## 6.2 Materials and methods

### 6.2.1 Design

The project investigated the acute effects of two blackcurrant extracts on cognition, blood platelet MAO-B activity, monoamines and associated metabolites, blood plasma prolactin and blood glucose. Drinks were matched for volume, taste, appearance and sugars but differed in the amount of total polyphenols. The study followed a double-blind, counterbalanced, placebo controlled, repeated measures design.

Participants were randomly allocated to treatment orders as selected through a Williams Latin Square (Williams, 1949)..

### 6.2.2 Participants

Thirty-nine healthy participants aged between 40 and 60 took part. Of these, 35 participants (9 male, 26 female) aged 53.5±6.03 with an average BMI of 24.4±0.46 completed the study.

*Table 6.1 Mean participant characteristics*

| Measure | Average measurement | SD | Range |
|---|---|---|---|
| Age (years) | 53.4 | 6 | 40-60 |
| Height (m) | 1.65 | 0.07 | 1.5-1.83 |
| Mass (kg) | 67.24 | 10.11 | 49-84 |
| BMI ($kg/m^2$) | 24.40 | 2.70 | 19-31 |

Participants were recruited from the general Newcastle area and received £120 on completion of the study. Participants were required to participate in three study visits with at least one week washout period between visits plus a 90 minute screening session. During the screening session, participants gave their signed consent to participate in the study and were screened for any contraindications to the study with the use an exclusion questionnaire (an example exclusion questionnaire can be seen in appendix I). Participants reported themselves to be healthy, not pregnant, non-tobacco users. Participants were not using dietary supplements or over the counter or recreational drugs (excluding the contraceptive pill), did not have any sensitivities to any of the study treatments and had a body mass index below $35kg/m^2$. Eligible

participants then practiced the study cognitive battery two times to assure familiarity with the study protocol and to ensure minimum scores on all tasks were obtained.

The study received ethical approval from the Northumbria University School of Life Sciences Ethics Committee and was conducted according to the Declaration of Helsinki (1964). All participants gave their informed consent before their inclusion in the study.

### 6.2.3 Treatments

Participants received three treatment drinks with at least one week washout in between visits. These drinks contained either 0mg of polyphenols (control) or 500mg of polyphenols from an anthocyanin enriched powdered blackcurrant extract (Delcyan$^{TM}$, Just The Berries, New Zealand (Delcyan)) or from a cold pressed blackcurrant juice drink, ((Blackadder cultivar, cultivated and processed in 2011 by Plant and Food Research Ltd, New Zealand (Blackadder Juice)). In each case, drinks comprised of 4.7g of glucose, 7.8g of fructose, 5.4g of Splenda® sweetener and 50ml of blackcurrant flavouring (Schweppes blackcurrant flavouring). The total volume of the drink was made up to 200ml with water. Unlike previous intervention chapters in this thesis, drinks were not calculated by kilo of body weight but were instead quantified at 500mg of polyphenols per drink. This was in light of findings from chapter three of this thesis, which highlighted a negligible dose response between 250mg and 500mg when standardised per 60kg of body weight and allowed the efficacy of a standard dose to be investigated, increasing ecological validity of the study.

*Table 6.2 Anthocyanins and other polyphenols in each of the intervention drinks(mg/60kg)*

| Treatment | Anthocyanins (mg) | Other phenolics (mg) | Total phenolics (mg) |
|---|---|---|---|
| Control | 0 | 0 | 0 |
| Juice | 372.8 | 127.2 | 500 |
| Delcyan$^{TM}$ | 483.2 | 16.8 | 500 |

*Table 6.3 Anthocyanins and other phenolic compounds in each of the treatment conditions.(mg per kilo of body weight, average dose given (mg) and dose range (mg))*

| For Treatment | Anthocyanins (mg/kg) | Anthocyanin average dose (mg) | Dose Range (mg) | Other polyphenols (mg/kg) | Other polyphenols average dose (mg) | Dose range (mg) | total polyphenols (mg/kg) | Total polyphenols average dose (mg) | Dose range (mg) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Blackadder | 6.21 | 417 | 308-324 | 2.11 | 141 | 104-178 | 8.33 | 560 | 414-703 |
| Delcyan™ | 8.05 | 541 | 400-679 | 0.28 | 18.82 | 13-23 | 8.33 | 560 | 415-703 |

### 6.2.4 Study tasks

Eleven cognitive measures and two mood scales were delivered using the Computerised Mental Performance Assessment System (COMPASS). Task stimulus was responded to using a peripheral response box (Cedrus RB-530) as shown in figure 2.1. The finger tap task was custom designed using Microsoft Visual Basic (version 1.5) and the digit symbol substitution task (DSST) was completed in pen and paper format. For the purpose of behavioural analysis, cognitive tasks were selected with the intention that a wide variety of cognitive functions could be assessed, including fine motor movement, verbal memory, retrieval memory, spatial memory, response inhibition, attention and cognitive flexibility. Progress through the battery of tasks was controlled by the participant with brief instructions given on screen prior to the start of each task.

**6.2.4.1 Finger tap***:* The finger tap task is one of the most frequently used paradigms to investigate human psychomotor function in neuro-imaging studies (Witt *et al.*, 2008) and is closely related to central dopaminergic tone (Jahanshahi *et al.*, 2010), with decrements in tapping performance observed in dopamine associated neurological diseases (Jahanshahi *et al.*, 2010). The constant finger tapping task, which has been shown to have good intra-person repeatability (Arias *et al.*, 2012), was used as a measure of psychomotor performance in the current study. Participants were required to press the space bar on the computer keyboard as many times as they could in 90 seconds, only using their dominant index finger. The outcome measure was number of taps per 90 seconds.

**6.2.4.2 Bond-Lader mood scales***:* Bond-Lader visual analogue mood scales (Bond & Lader, 1974). Scores from the 16 Bond-Lader visual analogue scales were combined as recommended by the authors to form three mood factors: ‘alert’, ‘calm’ and ‘content’.

**6.2.4.3 Visual analogue scales***:* Participants were asked to subjectively rate how physically energised, mentally energised and mentally fatigued they felt before and after the cognitive tasks. The scale was anchored “Not at all” and “extremely”. With higher scores representing more physically energised, mentally energised and fatigued.

**6.2.4.4 Picture presentation**: A series of 15 photographic images of everyday objects and scenes were presented on the screen at the rate of 1 every 3 seconds, with stimulus duration of 1 second, for the participant to remember.

**6.2.4.5 Word presentation**: Fifteen words, matched for frequency and concreteness, were presented in sequence on the screen for the participant to remember. Stimulus duration was 1 second, as was the inter-stimulus interval. Immediately after, a 60 second timer displayed on the screen.

**6.2.4.6 Immediate word recall:** As a measure of episodic memory, participants were faced with a 60 second clock and instructed to write down as many of the words presented during the word presentation as they could recall. The number of words correctly recalled were scored as a percentage and the number of incorrect recalls were recorded as errors.

**6.2.4.7 Digit symbol substitution working memory task**: (Weschler., 1958) This task is a timed paper and pencil task in which the participant was given 90 seconds to match the numbers 1 to 9 with a specific symbol. The task was scored as number of

correct responses. The DSST is a measure of working memory and speed of processing.

**6.2.4.8 Numeric working memory***:* Five digits were presented sequentially for the participant to hold in memory. This was followed by a series of 30 probe digits for each of which the participant decided whether or not it had been in the original series and pressed the 'YES' or 'NO' response button as appropriate as quickly as possible. This was repeated two further times with different stimuli and probe digits. Mean reaction times were measured in msec, and accuracy of responses to both original and novel (distractor) stimuli were recorded.

**6.2.4.9 Corsi blocks***:* A computerised adaptation of the Corsi blocks (Miner, 1971) task was used to assess visuo-spatial working memory. Nine blue squares were presented on a black background screen. The blue squares then changed to red and back to blue in a random sequence. Participants were asked to follow the sequence and respond via a mouse click. The task began at level four with four of the nine blue squares lighting up in sequence, and was repeated five times at each level. Task difficulty increased in increments with the number of blue squares lighting up as the levels increased. The task ended if the participant did not correctly recall 3 or more sequences on a level. The task was scored as span score derived from the average of the last 3 correctly completed trials.

**6.2.4.10 Peg and ball**: The peg and ball task is a measure of higher level executive function, planning and problem solving. Participants were presented with two configurations of three coloured balls (blue, green, red), a target configuration and a working configuration. The participant’s task was to rearrange the balls in the working configuration so that they matched the position of the balls in the target configuration, moving one ball that did not have a ball above it at a time. Participants were asked to work out the entire planning sequence and calculate the minimum number of moves

required prior to moving the balls using the mouse/cursor. Participants completed a total of 18 trials, comprising six trials each that could be solved in three, four and five moves, respectively, in ascending order of difficulty. Each trial generated scores for planning times prior to moving, time to complete and number of moves made above the minimum required.

**6.2.4.11 Digit vigilance**: A single target digit was randomly selected and constantly displayed to the right of the screen. A series of single digits were presented in the left of the screen at the rate of 80 per minute. The participant was required to press the target button on the peripheral response box as quickly as possible every time the digit in the series matched the target digit. The task lasted two minutes and there were 30 stimulus-target matches. Task outcomes were accuracy (%), reaction time for number of correct responses (msec) and number of incorrect responses.

**6.2.4.12 Stroop**: (Stroop 1935) Participants were presented with a written colour. The colour presented was written in a coloured font which could be the same as the written colour or different. Participants had to indicate the colour of the font using colour buttons on the peripheral response box. Task measures were accuracy (percent correct) and reaction time (msec).

**6.2.4.13 RVIP**: The RVIP task is a measure of sustained attention and working memory. The participant was required to monitor a continuous series of digits for targets of three consecutive odd or three consecutive even single digits. The single digits were presented at the rate of 100 per minute and the participant responded to the detection of a target string by pressing the space bar on the computer keyboard as quickly as possible. The task was continuous and lasted for 5 minutes, with 8 correct target strings being presented in each minute. The task was scored for percentage of

target strings correctly detected, average reaction time for correct detections (msec), and number of incorrect responses (false alarms).

**6.2.4.14 Delayed word recall:** The participant was given 60 seconds to write down as many of the words presented during the word presentation at the start of the cognitive battery. The number of words correctly recalled was recorded as a percentage of the total possible.

**6.2.4.15 Delayed picture recognition**: the original pictures plus 15 distractor pictures were presented one at a time in a randomised order. For each picture, participants indicated whether or not it was recognised as being from the original series by pressing the 'YES' or 'NO' button as appropriate and as quickly as possible. Mean reaction times were measured in msec, and accuracy of responses to both original and novel (distractor) stimuli were recorded.

**6.2.4.16 Delayed word recognition**: The original words plus 15 distractor words were presented one at a time in a randomised order. For each word the participant indicated whether or not it was recognised as being included in the original list of words by pressing the 'YES' or 'NO' button as appropriate and as quickly as possible. Mean reaction times were measured in msec, and accuracy of responses to both original and novel (distractor) stimuli were recorded. The tasks are presented in the order of completion in figure 6.1


*Figure 6.1- 35 minute cognitive battery. Numeric WM=numeric working memory, RVIP= rapid visual information processing, DSST= digit symbol substitution task.*

### 6.2.5 Blood analysis

Venous blood samples (2x5ml) were collected at baseline and six hours post supplementation of treatments. Samples were collected in 5ml BD vacutainers© (Becton, Dickinson and company, Plymouth, New Zealand). Both receptacles were treated with anticoagulants, one with LH and one with EDTA. Whole blood samples treated with LH and EDTA were prepared for storage using the methods described in section 2.2.5.

#### 6.2.5.1 MAO-B

MAO-B analysis was conducted as described in section 2.2.5.1. Some issues arose during analysis of platelet MAO-B analysis where several plates did not work. Therefore, the platelets of a subsection of only five participants were successfully analysed for MAO-B activity. Results must, therefore, be interpreted with caution.

#### 6.2.5.2 Prolactin

Prolactin analysis was measured by diagnostic Medlab, Auckland, New Zealand. Prolactin was analysed in 300µl of blood plasma collected in LH treated vacutainers. Twelve sets of blood samples were available for prolactin analysis, of which two participants were removed from analysis before unbinding due to readings above maximal “normal” levels during at least one visit.

#### 6.2.5.3 Blood glucose and lactate analysis

Twenty microliters of whole blood was collected in an end-to-end capillary (EKF Diagnostics) from a finger prick puncture at seven time points throughout each study session. Filled capillaries were immediately transferred into an EKF safe-lock cup prefilled with 1ml of haemolysis solution. The whole blood and haemolysis solution was then analysed using a Biosen C_line analyser (EKF diagnostics) for glucose (mmol/L) and lactate (mmol/L) within 30 minutes of collection. The manufacturer reports that the Biosen C_line analyser has a coefficient of variance (CV) of 1.5%. All participants who completed the study provided full sets of finger prick blood samples.

### 6.2.6 Procedure

Participants were required to attend the laboratory a total of four times. The first was a 90 minute screening visit to ensure eligibility, the second, third and fourth were study day visits lasting a total of eight hours. On study day visits participants arrived at the research centre at 8am after a 12 hour fast and firstly give a venous blood sample,

finger prick blood sample, and completed the baseline cognitive assessment. (Participants were given the option to opt out of venous blood sampling, which 23 participants opted to do, leaving 12 participants to give samples). Participants were then given their treatment drink which they had five minutes to drink. After a one hour resting absorption period participants completed the post-dose cognitive battery (figure 6.1) 60, 150, 240 and 360 minutes post-supplementation and gave blood glucose samples 50, 140, 180, 200, 230, and 350 minutes post-supplementation. A final venous blood sample was also taken 345 minutes post-supplementation from those participants who opted to do so. Lunch was administered three hours post-dose at ~12pm and comprised pasta and a tomato based pasta sauce. Participants were served 250g of pasta (finished wet weight including sauce providing 750kcals) along with a drink containing 10g of glucose, 50ml of Schweppes blackcurrant cordial and 200ml of water. Participants were instructed to eat all of the pasta and consume all of the drink. The 10g glucose dose was chosen as this is a high enough glucose load to initiate a glucose response, but not high enough to impact cognitive performance (Messier, 2004; Sünram-Lea *et al.*, 2011). Participants were permitted to leave the research centre during breaks between bouts of cognitive assessment with instructions not to participate in any physical activity other than slow/moderate walking and not to consume anything other than water. See figure 6.2 for a diagram of the study day running order.

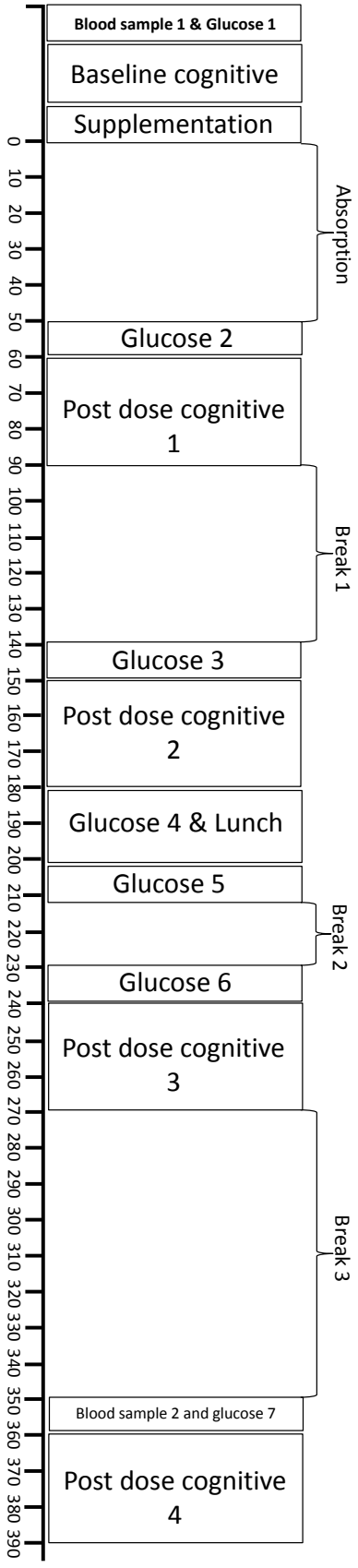



*Figure 6.2 – Study day running order.*

### 6.2.7 Statistical analysis

All ANOVAs were conducted in the SPSS statistics package using “change from baseline” data.

Repeated measures ANOVAs (General linear model); by treatment [control, juice and Delcyan$^{TM}$] × repetition [1 to 4] for all behavioural measures; and treatment [control, juice and Delcyan$^{TM}$] × epoch [1 to 6] for blood glucose and lactate; and treatment [control, juice and Delcyan$^{TM}$] for prolactin and MAO-B. Mauchly`s test of sphericity was used to assess equality of the variances of the differences between factors. Where sphericity had been violated, Huynh-Feldt corrections for non-sphericity were employed. Post hoc comparisons (pairwise) were implemented upon outcomes which evinced a p value less than 0.05 to ascertain any differences between treatments at each measurement. Partial Bonferroni corrections were applied to protect for error against multiple comparisons, therefore the p value was multiplied by the number of treatments being compared to control, in this case x 2. Adjusted p values are presented throughout.

## 6.3 Results

Prior to analysis of change from baseline data, mean pre-dose scores for all three treatments (control, Delcyan$^{TM}$, juice) for each outcome were subjected to a one way repeated measures ANOVA. No significant differences were found.

Mean pre-dose baseline and change from baseline scores for all behavioural and blood parameters are presented in tables 6.4 and 6.5. Unchanged “raw” data tables can be found in appendix X. Significant differences with interpretable effects of intervention are presented in figure 6.3. Only ANOVA results for measures which generated significant effects are reported below.

*Table 6.4 Mean pre-dose baseline and change from baseline scores, standard deviations and ANOVA outcomes for all behavioural parameters*

| Measure | N | Treatment | Baseline | | Repetition 1 | | Repetition 2 | | Repetition 3 | | Repetition 4 | | Effect of treatment | Treatment * repetition interaction |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | | |
| Finger tap number of taps | 34 | Control | 500.4 | 63.1 | 1.39 | 23.6 | 10.38 | 31.7 | 6.97 | 19.9 | 5.63 | 17.1 | F=0.69 p>0.1 | F=0.61 p>0.1 |
| | | Delcyan™ | 508.8 | 51.19 | 3.10 | 24.2 | 2.94 | 20.5 | -2.60 | 20.5 | -3.59 | 13.6 | | |
| | | Juice | 523.1 | 101.9 | 7.91 | 13.51 | 7.63 | 14.8 | 8.91 | 8.93 | 11.35 | 35.9 | | |
| Immediate word recall correct | 34 | Control | 6.03 | 0.61 | -0.07 | 0.95 | -0.11 | 0.98 | -0.14 | 1.13 | 0.07 | 0.95 | F=0.71 p>0.1 | F=0.39 p>0.1 |
| | | Delcyan™ | 6.09 | 0.51 | 0.21 | 0.84 | 0.14 | 1.13 | -0.18 | 0.90 | -0.21 | 0.84 | | |
| | | Juice | 6.31 | 0.53 | -0.03 | 0.87 | 0.18 | 0.90 | 0.11 | 0.98 | 0.03 | 0.87 | | |
| Immediate word recall incorrect | 34 | Control | 0.71 | 0.30 | -0.09 | 1.13 | -0.29 | 1.23 | 0.03 | 1.03 | 0.06 | 1.17 | F=1.76 p>0.1 | F=1.84 p=0.09 |
| | | Delcyan™ | 0.77 | 0.35 | -0.20 | 1.35 | 0.06 | 1.24 | 0.29 | 1.47 | 0.23 | 1.57 | | |
| | | Juice | 0.49 | 0.25 | 0.37 | 0.93 | 0.66 | 1.41 | 0.26 | 1.25 | 0.26 | 1.18 | | |
| DSST correct | 34 | Control | 57.77 | 4.05 | 2.51 | 5.27 | 2.54 | 5.64 | 3.69 | 6.62 | 2.60 | 4.96 | F=2.78 p>0.1 | F=4.74 p=0.037 |
| | | Delcyan™ | 54.91 | 2.69 | 4.23 | 3.81 | 6.34 | 6.52 | 5.54 | 5.80 | 6.29 | 5.44 | | |
| | | Juice | 55.91 | 3.81 | 3.71 | 6.58 | 3.49 | 6.60 | 5.57 | 6.29 | 6.94 | 8.54 | | |
| DSST incorrect | 34 | Control | 0.14 | 0.11 | -0.09 | 0.44 | -0.03 | 0.38 | -0.03 | 0.38 | -0.09 | 0.37 | F=1.62 p>0.1 | F=0.73 p>0.1 |
| | | Delcyan™ | 0.11 | 0.10 | 0.00 | 0.41 | 0.00 | 0.48 | -0.11 | 0.32 | -0.03 | 0.45 | | |
| | | Juice | 0.11 | 0.13 | 0.00 | 0.41 | 0.03 | 0.51 | 0.00 | 0.63 | -0.06 | 0.41 | | |
| Numeric working memory % correct | 34 | Control | 96.36 | 3.90 | -0.42 | 3.62 | 0.24 | 4.45 | -0.39 | 4.88 | -0.85 | 5.14 | F=0.24 p>0.1 | F=0.25 p>0.1 |
| | | Delcyan™ | 95.99 | 3.26 | 0.33 | 3.67 | 0.33 | 3.85 | -0.21 | 4.77 | 0.00 | 4.56 | | |
| | | Juice | 96.13 | 3.48 | 0.48 | 3.95 | 0.09 | 3.02 | -0.15 | 3.73 | -0.06 | 4.57 | | |
| Numeric working memory RT (msec) | 34 | Control | 860.5 | 210 | -24.82 | 126 | -18.55 | 134 | -24.36 | 133 | -25.91 | 114 | F=0.742 p>0.1 | F=0.87 p>0.1 |
| | | Delcyan™ | 834.3 | 231 | -11.36 | 131 | -31.85 | 110 | 20.58 | 310 | -10.21 | 155 | | |
| | | Juice | 867.8 | 255 | -57.21 | 100 | -47.18 | 122 | -42.67 | 146 | -89.06 | 124 | | |
| Corsi blocks RT (msec) | 34 | Control | 6424.48 | 1775 | -354.6 | 1678 | -582.5 | 1863 | -141.4 | 1596 | -610.4 | 1567 | F=0.83 p>0.1 | F=1.23 p>0.1 |
| | | Delcyan™ | 6273 | 1468 | 454.9 | 1428 | -75.65 | 1489 | -125.9 | 1605 | -450.5 | 1130 | | |
| | | Juice | 6360 | 1384 | -121.9 | 1275 | -299.8 | 1135 | -275.2 | 1445 | -376.4 | 1533 | | |
| Corsi blocks span | 34 | Control | 5.87 | 0.93 | -0.10 | 0.84 | -0.23 | 0.82 | -0.11 | 0.79 | -0.20 | 0.82 | F=0.66 p>0.1 | F=1.30 p>0.1 |
| | | Delcyan™ | 5.89 | 0.73 | 0.05 | 0.82 | 0.08 | 0.83 | 0.03 | 0.92 | -0.27 | 0.80 | | |
| | | Juice | 5.87 | 0.94 | 0.04 | 0.93 | 0.20 | 0.69 | -0.17 | 1.35 | -0.02 | 0.99 | | |
| Peg and ball thinking time | 34 | Control | 3129 | 1824 | -317.6 | 963 | -355.9 | 1023 | -510.6 | 880 | -453.8 | 1080 | F=1.539 p>0.1 | F=0.16 p>0.1 |
| | | Delcyan™ | 2939 | 1214 | -82.53 | 486 | -88.38 | 546 | -239.8 | 713 | -176.1 | 582 | | |
| | | Juice | 2934 | 1029 | -138.2 | 556 | -133.9 | 551 | -230.9 | 633 | -268.2 | 546 | | |
| Peg and ball working time (msec) | 34 | Control | 9623 | 2660 | -580.2 | 1498 | -818.0 | 1438 | -850.8 | 1246. | -825.2 | 1584 | F=0.24 p>0.1 | F=0.49 p>0.1 |
| | | Delcyan™ | 9467 | 2091 | -564.5 | 929 | -704.8 | 1033 | -743.8 | 1362 | -731.0 | 1466 | | |
| | | Juice | 9232 | 1952 | -438.0 | 1217 | -381.79 | 1386 | -716.9 | 1409 | -813.94 | 1581 | | |
| Peg and ball errors | 34 | Control | 3.88 | 4.29 | 0.12 | 4.58 | -0.91 | 3.38 | -0.21 | 3.10 | 0.09 | 4.00 | F=0.21 p>0.1 | F=1.11 p>0.1 |
| | | Delcyan™ | 4.35 | 3.89 | -1.26 | 4.22 | -1.65 | 3.95 | -0.29 | 4.31 | -1.03 | 5.22 | | |
| | | Juice | 3.18 | 3.20 | -0.18 | 4.06 | 0.62 | 3.95 | -0.21 | 3.72 | 0.21 | 3.71 | | |
| Digit vigilance % correct | 34 | Control | 95.29 | 4.70 | -2.55 | 9.04 | -1.93 | 9.55 | -4.62 | 9.52 | -1.41 | 8.44 | F=0.24 p>0.1 | F=0.81 p>0.1 |
| | | DelcyanTM | 93.33 | 16.1 | 0.93 | 19.1 | 1.45 | 17.61 | -3.97 | 24.3 | 0.21 | 18.8 | | |
| | | Juice | 95.75 | 5.37 | -1.76 | 9.35 | -1.90 | 8.84 | -2.45 | 7.70 | -0.21 | 6.28 | | |
| Digit vigilance RT (msec) | 34 | Control | 453.1 | 41.6 | 5.93 | 25.1 | 16.55 | 26.0 | 20.28 | 32.9 | 16.86 | 28.9 | F=0.37 p>0.1 | F=1.05 p>0.1 |
| | | Delcyan™ | 448.7 | 51.7 | 22.62 | 57.1 | 22.52 | 66.4 | 24.31 | 65.7 | 23.07 | 59.9 | | |
| | | Juice | 448.1 | 41.2 | 12.45 | 30.8 | 14.55 | 29.3 | 23.10 | 31.3 | 23.79 | 29.1 | | |
| Digit vigilance false alarms (number) | 29 | Control | 0.07 | 0.23 | 0.24 | 0.51 | 0.10 | 0.49 | 0.17 | 0.54 | 0.10 | 0.41 | F=0.57 p>0.1 | F=1.28 p>0.1 |
| | | Delcyan™ | 0.31 | 1.04 | 0.00 | 1.25 | -0.14 | 1.06 | 0.03 | 1.15 | -0.10 | 1.29 | | |
| | | Juice | 0.14 | 0.32 | -0.03 | 0.50 | 0.24 | 1.06 | 0.17 | 0.60 | 0.10 | 0.86 | | |
| Stroop accuracy (%) | 29 | Control | 98.38 | -2.35 | -0.15 | 2.42 | 0.35 | 2.18 | -0.59 | 2.42 | -0.24 | 2.48 | F=0.24 p>0.1 | F=0.49 p>0.1 |
| | | Delcyan™ | 98.38 | -1.99 | 0.32 | 2.02 | 0.38 | 1.96 | -0.06 | 1.87 | -0.03 | 1.71 | | |
| | | Juice | 98.48 | -1.62 | -0.03 | 1.81 | -0.06 | 2.01 | -0.18 | 1.88 | -0.32 | 2.12 | | |
| Stroop reaction time (msec) | 29 | Control | 810.0 | -177 | -18.76 | 106 | -18.76 | 135 | -28.29 | 115 | -33.03 | 146 | F=0.66 p>0.1 | F=1.83 p>0.1 |
| | | Delcyan™ | 788.2 | -131 | -29.38 | 72.7 | -18.15 | 84.0 | -1.21 | 208 | 11.41 | 212 | | |
| | | Juice | 799.4 | -137 | -35.68 | 66.9 | -34.65 | 91.4 | -14.24 | 105 | -63.26 | 82.6 | | |
| RVIP accuracy (%) | 29 | Control | 55.39 | 19.2 | 3.00 | 14.25 | 2.09 | 18.5 | 1.47 | 14.7 | 1.28 | 15.6 | F=0.86 p>0.1 | F=0.24 p>0.1 |
| | | Delcyan™ | 53.67 | 17.7 | 3.91 | 10.62 | 2.56 | 9.82 | 2.38 | 12.7 | 3.81 | 13.6 | | |
| | | Juice | 58.91 | 20.9 | 0.03 | 9.54 | 0.88 | 9.35 | -1.59 | 11.5 | -0.63 | 11.4 | | |
| RVIP false alarms (number) | 29 | Control | 1.31 | 1.2 | 0.34 | 1.72 | 0.25 | 1.83 | 0.03 | 1.67 | -0.19 | 1.54 | F=0.56 p>0.1 | F=0.94 p>0.1 |
| | | Delcyan™ | 1.13 | 1.2 | 0.34 | 1.52 | 0.06 | 1.37 | 0.03 | 1.44 | -0.19 | 1.33 | | |
| | | Juice | 1.59 | 1.8 | 0.00 | 2.37 | -0.59 | 1.95 | -0.16 | 1.64 | -0.22 | 1.81 | | |
| RVIP reaction time (msec) | 29 | Control | 501.5 | 49.9 | 3.69 | 35.1 | 5.28 | 41.6 | -3.63 | 32.4 | 3.34 | 43.1 | F=2.14 p>0.1 | F=1.10 p>0.1 |
| | | Delcyan™ | 507.2 | 46.8 | 0.06 | 31.8 | 4.59 | 32.7 | -0.22 | 39.3 | 5.50 | 42.9 | | |
| | | Juice | 513.3 | 50.8 | -8.50 | 31.8 | -7.47 | 29.7 | -6.38 | 34.1 | -19.25 | 35.5 | | |
| Delayed word recall % correct (number) | 35 | Control | 3.83 | 0.68 | -1.77 | 2.19 | -1.74 | 2.13 | -1.89 | 2.58 | -1.94 | 2.25 | F=0.89 p>0.1 | F=0.78 p>0.1 |
| | | Delcyan™ | 4.03 | 0.51 | -2.06 | 1.79 | -2.26 | 1.70 | -2.09 | 1.98 | -1.66 | 1.71 | | |
| | | Juice | 3.57 | 0.53 | -1.31 | 1.62 | -1.43 | 1.93 | -1.83 | 1.87 | -1.46 | 2.31 | | |
| Delayed word recall incorrect (number) | 35 | Control | 1.17 | 0.37 | 0.17 | 1.18 | 0.00 | 1.29 | 0.26 | 1.73 | -0.17 | 1.13 | F=4.10 p=0.02 | F=2.07 p=0.058 |
| | | Delcyan™ | 1.06 | 0.37 | 0.09 | 1.27 | 0.54 | 1.36 | -0.06 | 1.24 | 0.69 | 2.00 | | |
| | | Juice | 0.54 | 0.61 | 0.91 | 2.18 | 1.11 | 2.15 | 0.94 | 2.15 | 0.71 | 2.26 | | |
| Word recognition RT (msec) | 35 | Control | 972.9 | 218 | 39.29 | 114 | -3.74 | 170 | 33.60 | 209 | -17.26 | 170 | F=4.32 p=0.017 | F=0.89 p>0.1 |
| | | Delcyan™ | 933.3 | 189 | 46.94 | 161 | 34.23 | 133 | 29.00 | 177 | 29.80 | 194 | | |
| | | Juice | 992.1 | 245. | -17.71 | 225 | -23.06 | 194 | -47.00 | 200 | -86.83 | 169 | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Word recognition % correct | 35 | Control | 75.81 | 9.51 | -3.60 | 10.9 | -2.74 | 10.3 | -6.66 | 11.5 | -2.63 | 10.2 | F=1.37 p>0.1 | F=1.58 p>0.1 |
| | | Delcyan™ | 77.71 | 8.61 | -4.60 | 10.2 | -9.40 | 10.1 | -7.29 | 9.24 | -6.94 | 10.1 | | |
| | | Juice | 77.24 | 9.03 | -4.20 | 11.6 | -4.60 | 10.8 | -6.40 | 11.0 | -6.66 | 11.4 | | |
| Picture recognition RT (msec) | 35 | Control | 872.9 | 155 | 9.63 | 131 | 41.77 | 149 | 48.57 | 147 | 14.43 | 144 | F=0.89 p>0.1 | F=1.95 p=0.78 |
| | | Delcyan™ | 830.9 | 120 | 26.57 | 124 | 47.49 | 118 | 83.29 | 144 | 41.74 | 128.3 | | |
| | | Juice | 857.7 | 221 | 40.77 | 180 | -8.49 | 160 | 14.97 | 180 | 9.09 | 142 | | |
| Picture recognition % correct | 35 | Control | 94.95 | 6.39 | -0.46 | 6.11 | -1.94 | 4.63 | -1.91 | 6.72 | -2.77 | 6.45 | F=0.63 p>0.1 | F=1.08 p>0.1 |
| | | Delcyan™ | 95.43 | 5.11 | -0.97 | 6.20 | -0.80 | 5.99 | -3.17 | 7.51 | -3.74 | 7.48 | | |
| | | Juice | 95.43 | 4.37 | -2.49 | 6.39 | -0.86 | 5.22 | -1.77 | 5.71 | -3.37 | 7.13 | | |
| Bond-Lader alert (mm) | 35 | Control | 65.83 | 13.8 | -3.74 | 11.6 | -5.86 | 11.96 | -8.96 | 17.9 | -7.45 | 14.8 | F=0.13 p>0.1 | F=0.69 p>0.1 |
| | | Delcyan™ | 65.34 | 13.3 | -4.71 | 11.4 | -7.88 | 12.02 | -8.91 | 14.9 | -5.56 | 15.0 | | |
| | | Juice | 66.47 | 14.6 | -2.47 | 8.63 | -5.73 | 13.92 | -8.63 | 16.1 | -6.40 | 14.1 | | |
| Bond-Lader content (mm) | 35 | Control | 71.03 | 13.7 | 0.66 | 5.37 | 0.29 | 5.20 | 1.61 | 9.33 | 1.11 | 8.17 | F=1.00 p>0.1 | F=0.77 p>0.1 |
| | | Delcyan™ | 71.77 | 10.9 | -0.74 | 7.54 | -1.06 | 6.84 | -1.37 | 8.24 | 0.42 | 8.40 | | |
| | | Juice | 73.08 | 11.5 | -0.39 | 4.94 | -0.53 | 6.18 | 0.22 | 6.06 | 0.78 | 6.49 | | |
| Bond-Lader calm (mm) | 35 | Control | 61.81 | 14.2 | 2.63 | 10.8 | 1.67 | 9.88 | 6.01 | 11.6 | 3.89 | 9.48 | F=0.45 p>0.1 | F=1.95 p=0.07 |
| | | Delcyan™ | 63.10 | 15.1 | 2.86 | 13.3 | 2.67 | 13.0 | 1.71 | 13.8 | 1.41 | 10.9 | | |
| | | Juice | 63.39 | 13.6 | 0.30 | 8.21 | 2.97 | 11.1 | 3.79 | 11.2 | 1.23 | 9.31 | | |
| VAS fatigue (mm) | 35 | Control | 37.23 | 18.2 | 5.23 | 21.6 | 7.34 | 20.4 | 15.00 | 24.3 | 10.29 | 21.3 | F=0.27 p>0.1 | F=0.70 p>0.1 |
| | | Delcyan™ | 39.69 | 16.4 | 2.89 | 15.8 | 8.34 | 17.3 | 10.37 | 18.6 | 9.80 | 17.6 | | |
| | | Juice | 34.46 | 17.1 | 4.29 | 14.3 | 8.94 | 19.9 | 16.03 | 21.6 | 12.37 | 19.3 | | |
| VAS mentally energised (mm) | 35 | Control | 56.91 | 15.3 | -5.60 | 17.7 | -7.51 | 15.9 | -10.66 | 20.2 | -10.43 | 16.3 | F=0.39 p>0.1 | F=1.10 p>0.1 |
| | | Delcyan™ | 54.63 | 16.7 | -6.97 | 18.4 | -8.37 | 18.8 | -10.06 | 19.0 | -6.26 | 21.2 | | |
| | | Juice | 58.23 | 18.9 | -1.83 | 15.4 | -6.20 | 18.3 | -8.09 | 22.6 | -8.80 | 19.1 | | |
| VAS physically energised (mm) | 35 | Control | 58.46 | 15.2 | -2.40 | 13.3 | -4.57 | 15.2 | -9.31 | 21.2 | -7.23 | 17.3 | F=0.25 p>0.1 | F=1.25 p>0.1 |
| | | Delcyan™ | 56.89 | 15.9 | -3.80 | 19.1 | -7.06 | 19.1 | -7.66 | 15.2 | -2.74 | 21.4 | | |
| | | Juice | 57.03 | 17.8 | -7.09 | 20 | -10.89 | 18.7 | -11.86 | 24.9 | -11.83 | 23.2 | | |

*Table 6.5 Mean pre-dose baseline and change from baseline scores, standard deviations and ANOVA outcomes for all blood parameters*

| Measure | N | Treatment | Baseline | | 60 Minutes | | 140 minutes | | 180 minutes | | 200 minutes | | 230 minutes | | 350 minutes | | Effect of treatment | Treatment * repetition interaction |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | se | Mean | se | Mean | se | Mean | se | Mean | se | Mean | se | Mean | se | | |
| Blood glucose (mmol/L) | 35 | Control | 4.79 | 0.08 | 0.30 | 0.14 | -0.52 | 0.08 | -0.40 | 0.10 | 0.70 | 0.15 | 2.92 | 0.16 | 0.79 | 0.11 | F=0.35 p>0.1 | F=1.22 p>0.1 |
| | | Delcyan™ | 4.80 | 0.09 | 0.53 | 0.15 | -0.48 | 0.09 | -0.36 | 0.12 | 0.82 | 0.17 | 2.90 | 0.18 | 0.76 | 0.12 | | |
| | | Juice | 4.78 | 0.12 | 0.80 | 0.17 | -0.63 | 0.10 | -0.54 | 0.10 | 0.50 | 0.19 | 2.86 | 0.16 | 0.76 | 0.15 | | |
| Blood lactate (mmol/L) | 35 | Control | 1.47 | 0.13 | 0.56 | 0.15 | -0.13 | 0.11 | -0.33 | 0.15 | 0.08 | 0.16 | 0.35 | 0.16 | -0.22 | 0.15 | F=0.24 p>0.1 | F=1.14 p>0.1 |
| | | Delcyan™ | 1.35 | 0.15 | 0.50 | 0.17 | -0.05 | 0.16 | -0.21 | 0.16 | 0.13 | 0.17 | 0.30 | 0.14 | -0.05 | 0.16 | | |
| | | Juice | 1.36 | 0.12 | 0.79 | 0.12 | -0.03 | 0.13 | -0.24 | 0.11 | 0.22 | 0.15 | 0.55 | 0.13 | -0.12 | 0.15 | | |
| MAO-activity (nmol $H^2O^2$) | 5 | Control | 1192 | 446 | -72.96 | 400 | | | | | | | | | | | F=0.742 p>0.1 | |
| | | Delcyan™ | 1090 | 328 | -279.0 | 193 | | | | | | | | | | | | |
| | | Juice | 906.0 | 394 | 229.4 | 282 | | | | | | | | | | | | |
| Prolactin (mIU/L) | 11 | Control | 270.2 | 30.9 | 62.97 | 43.8 | | | | | | | | | | | F=1.05 p>0.1 | |
| | | Delcyan™ | 287.2 | 55.5 | 6.08 | 22.7 | | | | | | | | | | | | |
| | | Juice | 232.4 | 17.4 | 37.75 | 30.1 | | | | | | | | | | | | |

### 6.3.1 Behaviour

#### 6.3.1.1 Delayed word recall

The repeated measures ANOVA revealed a significant main treatment effect for the delayed word recall outcome incorrect responses [F(2,68)=4.096, p=0.021). Pairwise analysis revealed a significant increase in incorrect delayed word recalls after supplementation of the Blackadder juice group when compared to control (p=0.04). See figure 6.3a.

### 6.3.1.2 Word recognition

The repeated measures ANOVA revealed a significant treatment effect for the word recognition reaction time [F (1.63,55.66)=4.32, p=0.024). Pairwise analysis revealed no significant differences between either active treatment and placebo.

### 6.3.1.3 DSST

The repeated measures ANOVA revealed a significant treatment*repetition interaction for DSST correct responses [F(5.06,172.22)=2.92,p=0.047). Pairwise analysis revealed a significant increase in correct responses after supplementation of the Delcyan$^{TM}$ drink at repetition 2 (p=0.042) and 4 (p=0.02) when compared to control. See figure 6.3b.

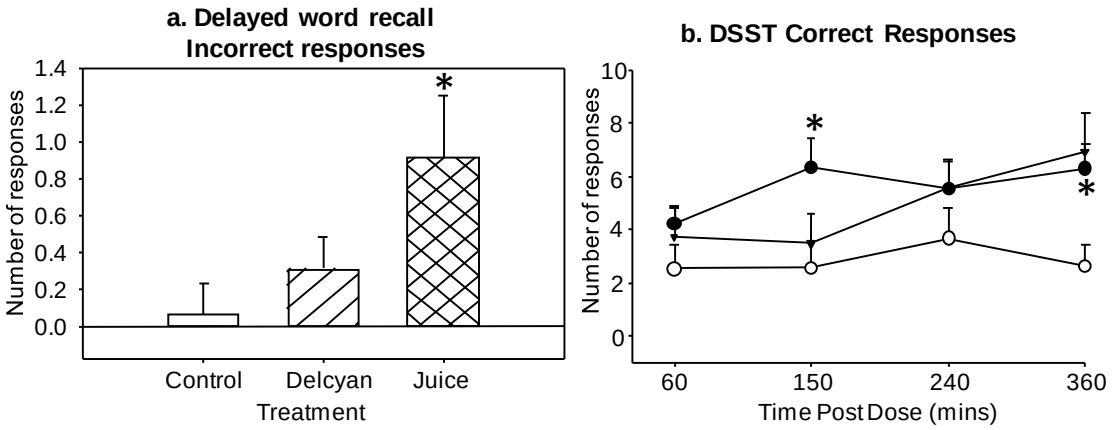



*Figure 6.3* Delayed word recall incorrect responses (a) and DSST correct responses (b). Time points which are significantly different to control are labelled. (*p<0.05)

## 6.4 Discussion

The main aims of the current study were to assess if acute supplementation of blackcurrant extracts can augment memory, attention and executive function in a middle aged population, likely to be exhibiting natural age related declines in cognitive function. To account for the biphasic nature of polyphenol pharmacodynamics, and to assess the time course of any behavioural effects, multiple cognitive assessments were undertaken throughout the day after consumption of the extracts. The study utilised two active treatments, Delcyan$^{TM}$ powdered extract and the Blackadder juice extract. Definitive effects upon memory and executive function were not evident after supplementation of the Delcyan$^{TM}$ or Blackadder juice blackcurrant in healthy middle aged adults.

Supplementation with the Delcyan$^{TM}$ treatment produced a significant increase in correct responses in the DSST task at post-dose repetition two, two hours post-dose, and repetition four, six hours post-dose, with an increase of ~6 correct responses at each time point when compared to control. As age related decreases in DSST scores are primarily related to decreased processing speed, rather than decrements in memory (Salthouse, 1992), positive acute effects of the Delcyan$^{TM}$ treatment upon the DSST task could be associated with an increase in processing speed, rather than a direct augmentation of working memory. Supplementation of the Blackadder juice drink produced an increase in incorrect responses during the delayed recall task. The main increase in incorrect responses came at repetition two, two hours post supplementation with a mean increase of ~1 incorrect response compared to control. Although only a trend on the main ANOVA, this decrement is partially supported by an increase in incorrect responses evident at repetition two of the immediate word recall task (with an increase of ~1 incorrect response) after supplementation of the Blackadder juice drink when compared to control. Both results indicate decrements in episodic memory after supplementation of the Blackadder juice drink in a middle aged cohort. This negative

impact is difficult to explain but it must be considered that, given the number of outcomes, these effects (both positive and negative) are highly likely to be type 1 errors.

A major factor driving the hypothesis of improved cognitive performance in middle aged adults after supplementation of the blackcurrant extracts, was the inhibition of MAO enzymes observed post consumption of the Blackadder juice extract in previous chapters of this thesis. This extract had no positive effect upon behavioural paradigms in the current study and also no inhibition of MAO-B was observed following either blackcurrant extracts when compared to control. This inhibition, by the Blackadder juice extract, has proven to be robust throughout earlier intervention chapters in this thesis. As the present study and the latter part of study four were run together, and results from all participants in chapter four show a powerful inhibition of MAO-B, there is no doubt that the compound or compounds driving the inhibition are present and active in the Blackadder juice extract. There are several reasons which could pertain to the null findings, these are 1) the sample size, 2) the administration of lunch and 3) the time of which the sample was taken. Due to issues with the MAO-B Amplex-Red assay, only five sets of blood samples were successfully analysed for MAO-B activity. Although the inhibition outlined in chapters two, three and five showed a robust effect of Blackadder juice extract upon MAO-B activity, the small N size cannot be ruled out as a potential reason for the lack of positive findings. Additionally, due to the time needed to be spent in the laboratory (8 hours) after an overnight fast, participants were fed a pasta meal 180 minutes post supplementation. Although food interactions with MAO inhibitors are well documented (i.e. the tyramine effect), no such information is available regarding food intake and potency reductions. It was, therefore, not thought that the lunch would impact the inhibition. The final issue is the timing of the post-dose blood sampling. In the current study, MAO-B activity was measured six hours post consumption of the blackcurrant extracts, whereas, previously the last time point where MAO-B was shown

to be significantly inhibited by supplementation of the Blackadder juice drink was at four hours post-dose (the last study day dose measured). However, as the level of inhibition was at such a level (100%) and as MAO-B activity had not fully returned to baseline after 24 hours, it was expected that the MAO inhibition would still be evident six hours post-dose. Although age related sensitivities to doses of pharmacological MAO inhibitors are not evident in the literature, it must also be noted that the inhibition of MAO by a blackcurrant extract has not been quantified in a middle aged cohort. Since the presence of central MAO-B increases at a rate of 7% per decade (Fowler *et al.*, 1997), a higher dose may be needed to elicit an inhibitory response in an older cohort. This highlights the need for further research focusing upon dose ranges and further post-dose time points, in a similarly designed study to chapter five of this thesis, to allow the efficacy of MAO inhibition via consumption of the Blackadder juice extract to be determined in a healthy middle aged population. Levels of blood plasma prolactin were not reduced in the current study after consumption of either blackcurrant extract. Plasma prolactin was shown to be reduced after consumption of the Blackadder blackcurrant juice extract standardised at a dose as low as 125mg of polyphenols in chapter three of this thesis. Therefore, the lack of a reduction after the consumption of the Blackadder juice treatment in the current study is more than likely associated with the null effects upon MAO-B.

Despite not being significant, it is important to discuss the fact that blood levels of glucose followed a similar pattern to that observed in chapter two and were ~0.5mmol/L higher after supplementation of the Blackadder juice drink 60 minutes post-supplementation of the Blackadder juice extract. Due to the number of fingertip blood samples taken throughout the session it was not possible to measure earlier time points in the present study, it was outlined in chapter five that this increase in blood glucose is a result of a lower post-prandial peak in blood glucose associated with the intervention. An aim of the study was to assess if a similar pattern was observed after a

glucose load at a later time point, in the case of this study, three hours post-supplementation of the study drinks. This pattern of glucose modulation was not observed a second time at the later post-supplementation, potentially highlighting that a glucose load needs to be co-consumed with phenolic compounds for the modulatory effect to be seen. A graphical representation of this data can be seen in appendix VII.

In conclusion, results from the current study reveal no reliable positive effects upon memory or psychomotor function of either the Blackadder or Delcyan$^{TM}$ treatment. This may outline the potential need for chronic consumption of polyphenol rich extracts for positive modulations to be observed. There were no measured effects upon MAO-B activity and blood plasma prolactin in a middle aged cohort six hours post supplementation. Caution must be used when interpreting the MAO-B data due to the discussed methodological issues. More research needs to be undertaken to measure the chronic effects of a blackcurrant extract upon cognitive performance in middle aged adults. Further examinations of the pharmacodynamics of MAO-B inhibition in a middle aged cohort also need to be carried out, so the MAO inhibitory efficacy of the Blackadder juice extract can be determined.

# CHAPTER 7. DISCUSSION

## 7.1 Summary of the objectives of the thesis

In recent times, self-medication through “off the shelf” herbal products, derived from functional foods, has increased to such a level that over 20% of the North American populous use them daily (Bent, 2008). There is, therefore, an increasing interest in the development of nutraceutical compositions which may improve health, wellbeing and mental performance in healthy cohorts. Natural products which enable improvements in learning, memory and alertness are, therefore, highly desirable for both their commercial and health impact. Within this field there is a growing body of literature suggesting the potential of foods rich in flavonoids, especially those present in berries, to exert a number of behavioural effects both in animal models (Joseph *et al.*, 1999; Sweeney *et al.*, 2002; Casadesus *et al.*, 2004; Bastianetto *et al.*, 2007; Williams *et al.*, 2008) and aged humans (Krikorian *et al.*, 2010a; Krikorian *et al.*, 2010b; Krikorian *et al.*, 2012). Although flavonoid compounds found in berries have shown promising results, definitive *in vivo* effects, especially in humans, are lacking. A growing discussion regarding potential *in vivo* mechanisms driving purported health promoting properties is also emerging in the literature; outlining their potential to exert clinically significant physiological effects (Matsumoto *et al.*, 2005b; Dreiseitel *et al.*, 2008; Rendeiro *et al.*, 2013). Although a potentially beneficial effect of flavonoid-rich blackcurrant consumption has been outlined in a patent application by Bormann and Shatton (1996), indicating that blackcurrant extracts improve attention in healthy adults; no published, peer reviewed reports of a modulation of human behaviour after consumption of blackcurrants are evident in the literature. Bormann and Shatton (1996) also outlined significant reductions in platelet MAO-B activity in the same patent; therefore, potentially highlighting a modulatory effect of blackcurrant extracts upon monoaminergic tone. The importance of this is highlighted by disorders in monoamines, such as dopamine, that have been shown to lead to decline in

neurocognitive functions, especially memory (Costa *et al.*, 2012), attention (Lahoste *et al.*, 1996) and cognitive flexibility (Cools *et al.*, 2010).

The main aim of this thesis was to assess the impact of blackcurrant extracts upon human cognitive performance and mood; two factors which have received little attention in the literature. Chapter two of this thesis compared the effects of two blackcurrant extracts, upon attention processes in healthy adults, utilising cognitive domains shown to be sensitive to flavonoid consumption in animal models and human interventions. Chapter three explored the acute impact of supplementation with differing doses of the Blackadder blackcurrant extract upon aspects of memory and learning in healthy young adults. The ability of the Blackadder juice extract to modulate cerebral haemodynamics was then explored in chapter four, assessing its acute effects during cognitive tasks known to activate the brain regions under investigation and during a resting absorption period. Chapter six further explored the impact of the two blackcurrant extracts employed in chapter two of this thesis upon memory and learning, utilising a healthy middle aged cohort.

A secondary aim of the thesis was to assess the physiological and biological impact of blackcurrant consumption. Throughout the behavioural intervention studies of this thesis, physiological mechanisms with the potential to modulate human behaviour were measured, with a particular focus upon MAO inhibition and blood glucose. Positive effects of the intervention were evident throughout. In order to create a concrete foundation for future methodologies to be based, chapter five solely focused upon biological parameters, utilising cannulation to assess the pharmacodynamics of these physiological mechanisms.

Two extracts were used at several points throughout this thesis. These extracts were, Delcyan$^{TM}$, a commercially available freeze-dried powdered extract and Blackadder

juice, a fresh from frozen crude cold pressed juice extract. Since berry cultivar and post-harvest preparation of food is an important factor underlying the phytochemical makeup of blackcurrants (Hollands *et al.*, 2008) and, therefore, potentially affects their ability to influence human behaviour, phenolic profiles of the extracts used in this thesis were assessed.

## 7.2 General summary of the findings

The remainder of this chapter will discuss the findings of each intervention chapter of this thesis with initial commentary focusing upon the pattern of behavioural results and how this relates to the literature. This will be followed by a depiction of the physiological mechanisms of action, which could potentially outline a basis for future research.

### 7.2.1 Behavioural effects

The primary aim of the thesis was to assess the impact of standardised blackcurrant extracts upon human behaviour. Data from the investigational chapters of this thesis give some indication of an augmentation of behaviour and cognitive performance in healthy humans after acute supplementation of the blackcurrant extracts. It must, however, be noted that there was no clear pattern of modulation. In chapter two, increased accuracy on the RVIP task was seen after supplementation of the Delcyan$^{TM}$ extract (containing ~500mg polyphenols) and reduced digit vigilance reaction times were seen after consumption of the Blackadder blackcurrant juice extract (500mg dose) in young healthy adults. Conversely, in chapter three, the Blackadder juice extract containing ~250mg of polyphenols led to a decrease in accuracy on a faster version of the digit vigilance task employed in chapter two. A significant increase in correct responses in the DSST task was shown in chapter 6 after supplementation of the Delcyan$^{TM}$ extract (500mg) in middle aged adults. Whereas, decrements in episodic memory in the form of increased word recall errors were found after supplementation of

the Blackadder juice extract. A summary of behavioural findings can be found in table 7.1.

*Table7.1 Significant effects of the different doses of the Blackadder and Delcyan™ extracts employed on cognitive and mood outcomes*

| Measure | | Blackadder Juice | | | Delcyan™ |
|---|---|---|---|---|---|
| | | 125mg | 250mg | 500mg | 500mg |
| Mood | Bond Lader Alert | - | - | - | - |
| | Bond Lader Content | - | - | - | - |
| | Bond Lader Calm | - | - | - | - |
| | Mental Fatigue | - | - | - | - |
| | Physical Fatigue | - | - | - | - |
| Attention | Simple RT | - | - | - | |
| | Digit vigilance accuracy (%) | - | - | - | - |
| | Digit vigilance false alarms (Number) | - | - | - | - |
| | Digit vigilance reaction time (msec) | - | - | ↑ Ch 1 | - |
| | RVIP accuracy (%) | - | - | - | ↑ Ch 1 |
| | RVIP false alarms | - | - | - | - |
| | RVIP reaction time (msec) | - | - | - | - |
| | Stroop accuracy (%) | | | - | - |
| | Stroop reaction time (msec) | | | - | - |
| Working Memory | Numeric Working Memory Accuracy | | | - | - |
| | Numeric working memory % correct | | | | |
| | Numeric working memory RT (msec) | | | | |
| | 3-back % correct | - | - | - | |
| | 3-back missed sequences | - | - | - | |
| | Serial 3 incorrect responses | | | - | |
| | Serial 3 correct responses | | | - | |
| | Serial 3 number of responses | | | - | |
| | Serial 7 incorrect responses | | | - | |
| | Serial 7 correct responses | | | - | |
| | Serial 7 number of responses | | | - | |
| | Corsi blocks span | - | - | - | - |
| | DSST correct | | | - | ↑ Ch 6 |
| | DSST incorrect | | | - | - |
| Secondary Memory | Picture Recognition Accuracy | | | - | - |
| | Picture Recognition RT | | | - | - |
| | Word recognition % correct | - | - | - | |
| | Word recognition reaction time | - | - | - | |
| | Immediate word recall % correct | - | - | - | - |
| | Immediate word recall error | - | - | - | - |
| | Delayed word recall % correct | - | - | - | - |
| | Delayed word recall error | - | - | ↓ Ch 6 | - |
| central exec | Stroop accuracy (%) | | | - | - |
| | Stroop reaction time (msec) | | | - | - |
| | Corsi blocks | - | - | - | - |
| | Logical reasoning reaction time | | | - | - |
| | Logical reasoning accuracy (%) | | | - | - |
| | Peg and ball thinking time | | | - | - |
| | Peg and ball working time (msec) | | | - | - |
| | Peg and ball errors | | | - | - |
| Psychomotor | Tracking accuracy (twips) | - | - | - | |
| | Finger tap - Number of taps | | | - | - |

*Where: ↑ or ↓ = significant improvement/impairment in the parameter tested, along with the chapter where the significant effect was found. Where: - = no effect found. Where ▭ = parameter not assessed at that dose.*

Initial findings were supportive of data outlined by Borman and Shatton (1996), where, in a patent application, it was reported that an undisclosed blackcurrant extract could improve aspects of attention in healthy young adults. Data from chapter two demonstrated a modulation of aspects of attention during mentally fatiguing cognitive assessment. Each blackcurrant extract caused a similar impact upon behavioural outcomes. An increase in accuracy was revealed during the RVIP task after supplementation with the Delcyan$^{TM}$ treatment, irrespective of task repetition, with no evidence of increased reaction times. With regards to the juice treatment, there was evidence of an attenuation of the increase in digit vigilance reaction times seen with repeated testing, with no evidence of decreased accuracy. The RVIP task contains a larger working memory element than the digit vigilance task, potentially indicating changes in working memory processing as well as attention. Working memory is a cognitive outcome which, in the literature, has been shown to be sensitive to flavonoid-rich cocoa (Scholey *et al.*, 2010) and ginkgo biloba (Rigney *et al.*, 1999) in healthy young adults. Non-significant trends towards a reduction in self-reported mental fatigue and increased feelings of alertness were also evident after the last repetition of the 70 minute cognitive battery following supplementation of the Delcyan$^{TM}$ extract. Although not significant, this data could provide support for a beneficial effect of standardised blackcurrant extracts upon cognitive performance during mentally fatiguing cognitive demand an outcome similar to that highlighted during and after a bout of sustained mental demand following supplementation of a flavonoid-rich cocoa (Scholey *et al.*, 2010). However, as described later in this chapter, these effects could not be replicated in further studies.

The initial intervention chapter of this thesis focused upon the effect of standardised blackcurrant extracts upon attention, as this is a cognitive domain most sensitive to dopaminergic tone (Nieoullon, 2002), and the only domain previously reported to be impacted by blackcurrant extract supplementation (Bormann & Schatton, 1996).

Positive findings in animal models, in limited published human epidemiologic observations and in intervention studies, outline positive effects of berries and their flavonoid constituents upon several aspects of memory (Joseph *et al.*, 1999; Joseph *et al.*, 2003; Casadesus *et al.*, 2004; Goyarzu *et al.*, 2004; Galli *et al.*, 2006; Williams *et al.*, 2008; Rendeiro *et al.*, 2013). However, only a limited number of these memory paradigms have been investigated in humans after consumption of flavonoid-rich berry extracts, with positive findings limited to improvements in verbal learning and recognition memory in aged adults (Krikorian *et al.*, 2010a; Krikorian *et al.*, 2010b). It must be noted that these studies have obvious methodological issues and results are based on data from small sample sizes. Evidence of a modulation of memory can, however, be drawn from studies investigating the effects of other flavonoid-rich foods in healthy cohorts such as cocoa (Scholey *et al.*, 2010; Field *et al.*, 2011) and ginkgo biloba (Kennedy *et al.*, 2000; Singh *et al.*, 2004). Further to this, published data outlines the impact of dose choice upon behavioural effects of flavonoid-rich cocoa in humans (Scholey *et al.*, 2010), anthocyanin supplementation in children (Whyte & Williams, 2012) and grape juice in rats (Shukitt-Hale *et al.,* 2006). In an attempt to assess the impact of blackcurrant supplementation upon human memory, chapter three explored the acute effects of three different doses of the Blackadder juice extract on episodic memory, working memory, memory span, visuo-spatial memory and verbal memory in healthy young adults at several different time points throughout the day. Drinks were reduced in dose sequentially from that shown to have an impact upon behaviour and biological mechanisms in chapter two of this thesis, and were standardised at 125mg, 250mg and 500mg of total polyphenols. Cognitive assessments were conducted 1 hour and 2.5 hours post supplementation. These time points were selected on the basis of findings from chapter two showing modulation of glucose uptake (60 mins) and MAO activity (150 mins) at these time points; as well as detectable levels of potentially active anthocyanin constituents of the extracts at 150 minutes. The Blackadder juice extract was found to have no positive impact upon aspects of memory measured at any dose

or at any time point. There was, however, a sustained reduction in digit vigilance correct responses after supplementation of the 250mg intervention. It is hypothesised that the lack of positive behavioural effects could be associated with the fact that the healthy adult cohort used were already performing at, or near to, their peak (Salthouse, 2009), and unlike the initial intervention study, participants were not mentally fatigued to a high enough level to reveal subtle cognitive benefits. These data, coupled with data from the literature, potentially highlight the need for a longer intervention or an older cohort before any positive modulations upon memory can be exhibited.

A limited number of articles exist supporting improvement of learning and memory in animal models and older human adults (60+). However, no such data are available in middle aged healthy adults, who are potentially exhibiting natural age related reductions in memory, and reaction times, when compared to the cohorts used in chapters two and three of this thesis (Salthouse, 2009). These age-related decrements are coupled with neurobiological markers such as; increased MAO activity, reduced cerebral blood flow and reduced monoaminergic tone, which are theorised to play a role in natural cognitive decline and could, therefore, be potential therapeutic targets to attenuate reductions in cognitive performance associated with age (Leenders *et al.*, 1990; Fowler *et al.*, 1997; Volkow *et al.*, 2000; Sheline *et al.*, 2002). These neurobiological mechanisms could potentially be modulated through the inhibition of MAO and increases in cerebral blood flow documented after consumption of the Blackadder blackcurrant juice extract in the intervention chapters of this thesis. Chapter six of this thesis, therefore, attempted to assess the acute impact of the Delcyan$^{TM}$ powdered extract and the Blackadder juice blackcurrant extracts upon memory, attention and executive processing in a middle aged cohort. The study utilised similar standardised cognitive tests as used in the previous intervention studies of this thesis as well as additional tasks that assessed mood, recall memory, working memory, cognitive flexibility, episodic memory and recognition memory over several acute post

supplementation time points (1.2.5, 4 and 6 hours post-dose). These time points were selected on the basis of when MAO activity is known to be inhibited, central blood flow is known to be modulated and when the “parent” compounds and biphasic metabolites of the blackcurrant extracts are known to be in the periphery. Although significant effects were observed in chapter six after supplementing healthy middle aged adults with the intervention drinks, no definitive pattern of cognitive modulation was observed and, given the number of cognitive outcomes, these findings must be interpreted with caution. A significant increase in correct responses at time point two (2 hours post-dose) and time point four (6 hours post-dose) in the DSST task were illustrated after supplementation of the Delcyan$^{TM}$ extract only. As age related decreases in DSST scores are associated with decreased processing speed, rather than decrements in memory (Salthouse, 1992), the effects of the Delcyan$^{TM}$ treatment upon the DSST are hypothesised to be associated with an increase in processing speed, rather than a direct augmentation of working memory. The only significant treatment effect upon memory performance after supplementation of the Blackadder juice extract was a decrement to the recall task scores, with increases in incorrect responses on the delayed recall task following supplementation of the Blackadder juice. This increase in incorrect responses was also evident at repetition two of the immediate word recall task after supplementation of the Blackadder juice drink when compared to a control but this was only a trend treatment*repetition interaction on the main ANOVA. Both results indicate decrements in verbal episodic memory after supplementation of the Blackadder juice drink, an outcome which has been shown to be improved after chronic supplementation of blueberries (Krikorian *et al.*, 2010b) and concord grapes (Krikorian *et al.*, 2010a) in aged adults.

To conclude, the behavioural findings from this thesis; initial findings would suggest that cognitive performance, particularly attention based processes and speed of processing can be positively impacted by the acute supplementation of blackcurrant

extracts. The pattern of evidence from the subsequent studies in this thesis are, however, not as clear with no replication of the aforementioned modulations upon attention and no definitive effects upon memory. Therefore, no real evidence of cognitive enhancement after supplementation of blackcurrant extracts is apparent from the intervention chapters of this thesis. The literature would suggest that for flavonoid-rich extracts to be effective as modulators of human cognitive performance chronic supplementation needs to be undertaken. The theory would, therefore, be that the long term consumption of blackcurrants in healthy humans would not improve cognitive performance from the basal point, but attenuate decrements in cognitive performance associated with natural ageing over the natural lifespan. Therefore, both short term chronic (several months) and long term chronic (several years) assessments need to be undertaken to establish if blackcurrant extracts can indeed slow the natural ageing process. It may also be the case that the doses used in the intervention chapters of this thesis were too low or post dose cognitive assessments were conducted too long after consumption. Higher doses or cognitive assessments pre-one hour post-dose could prove to be more effective. The possibility also remains that blackcurrant simply has no measurable effects on cognition in humans.

### 7.2.2 Physiological effects

A secondary aim of this thesis was to assess the physiological and haemodynamic effects of blackcurrant extracts. Throughout the behavioural intervention trials in this thesis, several physiological parameters, which could potentially impact human behaviour, were measured. These include central and peripheral haemodynamics and the regulation of blood glucose. A summary of physiological and bilological findings can be found in table 7.2

*Table 7.2 Significant effects of the different doses of the Blackadder and Delcyan™ extracts employed on physiological outcomes*

| Measure | | Blackadder Juice | | | Delcyan™ |
|---|---|---|---|---|---|
| | | 125mg | 250mg | 500mg | 500mg |
| Peripheral haemodynamics | Heart rate (BPM) | | | - | - |
| | Diastolic blood pressure (mmHg) | | | - | - |
| | Systolic blood pressure (mmHg) | | | - | |
| | DVP - SI | | | - | |
| | DVP - RI | | | - | |
| Central haemodynamics NIRS | Combined OxyHb | | | - | |
| | OxyHb LH | | | ↑Ch 4 | |
| | OxyHb RH | | | - | |
| | Combined DeoxyHb | | | ↓Ch 4 | |
| | DeoxyHb LH | | | - | |
| | DeoxyHb RH | | | - | |
| | Combined TotalHb | | | - | |
| | TotalHb LH | | | ↑Ch 4 | |
| | TotalHb RH | | | - | |
| Blood Parameters | Platelet MAO-B activity (nmol $H^2O^2$) | ↓Ch 3 | ↓Ch 3 | ↓Ch 2,3 & 5 | - |
| | Prolactin (mIU/L) | ↓Ch 3 | ↓Ch 3 | ↓Ch 2 & 5 | - |
| | Glucose (mmol/L) | | | ↑Ch 2 ↓Ch5 | - |
| | Lactate (mmol/L) | | | - | - |
| | Frap | - | - | - | |
| | Phenylethylamine (ng/ml) | | | - | - |
| | Dopamine (ng/ml) | | | - | - |
| | Serotonin (ng/ml) | | | - | - |
| | Normetanephrine (ng/ml) | | | ↑Ch 1 | - |
| | Noradrenaline (ng/ml) | | | - | - |
| | Adrenaline (ng/ml) | | | - | - |
| | DHPG (ng/ml) | | | ↓Ch 1 | - |
| | Homovanillic acid (ng/ml) | | | - | - |

*Where: ↑ or ↓ = significant increase/decrease in the parameter tested along with the chapter where the significant effect was found. Where: - = no effect found. Where ▭ = parameter not assessed at that dose.*

### 7.2.2.1 Haemodynamics

#### *Peripheral haemodynamics*

In the literature, there is some evidence towards a modulation of peripheral blood flow after supplementation of blackcurrants (Matsumoto *et al.*, 2005a; Matsumoto *et al.*, 2005b) and a plethora of data suggesting increases in peripheral blood flow after the consumption of flavonoid-rich cocoa (Matsumoto *et al.*, 2005a; Matsumoto *et al.*, 2005b; Heiss *et al.*, 2007; Faridi *et al.*, 2008a). Throughout this thesis, however, no observable impact of any of the study interventions upon peripheral haemodynamics was observed. These include; measures of, blood pressure, large artery stiffness and endothelial dependent vasodilation. In chapter two, blood pressure and heart rate were assessed 60 and 150 minutes post supplementation of the Blackadder juice and Delcyan™ extracts, with no significant effects found. These findings are consistent with

those of Jin *et al.,* (2011) who found no acute effect of a 20% blackcurrant extract upon blood pressure or heart rate. Berry anthocyanins have, however, been shown to induce endothelial dependent relaxation facilitated by up-regulation of eNOS (Andriambeloson *et al.*, 1998) and to inhibit iNOS (Chen *et al.*, 2001) *in vitro*. Therefore, pulse wave velocity, a measure of endothelial dependent vasodilation and large artery stiffness, which has been shown to be sensitive to NO synthase manipulation (Klemsdal *et al.*, 1994), administration of vasoactive drugs and vascular ageing (Takazawa *et al.*, 1998) was implemented in chapter five of this thesis. As with blood pressure, no significant effects were found after supplementation of the 500mg Blackadder juice drink (the only extract used in the study).

***Cerebral haemodynamics***

Modulations of central haemodynamics were observed after supplementation of the 500mg Blackadder blackcurrant juice extract, the only extract examined in this context. When compared to placebo, consumption of the Blackadder juice extract resulted in significant modulations in prefrontal cortex haemoglobin concentrations during resting absorption and cognitive task performance. Hemispheric differences in the modulation of haemodynamics were also illustrated. Increases in oxyhaemoglobin and total haemoglobin in the left hemisphere, but not the right, were seen during the cognitive task period after consumption of the blackcurrant drink when compared to control. No effect of task type was evident. The Blackadder juice extract also caused a significant reduction in deoxyhaemoglobin with no hemispheric differences being seen. Unlike oxyhaemoglobin, the modulation of deoxyhaemoglobin began 45 minutes post consumption of the blackcurrant juice, during the resting absorption period. Reductions in levels of deoxyhaemoglobin coincide with the potential appearance of berry anthocyanins in blood plasma, which previous research has shown to reach maximal concentrations at ˜1 hour post consumption (Matsumoto *et al.*, 2001). The significant or near to significant modulation of deoxyhaemoglobin continued throughout the

remaining 15 minutes of the absorption period and throughout the cognitive testing period; again, no effect of task type was evident.

The rise in total haemoglobin in response to post-dose tasks in chapter four, irrespective of intervention, reflects neurovascular coupling via the natural nitric oxide mediated drive for more fuel. It is possible that this is further up-regulated in the left hemisphere after consumption of the blackcurrant juice. Hemispheric differences found in the present study could, therefore, be indicative of the study intervention amplifying a natural difference in hemispheric activity in response to cognitive demand as previously shown during mental subtractions (Baurbaud *et al.,* 1999) and during the RVIP task (Coull *et al.,* (1996) employed.

As rate of utilisation of oxygen is slower than the rate at which it is provided, the relative decrease in deoxygenated haemoglobin during cognitive performance following blackcurrant is as expected, given the observed increase in oxygenated haemoglobin. However, the effects on deoxygenated haemoglobin do not show the lateralisation observed with oxygenated haemoglobin. Further to this, the pattern of effects upon deoxyhaemoglobin does not follow that of oxygenated blood flow. The effects on oxyhaemoglobin following control and Blackadder juice interventions exhibit a similar pattern until cognitive demand is imposed, whereas reductions in deoxyhaemoglobin seen after consumption of both treatments begin to diverge at 40 minutes post-dose. This divergence manifests as a rise in the control arm taking deoxyhaemoglobin back up to levels similar to these observed at the start of the session, whereas levels in the Blackadder juice arm continue to reduce throughout the remainder of the absorption period and throughout the cognitive assessment period. The time of onset and the lack of lateralisation of effects on deoxyhaemoglobin suggest that these are independent of effects on oxyhaemoglobin. A definitive mechanism behind this effect is not known, however, one tentative suggestion is, after consumption of the sugar matched drinks

more glucose is available for utilisation and, therefore, the ratio of lactate and glucose in neuronal tissue changes in favour of glucose, a ratio which could be exacerbated by the 12 hour fast prior to treatment consumption. As less oxygen is needed to utilise glucose as a fuel when compared to lactate (Larrabee, 1995), less oxygen may be needed to maintain cognitive activities post consumption of the sugar matched drinks, therefore, oxygen turnover is reduced. As glucose absorption is slowed after the consumption of the Blackadder juice extract and therefore glucose is available systemically for longer than control, this utilisation of glucose may continue throughout the cognitive assessment after consumption of the Blackadder juice extract but return to baseline 45 minutes post consumption of the control drink.

The blackcurrant components responsible for the effects upon oxyhaemoglobin and total haemoglobin are unknown. A factor which is further hindered by the absence of data assessing the pharmacodynamics of anthocyanins pre-one hours post consumption. As anthocyanins levels in blood plasma are either at or near to their maximal concentration one hour post consumption, it seems reasonable to assume that anthocyanins are present in plasma at the start of the cognitive battery and, therefore, could be modulating the effects seen on haemodynamic parameters. However, as discussed in section 1.2 of this thesis, blackcurrants contain conjugates of other flavonoids as well as phenolic acids. Phenolic acids have been shown to be present in the periphery 30 minutes after consumption of a blackcurrant drink (Jin *et al.*, 2011) and, therefore, cannot be ruled out as the compounds modulating the observed haemodynamic changes. To attempt to explore potential compounds driving these haemodynamic changes, a direction for future research would be to assess several different extract types with differing phenolic profiles at differing doses. Further to this, compounds in isolation could be assessed.

Reductions in cerebral blood flow are associated with natural ageing (Leenders *et al.*, 1990) and neurological disease (Dede *et al.*, 2007). Results from this thesis outline the ability of the Blackadder blackcurrant juice extract to increase cerebral blood flow in a young healthy population. Therefore, the effects of the Blackadder juice and other types of blackcurrant extracts upon brain function deserve further investigation.

#### 7.2.2.2 Glucose uptake

The findings from chapters two and five of this thesis demonstrate that the consumption of a blackcurrant extract can modulate blood glucose profiles. Data from chapter two demonstrates that the Blackadder juice treatment can increase capillary blood glucose at 60 and 150 minutes post supplementation when compared to control. A finding not present after the consumption of the Delcyan$^{TM}$ extract. This modulation of blood glucose is apparent despite all intervention drinks being sugar matched. A more thorough investigation of this glucose modulation was conducted in chapter five utilising an inlying cannula and nine post-dose blood samples over four hours. Chapter five showed that increases in blood glucose found in chapter two were a misrepresentation of the post-prandial blood glucose profile. Results showed that instead of increasing overall glucose absorption, the Blackadder blackcurrant juice drink reduces the post-prandial peak in blood glucose levels. This blunted peak is theorised to be due to reduced glucose uptake from the stomach to the blood stream, slowing glucose absorption and, therefore, resulting in a higher blood glucose reading one hour post supplementation when compared to placebo. The only statistically significant effect of the Blackadder juice extract upon blood glucose in chapter five was at 15 minutes post supplementation, where glucose levels were lower than when compared to control. However, a non-significant regulation of blood glucose occurred until 100 minutes post-dose, where it returned to a level similar to baseline and control. This phenomena of a blunted blood glucose peak is not unique to the consumption of blackcurrant extracts, it has also been outlined after the consumption of other phenolic-

rich meals such as cloudy apple juice (Johnston *et al.*, 2002) berry purée (Törrönen *et al.*, 2012) and cranberry juice (Wilson *et al.*, 2008b). On the most basic level, research has shown glucose to be a modulator of cognitive performance when high demand is placed on the brain (Donohoe & Benton, 2000; Kennedy & Scholey, 2000; Scholey et al.2001; Riby *et al.*, 2004; Scholey & Kennedy, 2004). As glucose is the primary fuel of the brain, it is hypothesised that this control of glucose uptake by phenolic compounds allows glucose to be available systemically for longer, which may have a positive impact on cognition during bouts of high demand.

Chapter six aimed to assess the impact of blackcurrant supplementation on glucose levels in a middle aged cohort, where glucose regulation might be impaired when compared to the young healthy cohorts used in the earlier interventions of this thesis (Shimokata *et al.,* 1991). Although not significant, blood glucose followed a similar pattern to that observed in chapter two with levels ~0.5mmol/L higher after supplementation of the Blackadder juice drink one hour post supplementation, an effect not observed after supplementation of the Delcyan$^{TM}$ extract. Although earlier time points were not measured, it was outlined in chapter five that this increase in blood glucose is a result of a lower post-prandial peak in blood glucose, associated with consumption of the intervention drink. The second aim, in terms of glucose modulation in chapter six was to assess if a similar pattern of glucose modulation was observable after a 10g glucose load at a later time point without co-consumption with polyphenols. This pattern of glucose modulation was not observed 200 minutes post supplementation of the blackcurrant extracts (10 minutes post consumption of the 10g glucose load). However, no significant effect was observed one hour post-dose so no conclusion can be drawn from the data available from chapter six. As mentioned earlier, it is noteworthy that an increase in blood glucose was documented in participants aged 18-35 in earlier chapters of this thesis but did not reach statistical significance in chapter six. This could be a consequence of reduced glucose regulation

in middle aged adults (Tuomilehto *et al.*, 2003), however, a more obvious explanation would be dose selection. Doses were standardised at 500mg of polyphenols per person, regardless of weight, reducing, on average, the amount of polyphenols per kg of body weight by 1mg compared to that in chapters one and five. Therefore, reducing the impact of the intervention upon blood glucose. In order to explore this further, it would be beneficial to examine these effects again in this population using the weight-linked doses employed in chapter two. It would also be beneficial to include administration of a later blood glucose load in young adults (18-30) in order to explore these effects in a population which have been shown to be sensitive to concomitant glucose and polyphenol consumption in the current thesis.

To conclude, the supplementation of the Blackadder juice extract but not the Delcyan$^{TM}$ extract significantly reduced the post-prandial glucose peak after consumption. This, therefore, highlights an impact of post-harvest preparation techniques upon blood glucose effects. The slowing of absorption is hypothesised to be facilitated by the inhibition of sodium glucose transporters by phenolic compounds (Manzano & Williamson, 2010) in the Blackadder juice extract, reducing gastric emptying and, therefore, attenuating the speed of transport of glucose to the blood stream. These phenolic acid compounds are in much lower quantities in the Delcyan$^{TM}$ extract which is theorised to be the reason why no effects were found on blood glucose after its consumption. Findings would highlight a possible role of some, but not all blackcurrant extracts as a glycaemic index lowering agent.

### 7.2.3 Biological mechanisms of action

The final aim of this thesis was to examine underlying mechanisms, in particular potential *in vivo* monoamine oxidase inhibitory properties of blackcurrants and associated biomarkers. Throughout the behavioural intervention trials in this thesis, several underlying biomarkers that could potentially impact human behaviour were

assessed. These include MAO inhibition, monoaminergic tone and prolactin secretion. Both isoforms of MAO were shown to be inhibited in healthy young adults after supplementation of the Blackadder juice extract but not the Delcyan$^{TM}$ extract. A modulation of monoaminergic tone and reductions in blood plasma prolactin were also observed after consumption of the Blackadder juice extract.

#### 7.2.3.1 Monoamine oxidase inhibition and associated monoaminergic tone

The initial intervention study of this thesis attempted to assess the impact of two blackcurrant extracts, with differing phenolic profiles, upon MAO-A and MAO-B. Data from this study demonstrated that platelet MAO-B activity was inhibited by 96% at 2.5 hours post consumption of the Blackadder juice extract when compared to control. This level of platelet MAO-B inhibition is similar to that of pharmacological MAO-B inhibitors currently prescribed as anti-Parkinsonian agents (Dingemanse *et al.*, 1997). A significant reduction in MAO-A activity (~35.5%) as indicated by reduced plasma DHPG levels (Koulu *et al.*, 1989; Zimmer, 1990), was also observed. Reductions in DHPG concentrations and platelet MAO-B activity in chapter two did not coincide with an accumulation of the measured associated monoamines (adrenalin, serotonin and dopamine), which is in line with previous research utilising acute administration of pharmaceutical MAO inhibitors (Eisler *et al.*, 1981; Illi *et al.*, 1996). However, an increase in normetanephrine was found after consumption of the Blackadder juice extract compared to control. This increase is theorised to be indicative of increased noradrenalin breakdown through COMT, due to inhibition of the MAO-A enzyme. The compounds which were previously theorised to be driving the reported inhibition of the MAO enzymes were anthocyanins (Dreiseitel *et al.*, 2009a). However, findings outlined in chapter two of this thesis demonstrate that this may not be the case. Despite blood plasma levels of all measured anthocyanins being higher after the consumption of the Delcyan$^{TM}$ extract when compared to the Blackadder juice extract, there was no significant inhibition of platelet MAO-B activity after consumption of the Delcyan$^{TM}$. This

highlights, that at least alone, the measured anthocyanins are unlikely to be the compound responsible for the inhibition of the enzyme. It is possible that another compound in the blackcurrant extract is the active inhibitor or an *in vivo* metabolite of a compound in the blackcurrant extract is inhibiting MAO *in vivo*. This, however, does not rule out potential for a synergy within the blackcurrant profile. For example, Dreiseitel *et al.,* (2009b) showed that anthocyanins have an affinity for breast cancer resistant protein (BCRP) efflux transporters in endothelial cell model (caco2 monolayers). This efflux away from the site of action may limit the bioavailability of anthocyanins in plasma and the brain, but could allow other compounds and metabolites in blackcurrants, which have potential to be MAO inhibitors, but have a lesser affinity to the efflux transporters to accumulate at the site of action, thereby, increasing their effectiveness.

Chapter three of this thesis assessed the sensitivity of the MAO-B inhibitory properties outlined in chapter two, to a range of doses of the Blackadder blackcurrant juice extract. Doses of 125mg, 250mg and 500mg of total polyphenols per 60kg of bodyweight were utilised. All blackcurrant drinks reduced MAO-B activity compared to placebo 120 minutes post supplementation. The 250mg and 500mg treatments inhibited MAO-B activity by ~80% and the 125mg treatment reduced activity by 68%. This limited dose effect of the Blackadder blackcurrant juice extract potentially further highlights that anthocyanins are not the inhibiting compound in the Blackadder juice extract. Dreiseitel *et al*., (2009a) showed that anthocyanins inhibited the MAO-B enzyme competitively. However, if the MAO-B inhibition was competitive, a larger effect of dose would be expected. This was further highlighted in chapter five where the pharmacodynamics of the inhibitory properties of the 500mg Blackadder juice extract were investigated, utilising intravenous cannulation and 10 post-dose blood samples over 24 hours. This pharmacodynamic investigation revealed a sustained reduction in MAO-B of 100% when compared to control. This reduction began 15 minutes post

supplementation (the first time post-dose measurement), continued throughout the subsequent measures and was still present at the final measurement on that day (four hours post-dose). This is a finding which is similar to pharmaceutical reversible MAO-B specific inhibitors such as the anti-Parkinsonian drug Lazamabemide. These data from chapter five of this thesis, and the lack of significant effects of the Blackadder juice extract upon MAO-B 24 hours post supplementation, outline a distinct reversible and complete inhibition of platelet MAO-B activity from the Blackadder juice extract.

In light of the robust findings above, it was surprising to find that in chapter six, there was no observable inhibition of MAO-B after supplementation of either the Blackadder juice extract or the Delcyan$^{TM}$ extract when compared to control within a cohort of healthy middle aged adults. There is no doubt that the active compound or compounds driving the inhibition are still present in the Blackadder juice extract, however, there are several methodological limitations as discussed in section 6.4, which could pertain to the null findings. Firstly, due to technical difficulties, only five sets of blood samples were successfully analysed for MAO-B activity, therefore, the statistical analysis may have been underpowered, however, findings do not follow the same pattern as previous studies. Secondly, due to the time needed to be spent in the laboratory after an overnight fast, participants were fed a pasta meal 180 minutes post supplementation. Food interactions with MAO inhibitors are well documented in terms of safety (i.e. the tyramine effect), however, no such information is available regarding food intake and potency reductions making the link between food intake and the null findings upon MAO activity unclear. Thirdly, MAO-B activity was measured six hours post consumption of the blackcurrant extracts, whereas the last time point where MAO-B was previously shown to be significantly inhibited by supplementation of the Blackadder juice drink was at four hours post-dose. However, the inhibition was at such a level (100%), that it was expected that the MAO inhibition would still be evident six hours post-dose. A final point to note is the increase in MAO activity with age. This

increase is reported to be at a rate of 7% per decade (Fowler *et al.*, 1997). As effects of the Blackadder juice extract have been shown after consumption of a dose standardised at 125mg/60kg of body weight in chapter two, these results and increase in MAO activity with age may highlight that a higher dose may be needed to elicit an inhibitory response in an older cohort. This highlights the need for further research focusing upon dose ranges and more time points, in a similarly designed study to chapter five of this thesis, to allow the efficacy of MAO inhibition via consumption of the Blackadder juice extract to be determined in a healthy middle aged and older population.

#### 7.2.3.2 Prolactin secretion

It is theorised that as MAO-B inhibition is associated with increased dopaminergic tone (Riederer & Youdim, 1986), which is the most important hypothalamic prolactin inhibiting factor (Fitzgerald & Dinan, 2008), inhibition of MAO-B by the Blackadder juice extract would impact dopaminergic tone and lower peripheral prolactin levels. The initial measurement of prolactin in the thesis was conducted in chapter two, with data revealing a potential lowering effect of the Blackadder juice extract. This potential modulation was illustrated in a between subjects fashion with a sample size too small for a reliable conclusion to be established. In chapter three, this potential prolactin lowering effect of the Blackadder blackcurrant juice extract was investigated further in a dose ranging study (125, 250 and 500mg of total polyphenols), this time in a larger cohort utilising a within subjects design. Peripheral prolactin was reduced after supplementation of all doses of the Blackadder blackcurrant juice drinks, but only statistically significantly reduced after consumption of the 125mg and 500mg polyphenol Blackadder blackcurrant juice drinks. The efficacy of the prolactin lowering effect of the Blackadder blackcurrant juice extract was further scrutinised in chapter five where the pharmacodynamics of the neuroendocrinological impact were investigated. A dose standardised at 500mg of total polyphenols was utilised and 10 post-dose blood

samples were extracted from an inlying cannula. Non-significant reductions in prolactin were seen 30 minutes post consumption of the Blackadder juice extract when compared to placebo. The first statistically significant area under the curve increment in prolactin levels between control and the Blackadder juice extract began 45 minutes post supplementation and continued until the last measured concentration at four hours post consumption of the intervention drinks. The maximal reduction appeared two hours post supplementation with 61% reduction being seen when compared to control. A reduction similar to that reported in chapter three, where a reduction of 55% was reported 120 minutes post supplementation of the same treatment. Prolactin levels had returned to a level similar to baseline 24 hours post supplementation of both intervention drinks. This finding was not seen in chapter six, where the effects of both the Blackadder blackcurrant juice drink and the Delcyan$^{TM}$ powdered extract had no significant effect at six hours post supplementation in a cohort of healthy middle aged adults. This result is unsurprising as there was no effect of the Blackadder juice intervention upon MAO activity, the underlying mechanism believed to be driving the prolactin lowering effect.

The neuroendocrinological impact of the Blackadder juice extract presented throughout the intervention chapters of this thesis is consistent with inhibition of prolactin secretion by the central D2 receptor agonist Bromocriptine, of which, 12mg reduces peripheral prolactin by ~60% two hours post-dose (Luciana *et al.*, 1998). These reductions in prolactin could have positive implications upon sexual health and libido and play a major role in alleviation of symptoms associated with hyperprolactinaemia (Buvat, 2003). More importantly, although it cannot be definitively proven from the body of evidence presented in this thesis, as predominant control of prolactin is via hypothalamic inhibition of lactotroph activity and the most important hypothalamic prolactin inhibiting factor is dopamine (Ben-Jonathan & Hnasko, 2001), results indicate the possibility of a centrally active inhibition of MAO after ingestion of the Blackadder

juice extract which could have implications in the treatment of neurodegenerative diseases and potentially help attenuate natural cognitive decline.

### 7.2.4 Summary of physiological effects and biological mechanisms

Physiological and biological mechanisms which have the potential to impact human behaviour were documented and quantified throughout this thesis. The efficacy of these mechanisms to impact human behaviour acutely after consumption of blackcurrant extracts, especially in the investigational chapters of this thesis are unclear. Results from chapter six showed that there is no clear pattern of effects upon memory and learning after consumption of blackcurrant extracts in a middle aged cohort. However, a lack of reliable acute improvements to cognitive performance following blackcurrant does not rule out the potential for promotion of long term maintenance of cognitive flexibility. For instance, mechanisms mediated by flavonoid consumption and related to hippocampal plasticity, such as up regulation of CREB (Brightwell et al., 2007) and increased levels of BDNF (Rendeiro *et al.*, 2013) in animal models, suggest that benefits may be conferred following chronic supplementation. As MAO inhibition in a young healthy cohort could not be replicated in a middle age cohort following blackcurrant and as MAO activity increases, and cerebral blood flow decreases, with age, there is an argument for the need of a larger dose in aged populations for an impact to be seen. If inhibition of MAO-A and MAO-B and modulation of cerebral blood flow, decrements in which have been shown to be implicated in cognitive decline, can be shown to be mediated in an older population, there is potential for blackcurrant consumption to attenuate natural age related cognitive decline. These potential mechanisms are shown below in figure 7.1. Finally, if the MAO inhibiting effects of the Blackadder juice can be proven to be centrally active, blackcurrant extracts could, potentially, be used as therapeutic agents in the treatment of neurological diseases such as Parkinson's, Huntington's and Alzheimer's disease.

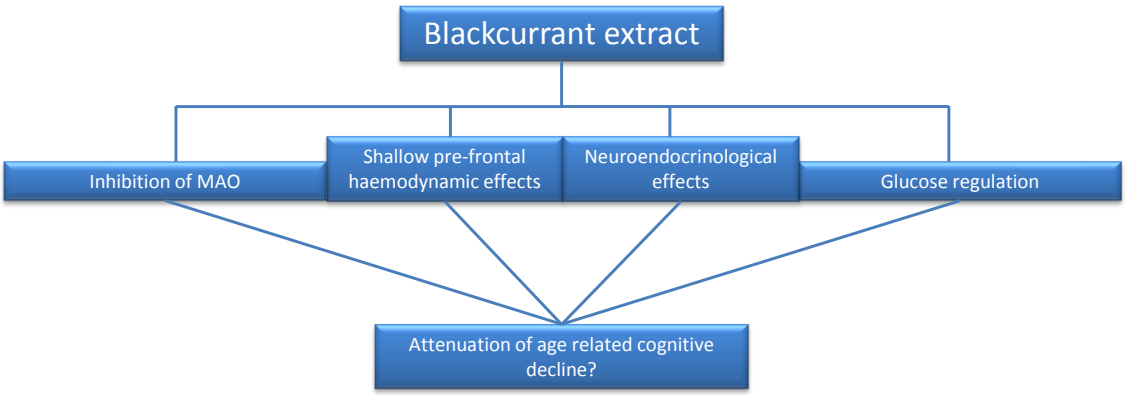



*Figure 7.1 Potential mechanisms which could synergistically impact natural cognitive decline.*

## 7.3 Potential methodological limitations

Although the intervention studies which make up this thesis provide novel findings with regards to the impact of acute blackcurrant consumption, it is sensible to acknowledge some methodological limitations.

A potential issue arises from the large amount of behavioural outcome measures used in chapters three and six of this thesis. Although the aim of said investigations was to acquire a broad understanding of the impact of blackcurrant extracts upon cognitive performance and educated decisions on task choice were made, there was inevitably an increased risk of detecting a significant effect by chance. In recognition of this potential limitation, interpretation and discussion of results throughout this thesis have been focused upon outcomes which fall into patterns, rather than discussing every result at length.

Throughout the thesis, blood glucose was measured using finger prick blood samples. Although this method has been used in many glucose studies to measure glycaemic response to a glucose challenge, more up to date methodologies are now available, offering continuous non-invasive methods of glucose quantification. Such methods as interstitial continuous glucose monitoring as used by Dye *et al.,* (2010) would allow for a full profile of the effects to be monitored during cognitive assessment.

In chapter six, MAO-B inhibition was measured in a very small sub sample (N=5 after errors with the assay) at a time point which had not previously been proven to be effective. Given the powerful inhibition (100%) shown in chapter five of this thesis after consumption of the Blackadder blackcurrant extract standardised at 500mg of polyphenols, it was deemed highly likely that the inhibition would still be evident six hours post-dose. Given that the effects had never been confirmed in the middle aged cohort used, and lunch was administered, an earlier time point where MAO is known to

be inhibited in younger participants should have been used. This would have allowed a direct comparison between the young and middle aged cohort.

Finally, there is no clear pattern of modulation upon cognitive or mood parameters assed in this thesis after consumption of either the Blackadder Juice or Delcyan™ blackcurrant extract. There are several methodological factors which could have impacted findings. Epidemiological studies have shown that diets habitually high in polyphenols are linked with an attenuation of cognitive decline associated with natural ageing in humans, when compared to diets lower in polyphenols (Letenneur *et al.*, 2007; Nurk *et al.*, 2009; Devore *et al.*, 2012; Kesse-Guyot *et al.*, 2012). Socio-economic factors have been shown to impact a person's habitual diet. For instance, a review of the literature by Darmon & Drewnowski (2008) showed that better quality diets are consumed by more educated and affluent people. Findings also show that people with a high socio-economic status consume a significantly higher quantity and variety of fruits and vegetables when compared to their counterparts in low socio-economic groups. In the intervention chapters of this thesis, no data was collected in regards to socio-demographics of the research cohort, a factor which could effect the habitual diet of participants. In addition habitual diets of participants were not assessed in this thesis, therefore, differences in participant diets cannot be ruled out as factors impacting observed results throughout. Further to this, the glycaemic index of an evening meal has been shown to significantly impact cognitive performance the next day (Lamport *et al.,* 2012). This is a factor which was not controlled for in the current thesis, therefore, the 12 hour fasting period prior to cognitive assessment may not have been sufficient of a control for this phenomena. It is also possible that geographical location could have played a role in the study findings in chapter two and lack of replication in chapter three. The initial intervention (chapter two) was conducted in healthy adults in New Zealand. In this population, improvements in attention based tasks were seen after consumption of the Blackadder juice extracts. In the second

intervention (chapter three), these results were not replicated, however, the study was conducted in Newcastle, England. Although it cannot be confirmed as participant diets were not recorded, it is theorised that a difference in habitual diet between countries could have impacted results.

Lastly, the gender of participants was not taken into consideration during the analysis of findings in chapters 2,3,4 and 6 of this thesis. Therefore, physiological and psychological differences between sexes of participants were not accounted for, potentially impacting study findings.

## 7.4 Recommendations for future research

Whilst the limitations described in section 7.3 highlight several methodological issues from this thesis which need to be addressed in future research, there is also considerable scope for future research which can be drawn from the intervention studies of this thesis.

The major recommendation would be to assess the impact of chronic supplementation of blackcurrant extracts in young, middle aged and elderly adults, utilising cognitive domains which have been shown to be sensitive in animal models. It would also be a recommendation to use a baseline measure and a control with several intervention testing sessions to assess the optimum time point, dose and potential ceiling effects, for example, measurements, taken at baseline and after acute supplementation, then monthly measurements until the end of the intervention period. These behavioural measures should be coupled with a non-invasive measure of cerebral blood flow, such as quantitative NIRS, (which allows the quantification of haemoglobin rather than an arbitrary value as seen in the continuous wave NIRS utilised in chapter four of this thesis, therefore, allowing for multiple measurements in a chronic study), and measurements of MAO activity to assess if intervention driven modulations of blood flow and MAO activity correlate with changes in cognitive performance. These

recommendations are, however, hindered by results highlighted in chapter two of this thesis where physiological (blood glucose) and biological (MAO) responses to the blackcurrant extracts differed after consumption of differently prepared extracts. The final recommendation would, therefore, be the measurement of peripheral and cerebral MAO activity after consumption of several blackcurrant extracts, assessing the impact of differing cultivars and post-harvest preparations. This would highlight if the inhibition is unique within cultivars, geographical growing locations and post-harvest preparations. It is acknowledged that the measurement of central MAO activity would involve expensive neuroimaging techniques such as positron emission tomography, a neuro-imaging technique which is capable of detecting radioactively labelled metabolically active substances which have been infused into the bloodstream. However, such research is essential to determine centrally active MAO effects.

In addition to results from this thesis, the above suggested research would create a foundation for future studies investigating the impact of blackcurrant extracts upon cognitive performance and potentially symptoms of neurodegenerative disease to be based.

### 7.5 General conclusion

The main aim of this thesis was to assess the impact of standardised blackcurrant extracts upon human behaviour in healthy participants. Single doses of each of the blackcurrant extracts used in this thesis yielded some positive results with effects of post-harvest extraction evident. Increases in attention processing were observed in young healthy adults, with limited effects being evident upon any other cognitive paradigm. The overarching recommendation for future research would be a chronic study assessing the effects of standardised blackcurrant extracts on a broad range of cognitive outcomes, especially memory, attention and executive functioning in young, middle aged and elderly cohorts.

Underlying physiological mechanisms with potential to modulate human behaviour were also evaluated throughout the thesis. Modulations of post-prandial glucose and shallow pre-frontal cortical haemodynamics were seen, but most striking were robust inhibitions of peripheral MAO in young healthy cohorts, at a level great enough to modulate hormone secretion.

In summary the original contribution to knowledge arriving from the intervention chapters in this thesis are:

- A suggestion of enhanced cognitive performance in young healthy adults after the acute administration of blackcurrant extracts
- Confirmation of the monoamine oxidase inhibitory properties of blackcurrant extracts; including the first demonstration of an impact of post-harvest processing techniques upon *in vivo* effects
- The first demonstration of the effect of blackcurrant consumption upon peripheral prolactin, potentially outlining modulations of CNS monoaminergic tone
- The first demonstration of modulations in cerebral blood flow after the consumption of a blackcurrant extract
- The first demonstration of the modulation of blood glucose after the ingestion of blackcurrant extracts; including demonstration of the impact of post-harvest processing upon this effect

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# APPENDICES

## Appendix I - Example screening questionnaire

brain, performance and nutrition research centre
food and diet neuroscience at northumbria university

### General Exclusion Criteria

Our research focuses on the interaction between nutrition and behaviour. Below is a list of criteria relating to how the brain works, how your body deals with ingested substances (food or otherwise), and some general health criteria. If any apply to you, you will not be eligible to take part in the research study. If you are unsure or wish to discuss any of these points with the researcher then you are welcome to do so.

1. You have learning difficulties, dyslexia or colour blindness
2. You have visual impairment that cannot be corrected with glasses or contact lenses
3. You have a history of neurological or psychiatric illness (excluding depressive illness and anxiety)
4. You have a current diagnosis of depression and/or anxiety
5. You have a history or current diagnosis of drug/alcohol abuse
6. You have frequent migraines that require medication.
7. You have any disorders of the blood
8. You have a heart disorder or history of vascular illness
9. You have diabetes
10. You have any medical condition that affects absorption of food components
11. You have a history of kidney or liver disease
12. You have an over- or underactive thyroid gland
13. You are pregnant or trying to become pregnant.
14. You are lactating
15. You have any food intolerances/sensitivities
16. You have any known active infections
17. You are HIV antibody positive or you think that you may be HIV positive
18. You currently have, have ever had, or think you may be at risk of hepatitis
19. You have ever had breast cancer and/or a mastectomy
20. You have haemophilia or any similar clotting disorder

## Appendix II - Example Case Report form

Participant initials:
Participant no.:

Study code: 28AI1

<The Effect of Fruit Drinks on Mood and Cognition in Healthy Adults>

CASE REPORT FORM

Sponsor: Plant & Food Research

Study Site: Plant & Food Research,
Mt Albert
Auckland
New Zealand



Final Version Date: 22/06/2010

**CRF completion Instructions**

When completing the CRF please ensure:

- Black ink should be used.
- Each section is completed fully.
- Any corrections made to any data in the CRF are initialled and dated.
- The consent form is signed, dated and the name of signatory is clearly printed by all parties.
- The date of consent recorded in the CRF is the date the Participant signs the consent form.

**Taking Consent**

Participant must be eligible and have given consent before entering the study.

For the Participant to give consent they must sign and date two original consent forms after they have completely read the Participant information sheet and have fully understood what the study entails. The Participant must clearly print their name on the consent and the date must be the date the Participant signs the form. No study procedures can occur prior to the Participant signing the consent form.

As well as the Participant signing the consent form, it is necessary for the person explaining the study to the Participant to sign the consent form. By signing the consent form the person explaining the study confirms that they have witnessed the Participant give consent and that the Participant fully understands what the study entails.

Please ensure that two copies of the consent form are signed – one copy to be given to the Participant, one copy to remain at Plant & Food Research (this copy should be stored in a locked filing cabinet, separate from all participant data).

VISIT 0 - SCREENING  Date: D D M M Y Y Y Y

Participant initials:
Participant number:

PARTICIPANT ELIGIBILITY CHECKLIST - TRIAL ENTRY

| Please tick: | YES | NO |
|---|---|---|
| Has the Participant given written informed consent? | ☐ | ☐ |
| Is the Participant: | | |
| In good health? | ☐ | ☐ |
| Aged between 18 and 35 years? | ☐ | ☐ |
| Proficient in English equivalent to a native English speaker? | ☐ | ☐ |
| Orientated to person, place and time and has the ability to communicate with study staff? | ☐ | ☐ |
| Motivated to participate in and complete the study as instructed and to attend visit in a well-rested state? | ☐ | ☐ |
| Does the Participant: | | |
| Intend to comply with the study tobacco restriction? | ☐ | ☐ |
| Intend to comply with the study caffeine restriction? | ☐ | ☐ |
| Intend to comply with the study alcohol restriction? | ☐ | ☐ |
| Intend to comply with restriction of dietary/ supplement intake? | ☐ | ☐ |
| Intend to comply with the 12 hour fasting restriction prior to each study session? | ☐ | ☐ |
| Have normal or corrected-to-normal vision? | ☐ | ☐ |

**If NO the Participant is ineligible for the trial. Please only complete pages S1 – S5.**

VISIT 0 - SCREENING

Date: D D M M Y Y Y Y

Participant initials:

Participant number:

| | Please tick: YES | NO |
|---|---|---|
| Is the Participant: | | |
| Pregnant or seeking to become pregnant? | ☐ | ☐ |
| Does the Participant: | | |
| Currently take a vitamin complex or herbal/ dietary supplement? | ☐ | ☐ |
| Currently take a pharmaceutical product/medicine (except contraception?) | ☐ | ☐ |
| Have any known allergies or intolerance to any ingredients in the study preparation? | ☐ | ☐ |
| Have any serious disorder that might interfere with the Participation in the test? | ☐ | ☐ |
| Have a Body Mass Index (BMI) above 40 kg/$m^2$ (severely obese)? | ☐ | ☐ |
| Smoke or consume any tobacco products (even occasionally)? | ☐ | ☐ |
| Currently abuse drugs or alcohol? | ☐ | ☐ |
| Have (or have a history of) head trauma, migraines, gastric problems, learning difficulties, dyslexia, colour blindness or ADHD? | ☐ | ☐ |
| Do you have allergies to **ANY** food product? | ☐ | ☐ |

**If YES the Participant is ineligible for the trial.**
**At Screening visit, please only complete pages S1 – S5.**

VISIT 0 - SCREENING Date: D D M M Y Y Y Y

Participant initials:
Participant number:

DOCUMENTATION OF INFORMED CONSENT

**IMPORTANT: Informed consent must be obtained from the Participant BEFORE any trial procedures are started.**

Has the Participant's written informed consent been obtained?

Yes ☐
No ☐ (Please tick)

If NO: The Participant is not eligible for the trial

If YES: Keep the site consent form with the Participant's notes

Date of Consent: D D M M Y Y Y Y

Is the Participant eligible for this trial?

Yes - Eligible ☐
No - Screen failure ☐ (Please tick)

If NO: please state main reason:

Fails to meet inclusion / exclusion criteria ☐
Participant has withdrawn consent ☐ (Please tick)

_____ Date: D D M M Y Y Y Y
RR's signature

**If the Participant does not satisfy ALL of the eligibility criteria or has withdrawn consent then please only complete pages S1 – S5. If the Participant satisfies all of the eligibility criteria and has provided appropriate consent please proceed.**

VISIT 0 - SCREENING Date: D D M M Y Y Y Y

Participant initials:
Participant number:

PARTICIPANT DEMOGRAPHICS

Date of Birth: D D M M Y Y Y Y

Age: Years_____ Months_____

Sex: male (M) ☐ or female (F) ☐

Race:
- Maori ☐
- Black ☐
- Oriental ☐
- Caucasian ☐
- Other ☐ please specify _____

Does the Participant require glasses/contact lenses to use a computer?

YES ☐
NO ☐
(Please tick)

Which hand does the Participant use to write with? RIGHT ☐ LEFT ☐

Is the participant vegetarian? Yes ☐ No ☐

How many portions of fruit and vegetables does the participant eat in a typical day? [Portion= one piece of fruit, a handful of vegetables or a glass of fresh fruit juice (each additional glass of juice does not count as extra)]

_____ portion(s).

How many years of full time education has the participant had? _____

What is the highest level of qualification achieved?_____

Height ☐.☐☐ m

Weight ☐☐☐.☐☐ kg

BMI ☐☐.☐ kg/m$^2$

Blood Pressure

Systolic ☐☐☐

Diastolic ☐☐☐

Heart Rate ☐☐☐ BPM

CONCOMITANT MEDICATION:

Is the Participant receiving any concomitant medications, therapies and/or vitamin supplementation?

Yes ☐
No ☐ (Please tick)

If YES: Please complete the concomitant medication record on page 17.

MEDICAL HISTORY (Within the past 5 years)

| Specify Diagnosis | 1 = Past<br>2 = Present | Severity<br>1 = mild<br>2 = moderate<br>3 = severe | Concomitant Treatment<br>1 = Yes *<br>2 = No | Details |
|---|---|---|---|---|
| 1. | | | | |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |
| 5. | | | | |
| 6. | | | | |
| 7. | | | | |
| 8. | | | | |
| 9. | | | | |
| 10. | | | | |

* If Yes please complete concomitant medication record on page 17.

**Please note that the volunteer may not eligible to participate if taking or intending to take any prescription pharmaceutical product during the study (except for contraception for females and some topically applied therapeutic agents). Please refer to the protocol for the specific guidelines for the study.**

Participant initials: ☐☐☐
Compass Number: ☐☐☐
Participant Number: ☐☐☐

STUDY DAY 1 Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

**Study Day 1**

Participant number allocated: ☐☐☐

Have there been any changes to the subject's concomitant medications, therapies and/or vitamin supplementation since their last visit?

No ☐ Yes ☐ (if Yes complete concomitant medication record)

Has the participant experienced any adverse events (illness) since the last visit?

No ☐ Yes ☐ (if Yes complete adverse event record)

Has the participant fasted from 8pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Has participant confirmed they have consumed no caffeine or alcohol since 8pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

**Baseline**

**Samples**

**What were the Participant's blood pressure and heart rate?**

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

**What was the Participant's blood glucose level?**

☐☐ Mmol/L

**Has 10 mL of venous blood been taken?**

No ☐ Yes ☐ (if No please give a reason)

Participant initials: ☐☐☐
Compass Number: ☐☐☐
Participant Number: ☐☐☐

STUDY DAY 1 Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

**Mood Assessment** (Please tick when completed)

Mood Scales ☐

**Baseline Cognitive Tasks:**

Digital Vigilance ☐

Stroop ☐

RVIP ☐

Logical Reasoning ☐

**Treatment**: Treatment taken ☐ Time taken _____

**Post dose Blood Glucose, Blood Pressure and Heart Rate(60 minutes post treatment)**

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

**Post Dose Cognitive Tasks (70 minutes post treatment):**

Mood Scales ☐

Repetition 1 ☐

Repetition 2 ☐

Repetition 3 ☐

Repetition 4 ☐

Repetition 5 ☐

Repetition 6 ☐

Repetition 7 ☐

Logical Reasoning ☐

Mood Scales ☐

Participant initials: | | |
Compass Number: | | |
Participant Number: | | |

STUDY DAY 1 Date: D D M M Y Y Y Y | | | | | | | | |

**What were the Participant's blood pressure and heart rate?**

Blood Pressure

Systolic | | | | MM Hg

Diastolic | | | | MM Hg

Heart Rate | | | | BPM

**What was the Participant's blood glucose level?**

| | | Mmol/L

**Has 10 mL of venous blood been taken?**

No ☐ Yes ☐ (if No please give a reason)

_____

_____

_____

_____ Date: D D M M Y Y Y Y | | | | | | | | |

RR's signature

Participant initials: ☐☐☐
Compass Number: ☐☐☐
Participant Number: ☐☐☐

STUDY DAY 2 Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

**Study Day 2**

Have there been any changes to the subject's concomitant medications, therapies and/or vitamin supplementation since their last visit?

No ☐ Yes ☐ (if Yes complete concomitant medication record)

Has the participant experienced any adverse events (illness) since the last visit?

No ☐ Yes ☐ (if Yes complete adverse event record)

Has the participant fasted from 8pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Has participant confirmed they have consumed no caffeine or alcohol since 8pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

**Baseline**

**Samples**

**What were the Participant's blood pressure and heart rate?**

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

**What was the Participant's blood glucose level?**

☐☐ Mmol/L

**Has 10 mL of venous blood been taken?**

No ☐ Yes ☐ (if No please give a reason)

_____

_____

_____

Participant initials: ☐☐☐
Compass Number: ☐☐☐
Participant Number: ☐☐☐

STUDY DAY 2 Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

**Mood Assessment** (Please tick when completed)

Mood Scales ☐

**Baseline Cognitive Tasks:**

Digital Vigilance ☐

Stroop ☐

RVIP ☐

Logical reasoning ☐

**Treatment**: Treatment taken ☐ Time taken _____

**Post dose Blood Glucose, Blood Pressure and Heart Rate**

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

**Post Dose Cognitive Tasks (70 minutes post treatment):**

Mood Scales ☐

Repetition 1 ☐

Repetition 2 ☐

Repetition 3 ☐

Repetition 4 ☐

Repetition 5 ☐

Repetition 6 ☐

Repetition 7 ☐

Logical Reasoning ☐

Mood Scales ☐

Participant initials: ☐☐☐
Compass Number: ☐☐☐
Participant Number: ☐☐☐

STUDY DAY 2 Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

**What were the Participant's blood pressure and heart rate?**

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

**What was the Participant's blood glucose level?**

☐☐ Mmol/L

**Has 10 mL of venous blood been taken?**

No ☐ Yes ☐ (if No please give a reason)

_____

_____

_____

_____
RR's signature

Date: D D M M Y Y Y Y ☐☐☐☐☐☐☐☐

Study Day 3: Date: D D M M Y Y Y Y

Participant initials:
Participant number:

**Study Day 3**

Have there been any changes to the subject's concomitant medications, therapies and/or vitamin supplementation since their last visit?

No ☐ Yes ☐ (if Yes complete concomitant medication record)

Has the participant experienced any adverse events (illness) since the last visit?

No ☐ Yes ☐ (if Yes complete adverse event record)

Has the participant fasted from 8pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

Has participant confirmed they have consumed no caffeine or alcohol since 8pm yesterday? (If No re-schedule their study day)

No ☐ Yes ☐

**Baseline**

**Samples**

**What were the Particpant's blood pressure and heart rate?**

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

**What was the Participant's blood glucose level?**

☐☐ Mmol/L

**Has 10 mL of venous blood been taken?**

No ☐ Yes ☐ (if No please give a reason)

Study Day 3: Date: D D M M Y Y Y Y

Participant initials:
Participant number:

**Mood Assessment** (Please tick when completed)

Mood Scales ☐

**Baseline Cognitive Tasks:**

Digital Vigilance ☐

Stroop ☐

RVIP ☐

Logical Reasoning ☐

**Treatment**: Treatment taken ☐ Time taken _____

**Post dose Blood Glucose, Blood Pressure and Heart Rate**

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

**Post Dose Cognitive Tasks (70 minutes post treatment):**

Mood Scales ☐

Repetition 1 ☐

Repetition 2 ☐

Repetition 3 ☐

Repetition 4 ☐

Repetition 5 ☐

Repetition 6 ☐

Repetition 7 ☐

Logical Reasoning ☐

☐

Study Day 3: Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Mood Scales

**What were the Participant's blood pressure and heart rate?**

Blood Pressure

Systolic ☐☐☐ MM Hg

Diastolic ☐☐☐ MM Hg

Heart Rate ☐☐☐ BPM

**What was the Participant's blood glucose level?**

☐☐ Mmol/L

**Has 10 mL of venous blood been taken?**

No ☐ Yes ☐ (if No please give a reason)

_____

_____

_____

_____ Date: D D M M Y Y Y Y

RR's signature

Study Day 3:

Date: D D M M Y Y Y Y

Participant initials:
Participant number:

TRIAL OUTCOME (please tick appropriate box):

Completed trial: ☐

If trial not completed, please complete:

Date of subject withdrawal from the study: D D M M Y Y Y Y

Provide main reason for premature termination (one reason only):

| | | | |
|---|---|---|---|
| adverse event | ☐ | withdrawn consent | ☐ |
| did not co-operate | ☐ | refused treatment | ☐ |
| administrative reason | ☐ | lost to follow-up | ☐ |
| protocol violation | ☐ | other | ☐ |

if other please specify: _____

_____ Date: D D M M Y Y Y Y

Investigator's signature

CONCOMITANT MEDICATION RECORD

Date: D D M M Y Y Y Y

Participant initials:
Participant number:

Please complete the following information fully for any concomitant medication, therapies and/or vitamin supplementation:

*** If Indication is due to a new/worsening AE, please complete the AE form (pg 18).**

| Concomitant Treatment (please use generic name) | Indication | Single Dose | Total Daily Dose | Units | Frequency (e.g. BID, PRN) | Route code | Start and Stop Dates DD MM YY |
|---|---|---|---|---|---|---|---|
| 1. | | | | | | | Start: / / |
| | | | | | | | Stop: / / |
| 2. | | | | | | | Start: / / |
| | | | | | | | Stop: / / |
| 3. | | | | | | | Start: / / |
| | | | | | | | Stop: / / |
| 4. | | | | | | | Start: / / |
| | | | | | | | Stop: / / |
| 5. | | | | | | | Start: / / |
| | | | | | | | Stop: / / |
| 6. | | | | | | | Start: / / |
| | | | | | | | Stop: / / |
| 7. | | | | | | | Start: / / |
| | | | | | | | Stop: / / |
| 8. | | | | | | | Start: / / |
| | | | | | | | Stop: / / |
| 9. | | | | | | | Start: / / |
| | | | | | | | Stop: / / |

ADVERSE EVENTS

Date: D D M M Y Y Y Y

Participant initials:
Participant number:

## Adverse Events

**Were there any Adverse Events?** ☐ 1 NO ☐ 2 YES, please complete all sections below, cross appropriate number.

| | if yes* | | Date | Severity | Relation to Study Drug | Action(s) Taken *(several statements are possible)* | Outcome of Event |
|---|---|---|---|---|---|---|---|
| **Adverse Event, specify**<br>Please list ONE event per line. | **Serious**<br>1 No<br>2 Yes * | **Reason** *(several statements are possible)*<br>1. Results in Death<br>2. Life-threatening<br>5. Hospitalization – new / prolonged<br>6. Congenital anomaly/birth defect<br>10. Persistent or significant disability/incapacity<br>11. Important medical event | **Start / Stop**<br>If ongoing update at next visit<br>dd / mm / yy | 1. Mild<br>2. Moderate<br>3. Severe | **Possible?**<br>1. No<br>2. Yes | 1. None<br>2. Dose of study drug reduced<br>3. Study drug discontinued and restarted<br>4. Study drug discontinued permanently<br>5. Remedial drug therapy, specify on concomitant medication page<br>6. Other (specify below)<br>7. Infusion rate of study drug educed<br>8. Hospitalization required or prolonged | 1. Resolved<br>2. Improved<br>3. Unchanged<br>4. Worsened<br>6. Death<br>7. Insufficient Follow-up |
| 1. | 1 2* ☐☐ | 1 2 5 6 10 11 ☐☐☐☐☐☐ | / / / / | 1 2 3 ☐☐☐ | 1 2 ☐☐ | 1 2 3 4 5 6 7 8 ☐☐☐☐☐☐☐☐ | 1 2 3 4 6 7 ☐☐☐☐☐☐ |
| 2. | 1 2* ☐☐ | 1 2 5 6 10 11 ☐☐☐☐☐☐ | / / / / | 1 2 3 ☐☐☐ | 1 2 ☐☐ | 1 2 3 4 5 6 7 8 ☐☐☐☐☐☐☐☐ | 1 2 3 4 6 7 ☐☐☐☐☐☐ |
| 3. | 1 2* ☐☐ | 1 2 5 6 10 11 ☐☐☐☐☐☐ | / / / / | 1 2 3 ☐☐☐ | 1 2 ☐☐ | 1 2 3 4 5 6 7 8 ☐☐☐☐☐☐☐☐ | 1 2 3 4 6 7 ☐☐☐☐☐☐ |
| 4. | 1 2* ☐☐ | 1 2 5 6 10 11 ☐☐☐☐☐☐ | / / / / | 1 2 3 ☐☐☐ | 1 2 ☐☐ | 1 2 3 4 5 6 7 8 ☐☐☐☐☐☐☐☐ | 1 2 3 4 6 7 ☐☐☐☐☐☐ |

Further Details of Adverse Events: _____

_____

D D M M Y Y Y Y

_____ Investigator's Signature Date: ☐☐☐☐☐☐☐☐

## Appendix III - Amplex red MAO-B assay

### Stock Solution Preparation

**1.1** Prepare a ~20 mM stock solution of the Amplex Red reagent by allowing one vial of the Amplex Red reagent (Component A) and the DMSO (Component B) to warm to room temperature. Just prior to use, dissolve the contents of one vial of Amplex Red reagent (1mg) in 200µl DMSO. Each vial of Amplex Red reagent provides sufficient material for approximately 100 assays of 200µl each. This stock solution should be stored frozen at ≤–20°C, protected from light.

**1.2** Prepare a 1X working solution of Reaction Buffer by adding 5ml of 5X Reaction Buffer stock solution (Component E) to 20ml of deionized water (dH2O). This 25ml volume of 1X Reaction Buffer is sufficient for approximately 100 assays of 200µl each, with a 5ml excess for making stock solutions and dilutions.

**1.3** Prepare a 200U/ml stock solution of horseradish per oxidase (HRP) by dissolving the entire vial of HRP (Component C) in 1.0ml of 1X Reaction Buffer. After use, the remaining solution should be divided into small aliquots and stored frozen at ≤–20°C.

**1.4** Prepare a 20mM H2O2 working solution by diluting the ~3% H2O2 stock solution (Component D) into the appropriate volume of 1X Reaction Buffer. The actual H2O2 concentration is indicated on the components label. For instance, a 20 mm H2O2 working solution can be prepared from a 3.0% H2O2 stock solution by diluting 23µl of 3.0% H2O2 into 977µl of 1X Reaction Buffer. Please note that although the ~3% H2O2 stock solution has been stabilized to slow degradation, the 20 mm H2O2 working solution will be less stable and should be used promptly.

1ml of 20mM $H_2O_2$ solution = 20.6 µl of $H_2O_2$ + 979.4 µl of buffer.

Below is a table of dilutions for standards.

| | Dilution | Stock | | Buffer |
|---|---|---|---|---|
| A | 10µm | 5µm | | 9.995ml |
| B | 7.5µm | 750.2µm of | A | 249.8ml |
| C | 5µm | 500µm of | A | 500ml |
| D | 2.5µm | 500µm of | C | 500ml |
| E | 1000nm | 100µm of | A | 900ml |
| F | 100nm | 100µm of | E | 900ml |
| G | 10nm | 100µm of | F | 900ml |

**1.5** Prepare 100mm stock solutions of the two amine oxidase substrates (benzylamine and tyramine, Components F and G) by adding 1.2ml dH2O directly to the individual substrate vials. Substrate stock solutions should be stored at ≤–20°C.

**1.6** Prepare 0.5 mm stock solutions of each of the MAO inhibitors (clorgyline and pargyline, Components H and I) by adding 1.0 ml dH2O directly to the individual vials. Inhibitor stock solutions should be stored at ≤–20°C.

1:10 dilution for 1.e 10 µl 1µl clorgyline to 9 µl of $dH_2O$.

**1.7** If desired, prepare a 2 mm stock solution of resorufin by adding 1ml dH2O directly to the vial of resorufin solid (Component J). This solution can be used to prepare a standard curve to determine the moles of product produced in the Amplex Red reaction. This stock solution should be stored frozen at ≤–20°C,protected from light.

**Amine Oxidase Assay**

**2.1** Dilute the blood platelets in 1X Reaction Buffer. A volume of 50µl will be used for each reaction. One of the MAO inhibitors (at a concentration of 1µm) can be included, if desired, by adding the equivalent of 0.2µl of the 0.5 mm inhibitor stock solution (prepared in step 1.6) to each 100µl volume of diluted sample and pre-incubating the sample (e.g. 30 minutes at room temperature). Please note that amine oxidase and inhibitor concentrations will be twofold lower in the final reaction volume.

**2.2** Prepare a positive control by diluting the 20 mm H2O2 working solution to 10µm in 1X Reaction Buffer. Use 1X Reaction Buffer without H2O2 as a negative control.

**2.3** Pipette 100µl of the diluted amine oxidase samples and controls into separate wells of a microplate.

**2.4** Prepare a working solution of 400µm Amplex Red reagent containing 2 U/ml HRP and 2 mm substrate by adding 200µl of Amplex Red reagent stock solution (prepared in step 1.1), 100µl of the HRP stock solution (prepared in step 1.3) and 200µl of substrate stock solution (prepared in 1.5) to 9.5 ml 1X Reaction Buffer. This 10ml volume is sufficient for ~100 assays. Note that final concentrations of each component will be twofold lower in the final reaction volume.

For 10 ml of solution:
200µl Amplex red
100µl HRP
200µl Substrate
9.5ml buffer

**2.5** Begin the reactions by adding 100µl of the Amplex Red reagent/HRP/substrate working solution to each micro plate well containing the amine oxidase samples and controls.

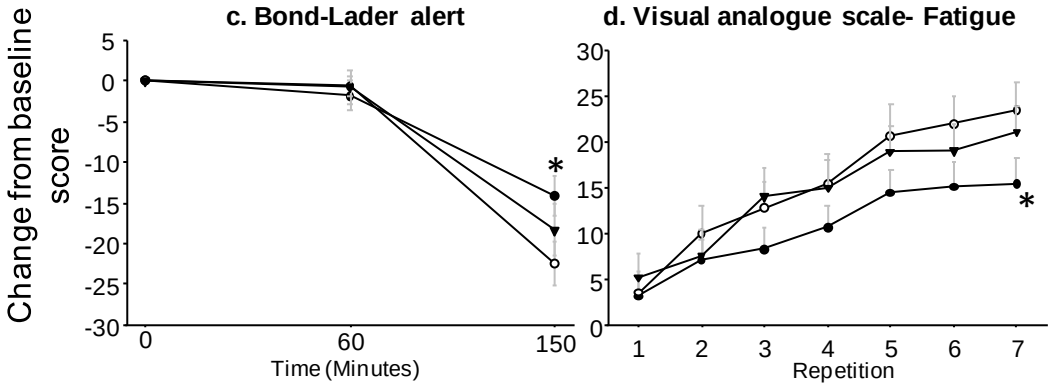
**2.6** Incubate the reactions for 30 minutes or longer at room temperature, protected from light. Because the assay is continuous (not terminated), fluorescence may be measured at multiple time points to follow the kinetics of the reactions.

**2.7** If desired, prepare a resorufin standard curve: Dilute the appropriate amount of 2 mm resorufin stock solution in 1X Reaction Buffer to yield resorufin solution ranging from 0 to 20µm resorufin. Pipette 200µl of each resorufin standard into individual (empty) wells of a microplate at any time prior to measuring fluorescence.

**2.8** Measure the fluorescence in a fluorescence micro plate reader using excitation in the range of 530–560 nm and emission detection at ~590 nm (see Figure 1).

**2.9** For each point, correct for background fluorescence by subtracting the values derived from the no–amine oxidase control.

## Appendix IV- Mental fatigue & Bond-Lader alert



## Appendix VI- FRAP assay

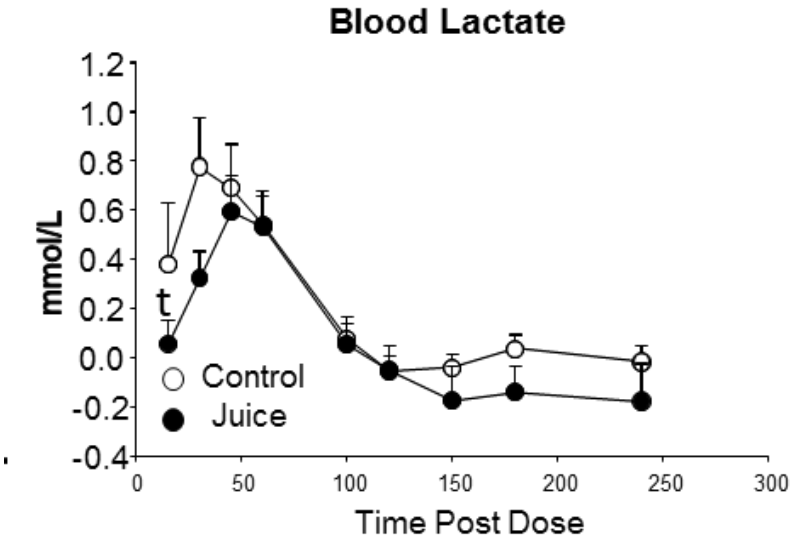
Sodium acetate trihydrate and iron (III) chloride hexahydrate (ferric chloride) were obtained from Sigma-Aldrich, Poole, Dorset, U.K. Glacial acetic acid was obtained from BDH Laboratory Supplies. 2,4,6-Tripyridyl-s-triazine (TPTZ) was obtained from Fluka Chemicals, Switzerland. Hydrochloric acid (HCl) was obtained from Merck, Darmstadt, Germany.

A Cecil Instruments 1000 series (Cambridge, England) UV/Visible spectrophotometer was used to measure the absorbance readings. The spectrophotometer was set to read at 593nm absorbency and a 3.0ml sample of FRAP was used as a blank to zero the equipment.

Acetate buffer (300 mM) was prepared from 1.55g sodium acetate trihydrate mixed with 8ml glacial acetic acid and made up to 500ml with distilled water; 10mM TPTZ solution was prepared in 40mM HCl; Ferric chloride was dissolved in deionised water to generate a 20 mM solution. These solutions were mixed in the ratio 10:1:1 to give the working FRAP reagent. FRAP reagent was prepared fresh daily.

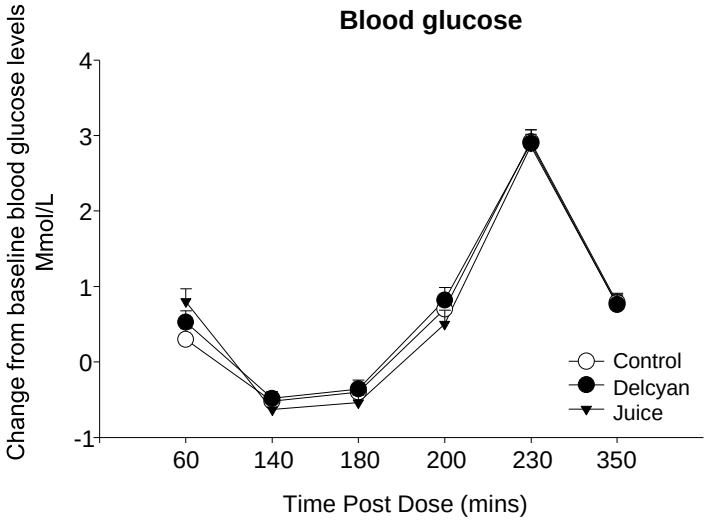
100µl of plasma or standard was added to 3.0ml of FRAP solution.. The cuvette was vortexed and a reading was taken. The sample was then incubated at 37ºC. At 4 minutes, the sample was removed from the incubator and a second reading was taken. FRAP values were obtained by comparing the absorbance at 593nm of the sample and the blank reagent (Benzie & Strain, 1999).

## Appendix VII- change from baseline post-dose lactate levels




*Mean change from baseline post-dose lactate levels indicating no effects of treatment following consumption of the Blackadder blackcurrant extract and control intervention drinks. (t=p<0.1).*

## Appendix VIII change from baseline post-dose glucose levels




*Mean change from baseline post-dose glucose levels indicating no effects of treatment after a glucose load 200 minutes post supplementation (chapter six)*

# Appendix IX- NIRS resting baseline scores

*8.1 Mean scores (mmol/L) and, standard deviations (SD) for left hemisphere (LH), right hemisphere (RH) and hemispheres combined (combined) for NIRS outcomes total-HB Oxy-HB and deoxy-HB during the resting baseline measurement*

| Measure | N | Treatment | Resting Baseline | |
|---|---|---|---|---|
| | | | Mean | SD |
| Combined OxyHb | 20 | Control | -1.545 | 3.542 |
| | | Juice | -1.249 | 2.688 |
| OxyHb LH | 20 | Control | -1.489 | 3.360 |
| | | Juice | -1.035 | 2.948 |
| OxyHb RH | 20 | Control | -1.601 | 4.186 |
| | | Juice | -1.463 | 3.097 |
| Combined DeoxyHb | 20 | Control | 0.081 | 1.045 |
| | | Juice | 0.091 | 1.179 |
| DeoxyHb LH | 20 | Control | 0.296 | 1.165 |
| | | Juice | 0.021 | 1.487 |
| DeoxyHb RH | 20 | Control | -0.132 | 1.248 |
| | | Juice | 0.162 | 1.153 |
| Combined TotalHb | 20 | Control | -1.627 | 3.689 |
| | | Juice | -1.340 | 3.024 |
| TotalHb LH | 20 | Control | -1.785 | 3.416 |
| | | Juice | -1.056 | 3.488 |
| TotalHb RH | 20 | Control | -1.469 | 4.313 |
| | | Juice | -1.625 | 3.008 |

*Data presented In this table is arbitrary and, therefore ,does not represent actual levels of haemoglobin at baseline.*

# Appendix X – Raw Mean pre-dose baseline, post dose scores and standard deviations for all outcomes

## Chapter 2

*Table 9.1 Mean pre-dose baseline, post dose scores and standard deviations for Bond-Lader mood scales following supplementation of the control, Delcyan™ and Blackadder juice drinks.*

| Measure | N | Treatment | Baseline | | Repetition 1 | | Repetition 2 | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD |
| Bond-Lader calm | 33 | Control | 58.71 | 15.05 | 58.06 | 11.02 | 63.23 | 20.13 |
| | | Delcyan™ | 61.06 | 16.35 | 57.83 | 11.31 | 63.51 | 14.53 |
| | | Juice | 60.11 | 15.08 | 62.35 | 14.32 | 64.55 | 18.43 |
| Bond-Lader content | 33 | Control | 69.33 | 10.2 | 67.4 | 10.08 | 58.95 | 13.78 |
| | | Delcyan™ | 70.16 | 11.41 | 69.76 | 5.58 | 63.74 | 10.13 |
| | | Juice | 69.01 | 11.09 | 69.09 | 5.79 | 62.76 | 12.99 |
| Bond-Lader alert | 33 | Control | 61.31 | 12.6 | 59.5 | 10.83 | 38.82 | 15.95 |
| | | Delcyan™ | 62.99 | 15.32 | 62.37 | 6.51 | 48.83 | 13.99 |
| | | Juice | 62.83 | 13.15 | 62.07 | 11.45 | 44.46 | 18.54 |

Table 9.2 Mean pre-dose baseline, post dose scores and standard deviations for each cognitive condition following supplementation of the control, Delcyan™ and Blackadder juice drinks.

| Measure | N | Treatment | Baseline | | Repetition 1 | | Repetition 2 | | Repetition 3 | | Repetition 4 | | Repetition 5 | | Repetition 6 | | Repetition 7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Digit vigilance accuracy (%) | 33 | Control | 97.03 | 4.3 | 95.97 | 3.24 | 93.85 | 7.73 | 92.16 | 10.26 | 89.96 | 7.48 | 89.54 | 9.41 | 88.27 | 9.88 | 90.01 | 8.74 |
| | | Delcyan™ | 97.5 | 3.22 | 95.51 | 6.26 | 92.99 | 8.68 | 91.28 | 13.51 | 91.69 | 9.56 | 91.67 | 8.64 | 92.05 | 7.92 | 90.59 | 10.23 |
| | | Juice | 96.45 | 3.83 | 95.04 | 8.46 | 93.56 | 7.04 | 91.16 | 10.69 | 89.82 | 10.87 | 91.05 | 7.62 | 88.24 | 12.77 | 91.74 | 7.92 |
| Digit vigilance false alarms (Number) | 33 | Control | 0.91 | 1.34 | 1.40 | 1.22 | 1.43 | 1.82 | 1.89 | 1.51 | 2.60 | 2.25 | 2.57 | 1.85 | 2.83 | 2.24 | 2.43 | 2.02 |
| | | Delcyan™ | 0.8 | 0.8 | 1.03 | 1.12 | 1.51 | 1.62 | 1.80 | 1.95 | 1.80 | 1.91 | 2.37 | 1.90 | 1.86 | 1.56 | 2.06 | 2.10 |
| | | Juice | 0.86 | 1.14 | 1.14 | 1.53 | 1.78 | 1.77 | 2.28 | 2.48 | 2.42 | 2.17 | 2.28 | 1.85 | 2.69 | 2.57 | 2.28 | 2.25 |
| Digit vigilance reaction time (msec) | 33 | Control | 414.1 | 29.28 | 434.2 | 32.39 | 442.2 | 32.30 | 444.4 | 32.43 | 457.6 | 34.41 | 450.9 | 32.29 | 458.7 | 32.22 | 457.1 | 32.21 |
| | | Delcyan™ | 413.5 | 28.64 | 427.8 | 29.01 | 431.5 | 29.55 | 448.8 | 35.02 | 445.1 | 33.87 | 448.3 | 31.81 | 445.8 | 29.95 | 452.1 | 29.48 |
| | | Juice | 422.1 | 31.03 | 427.9 | 28.13 | 440.9 | 33.97 | 448.3 | 34.08 | 447.7 | 30.29 | 450.6 | 31.97 | 455.5 | 30.13 | 451.0 | 32.80 |
| RVIP accuracy (%) | 32 | Control | 65.86 | 15.26 | 64.61 | 11.47 | 59.14 | 11.84 | 62.27 | 12.76 | 58.28 | 12.32 | 57.27 | 16.82 | 57.73 | 11.55 | 55.23 | 14.84 |
| | | Delcyan™ | 64.53 | 17.59 | 64.30 | 9.38 | 62.97 | 10.04 | 66.48 | 10.79 | 62.81 | 11.80 | 62.81 | 10.73 | 62.97 | 10.79 | 62.50 | 16.08 |
| | | Juice | 70.47 | 14.6 | 65.16 | 12.08 | 63.91 | 12.84 | 62.89 | 14.11 | 59.77 | 13.17 | 59.92 | 13.81 | 60.63 | 13.51 | 61.64 | 14.31 |
| RVIP false alarms | 32 | Control | 4 | 4.7 | 2.97 | 3.76 | 3.06 | 3.43 | 2.72 | 3.21 | 3.75 | 3.56 | 3.25 | 4.13 | 3.03 | 3.36 | 3.28 | 3.39 |
| | | Delcyan™ | 2.88 | 4.88 | 2.63 | 3.41 | 2.69 | 3.76 | 2.63 | 4.44 | 2.50 | 4.29 | 2.91 | 3.95 | 2.63 | 4.54 | 3.16 | 5.29 |
| | | Juice | 2.81 | 3.35 | 1.93 | 2.55 | 2.40 | 2.87 | 3.19 | 2.09 | 3.47 | 3.74 | 2.68 | 2.78 | 2.09 | 3.20 | 2.56 | 2.86 |
| RVIP reaction time (msec) | 32 | Control | 484.4 | 43.93 | 483.53 | 29.83 | 491.6 | 31.80 | 497.1 | 31.23 | 493.8 | 32.70 | 495.3 | 35.65 | 493.2 | 40.32 | 504.1 | 44.98 |
| | | Delcyan™ | 479.8 | 35.2 | 487.51 | 34.25 | 494.2 | 37.04 | 488.2 | 31.31 | 490.5 | 35.15 | 494.1 | 37.62 | 484.3 | 37.58 | 492.3 | 35.15 |
| | | Juice | 484.6 | 40.58 | 483.22 | 26.74 | 500.5 | 32.19 | 495.7 | 34.31 | 499.0 | 40.99 | 496.6 | 35.43 | 492.1 | 35.99 | 494.9 | 43.18 |
| Stroop accuracy (%) | 35 | Control | 98.88 | 2.14 | 98.72 | 2.28 | 99.02 | 1.61 | 99.03 | 2.43 | 98.15 | 2.86 | 98.21 | 2.73 | 98.37 | 2.53 | 98.59 | 2.29 |
| | | Delcyan™ | 99.14 | 1.61 | 98.83 | 1.91 | 98.83 | 2.05 | 99.21 | 1.90 | 99.18 | 1.59 | 98.72 | 1.83 | 99.23 | 1.69 | 98.42 | 2.27 |
| | | Juice | 98.9 | 2.77 | 98.33 | 2.97 | 98.64 | 2.11 | 98.93 | 2.06 | 98.90 | 2.49 | 98.63 | 2.51 | 98.62 | 2.29 | 98.40 | 3.01 |
| Stroop reaction time (msec) | 35 | Control | 797.4 | 105.9 | 788.37 | 51.78 | 793.2 | 49.64 | 786.1 | 54.52 | 812.3 | 60.77 | 807.9 | 59.67 | 851.2 | 174.70 | 816.0 | 64.57 |
| | | Delcyan™ | 801.5 | 124.4 | 785.64 | 49.59 | 799.2 | 57.42 | 827.9 | 157.30 | 799.9 | 57.53 | 790.1 | 120.66 | 807.5 | 60.99 | 798.2 | 72.49 |
| | | Juice | 788.5 | 115.1 | 772.81 | 70.97 | 780.6 | 81.20 | 793.8 | 102.10 | 813.5 | 121.70 | 786.1 | 70.25 | 808.2 | 95.64 | 796.3 | 79.30 |
| VAS fatigue (mm) | 33 | Control | 35.06 | 19.23 | 38.61 | 13.13 | 45.09 | 17.41 | 47.79 | 16.19 | 50.45 | 18.22 | 55.64 | 19.31 | 57.00 | 17.08 | 58.39 | 18.22 |
| | | Delcyan™ | 34.42 | 19.97 | 37.57 | 10.48 | 41.45 | 12.57 | 42.72 | 12.93 | 45.06 | 13.55 | 48.81 | 14.88 | 49.45 | 15.68 | 49.72 | 16.53 |
| | | Juice | 32.61 | 17.69 | 37.79 | 15.47 | 40.13 | 15.99 | 46.64 | 17.30 | 47.52 | 17.48 | 51.52 | 15.99 | 51.58 | 16.70 | 53.58 | 16.54 |
| VAS difficulty (mm) | 33 | Control | 48.97 | 15.48 | 51.91 | 14.29 | 59.94 | 20.25 | 66.21 | 14.14 | 69.73 | 18.76 | 74.00 | 17.67 | 74.52 | 18.86 | 78.21 | 17.73 |
| | | Delcyan™ | 40.12 | 18.97 | 45.03 | 10.85 | 53.00 | 14.57 | 55.12 | 12.78 | 57.73 | 14.83 | 61.36 | 15.25 | 62.57 | 15.95 | 63.24 | 16.04 |
| | | Juice | 39.85 | 20.63 | 47.55 | 18.05 | 55.52 | 21.86 | 57.64 | 18.51 | 60.43 | 17.26 | 64.67 | 19.43 | 66.58 | 21.01 | 67.33 | 20.04 |
| Logical reasoning reaction time | 33 | Control | 3977 | 1330 | 3,840.30 | 797.5 | | | | | | | | | | | | |
| | | Delcyan™ | 3834 | 1132 | 3,725.10 | 964.1 | | | | | | | | | | | | |
| | | Juice | 3963 | 1266 | 3,705.30 | 750.1 | | | | | | | | | | | | |
| Logical reasoning accuracy (%) | 33 | Control | 86.48 | 15.88 | 87.87 | 22.97 | | | | | | | | | | | | |
| | | Delcyan™ | 86.48 | 15.85 | 86.66 | 8.34 | | | | | | | | | | | | |
| | | Juice | 86.3 | 16.93 | 87.82 | 11.25 | | | | | | | | | | | | |

*Table 9.3 Mean pre-dose baseline, post dose scores and standard deviations for each physiological parameter following supplementation of the control, Delcyan™ and Blackadder juice drinks.*

| Measure | N | Treatment | Baseline | | Repetition 1 | | Repetition 2 | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD |
| Heart rate (BPM) | 35 | Control | 69.60 | 11.76 | 67.34 | 10.88 | 62.14 | 9.91 |
| | | Delcyan™ | 72.14 | 12.07 | 67.94 | 12.16 | 64.00 | 12.00 |
| | | Juice | 72.46 | 11.89 | 68.37 | 10.02 | 62.26 | 9.31 |
| Diastolic blood pressure (mmHg) | 35 | Control | 76.83 | 7.64 | 74.66 | 7.96 | 77.54 | 8.66 |
| | | Delcyan™ | 76.89 | 8.31 | 74.97 | 8.22 | 76.77 | 7.82 |
| | | Juice | 74.63 | 7.32 | 75.17 | 7.19 | 74.63 | 9.37 |
| Systolic blood pressure (mmHg) | 35 | Control | 121.30 | 12.62 | 118.23 | 10.51 | 121.03 | 11.41 |
| | | Delcyan™ | 122.70 | 13.65 | 120.54 | 12.07 | 122.14 | 13.73 |
| | | Juice | 122.90 | 11.74 | 121.03 | 13.91 | 119.37 | 13.89 |
| Glucose (mmoL) | 35 | Control | 4.95 | 0.53 | 4.75 | 0.77 | 5.29 | 0.78 |
| | | Delcyan™ | 4.93 | 0.39 | 4.95 | 0.68 | 4.54 | 0.36 |
| | | Juice | 4.96 | 0.48 | 4.52 | 0.46 | 4.74 | 0.61 |
| Prolactin (IU/L) | 8 | Control | 271.60 | 70.52 | 169.29 | 50.36 | | |
| | 7 | Delcyan™ | 315.40 | 135.90 | 194.14 | 94.37 | | |
| | 5 | Juice | 353.40 | 105.30 | 146.00 | 48.53 | | |
| MAO-B (nmol $H^2O^2$) | 8 | Control | 258.00 | 112.30 | 269.33 | 106.76 | | |
| | | Delcyan™ | 220.60 | 131.20 | 142.55 | 105.24 | | |
| | | Juice | 273.10 | 114.10 | 5.68 | 6.75 | | |
| Total anthocyanins (nM/L) | 17 | Control | 0 | 0 | 0.23 | 0.05 | | |
| | | Delcyan™ | 0 | 0 | 22.19 | 5.57 | | |
| | | Juice | 0 | 0 | 15.20 | 3.89 | | |
| Phenylethylamine (ng/ml) | 17 | Control | 0.05 | 0.18 | 0.05 | 0.20 | | |
| | | Delcyan™ | 0.04 | 0.14 | 0.05 | 0.18 | | |
| | | Juice | 0.09 | 0.35 | 0.09 | 0.35 | | |
| Dopamine (ng/ml) | 17 | Control | 0.02 | 0.02 | 0.02 | 0.02 | | |
| | | Delcyan™ | 0.02 | 0.01 | 0.02 | 0.01 | | |
| | | Juice | 0.02 | 0.01 | 0.02 | 0.01 | | |
| Serotonin (ng/ml) | 17 | Control | 1.9 | 1.34 | 1.82 | 1.57 | | |
| | | Delcyan™ | 2.37 | 2.06 | 2.01 | 1.83 | | |
| | | Juice | 2.23 | 2.3 | 1.91 | 1.83 | | |
| Normetanephrine (ng/ml) | 17 | Control | 0.05 | 0.02 | 0.06 | 0.03 | | |
| | | Delcyan™ | 0.06 | 0.03 | 0.07 | 0.03 | | |
| | | Juice | 0.05 | 0.02 | 0.07 | 0.03 | | |
| Noradrenaline (ng/ml) | 17 | Control | 0.3 | 0.1 | 0.38 | 0.18 | | |
| | | Delcyan™ | 0.35 | 0.1 | 0.41 | 0.16 | | |
| | | Juice | 0.33 | 0.11 | 0.37 | 0.15 | | |
| Adrenaline (ng/ml) | 17 | Control | 0.02 | 0.02 | 0.03 | 0.03 | | |
| | | Delcyan™ | 0.02 | 0.03 | 0.02 | 0.03 | | |
| | | Juice | 0.02 | 0.02 | 0.02 | 0.02 | | |
| DHPG (ng/ml) | 17 | Control | 1.61 | 0.36 | 1.71 | 0.47 | | |
| | | Delcyan™ | 1.6 | 0.34 | 1.70 | 0.45 | | |
| | | Juice | 1.63 | 0.37 | 1.34 | 0.44 | | |
| Homovanillic acid (ng/ml) | 17 | Control | 21.42 | 12.21 | 19.42 | 12.02 | | |
| | | Delcyan™ | 27.45 | 16.7 | 23.62 | 14.62 | | |
| | | Juice | 17.12 | 8.01 | 14.47 | 7.18 | | |

*Table 9.4 Mean pre-dose baseline, post dose scores and standard deviations for each mood parameter over both measured assessments*

| Measure | N | Treatment | Baseline | | Session 1 (60 minutes) | | | | | | | | Session 2 (150 minutes) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Repetition 1 | | Repetition 2 | | Repetition 3 | | Repetition 4 | | Repetition 1 | | Repetition 2 | | Repetition 3 | | Repetition 4 | |
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| VAS mentally energised | 32 | Control | 45.06 | 11.93 | 45.88 | 13.54 | 41.97 | 12.25 | 39.88 | 16.22 | 37.56 | 16.64 | 46.44 | 15.16 | 42.84 | 17.95 | 39.47 | 16.45 | 41.66 | 16.44 |
| | | 125mg | 46.91 | 11.47 | 47.38 | 13.60 | 42.47 | 16.51 | 43.03 | 16.61 | 40.56 | 16.64 | 47.53 | 14.60 | 44.56 | 16.73 | 46.28 | 16.81 | 47.47 | 17.92 |
| | | 250mg | 46.13 | 15.55 | 46.50 | 16.09 | 44.69 | 14.44 | 43.91 | 18.31 | 40.84 | 17.67 | 44.72 | 17.68 | 42.97 | 19.81 | 42.44 | 17.73 | 40.06 | 18.34 |
| | | 500mg | 44.63 | 12.47 | 47.38 | 13.60 | 42.47 | 16.51 | 43.03 | 16.61 | 40.56 | 16.64 | 47.53 | 14.60 | 44.56 | 16.73 | 46.28 | 16.81 | 47.47 | 17.92 |
| VAS motivation | 32 | Control | 47.88 | 14.66 | 44.41 | 13.34 | 41.22 | 11.64 | 37.81 | 13.91 | 37.88 | 13.95 | 46.03 | 11.85 | 42.06 | 14.74 | 41.31 | 15.26 | 40.88 | 15.89 |
| | | 125mg | 49.78 | 10.25 | 47.28 | 14.92 | 42.41 | 17.46 | 40.06 | 16.55 | 40.38 | 16.72 | 47.94 | 15.86 | 42.22 | 18.80 | 42.66 | 18.13 | 44.25 | 18.27 |
| | | 250mg | 47.56 | 15.96 | 48.66 | 17.23 | 43.84 | 18.88 | 40.09 | 18.46 | 38.75 | 18.62 | 44.50 | 16.86 | 40.16 | 19.88 | 37.50 | 18.14 | 38.56 | 19.18 |
| | | 500mg | 49.5 | 12.56 | 47.28 | 14.92 | 42.41 | 17.46 | 40.06 | 16.55 | 40.38 | 16.72 | 47.94 | 15.86 | 42.22 | 18.80 | 42.66 | 18.13 | 44.25 | 18.27 |
| VAS physically energised | 32 | Control | 48.97 | 13.05 | 46.38 | 12.31 | 42.50 | 13.80 | 39.88 | 18.11 | 40.00 | 16.15 | 49.09 | 11.37 | 43.06 | 16.38 | 43.25 | 16.19 | 41.66 | 16.39 |
| | | 125mg | 49.31 | 11.03 | 48.44 | 13.15 | 41.28 | 18.30 | 42.84 | 17.99 | 38.97 | 17.04 | 49.03 | 13.66 | 45.50 | 17.80 | 46.69 | 15.69 | 45.56 | 17.17 |
| | | 250mg | 45.47 | 13.35 | 48.88 | 16.78 | 43.28 | 19.18 | 43.09 | 18.87 | 42.53 | 19.44 | 46.59 | 14.20 | 41.88 | 18.47 | 42.16 | 18.36 | 41.41 | 17.19 |
| | | 500mg | 46.81 | 11.8 | 48.44 | 13.15 | 41.28 | 18.30 | 42.84 | 17.99 | 38.97 | 17.04 | 49.03 | 13.66 | 45.50 | 17.80 | 46.69 | 15.69 | 45.56 | 17.17 |
| STA-I | 32 | Control | 31.31 | 1.32 | 30.48 | 7.18 | | | | | 33.72 | 8.51 | 31.20 | 8.05 | | | | | 32.65 | 8.61 |
| | | 125mg | 32.1 | 1.23 | 32.00 | 8.12 | | | | | 33.63 | 8.40 | 32.03 | 8.48 | | | | | 32.91 | 8.03 |
| | | 250mg | 31.53 | 1.13 | 31.19 | 7.06 | | | | | 33.41 | 8.61 | 31.38 | 7.57 | | | | | 33.41 | 8.67 |
| | | 500mg | 31.31 | 1.32 | 31.00 | 7.12 | | | | | 33.19 | 7.64 | 31.53 | 7.41 | | | | | 32.63 | 8.08 |
| Bond-Lader calm | 32 | Control | 62.84 | 11.71 | 58.34 | 12.60 | | | | | 61.34 | 12.32 | 59.48 | 12.14 | | | | | 61.34 | 11.42 |
| | | 125mg | 62.97 | 11.44 | 61.50 | 13.17 | | | | | 62.42 | 10.39 | 61.48 | 12.26 | | | | | 59.58 | 10.10 |
| | | 250mg | 64.23 | 10.8 | 61.95 | 10.21 | | | | | 62.67 | 11.58 | 61.73 | 12.19 | | | | | 60.33 | 11.34 |
| | | 500mg | 63.34 | 11.69 | 57.89 | 9.19 | | | | | 58.94 | 10.38 | 59.98 | 8.94 | | | | | 58.59 | 9.89 |
| Bond-Lader content | 32 | Control | 61.99 | 13.51 | 61.56 | 13.24 | | | | | 60.50 | 13.45 | 61.71 | 12.85 | | | | | 61.18 | 13.33 |
| | | 125mg | 62.41 | 12.73 | 62.61 | 12.29 | | | | | 60.66 | 13.36 | 63.49 | 12.56 | | | | | 63.52 | 11.94 |
| | | 250mg | 63.54 | 12.73 | 64.24 | 13.00 | | | | | 61.67 | 10.89 | 63.98 | 10.28 | | | | | 62.37 | 12.16 |
| | | 500mg | 63.15 | 11.37 | 61.26 | 12.84 | | | | | 58.13 | 13.36 | 61.96 | 11.62 | | | | | 61.15 | 14.15 |
| Bond-Lader alert | 32 | Control | 52.89 | 13.57 | 54.64 | 13.99 | | | | | 46.56 | 14.22 | 52.24 | 13.64 | | | | | 44.91 | 15.44 |
| | | 125mg | 55.73 | 12.32 | 57.36 | 10.57 | | | | | 47.79 | 12.19 | 55.67 | 13.26 | | | | | 52.57 | 13.45 |
| | | 250mg | 53.53 | 14.71 | 52.66 | 13.27 | | | | | 44.71 | 13.52 | 52.48 | 10.97 | | | | | 50.06 | 12.04 |
| | | 500mg | 51.63 | 11.24 | 54.62 | 12.26 | | | | | 45.97 | 12.61 | 53.39 | 11.02 | | | | | 48.36 | 13.18 |

*Table 9.5 Mean pre-dose baseline, post dose scores and standard deviations for each physiological parameter over both measured epochs*

| Measure | N | Treatment | Baseline | | Post Dose | |
|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD |
| Platelet MAO-B | 8 | Control | 2517 | 1556 | 2517 | 856.1 |
| | | 125mg | 2391 | 1600 | 2391 | 1219 |
| | | 250mg | 2366 | 1897 | 2366 | 905.4 |
| | | 500mg | 2276 | 1319 | 2276 | 1053 |
| FRAP | 10 | Control | 859.9 | 110.7 | 935.4 | 286.7 |
| | | 125mg | 917.7 | 113.1 | 873.5 | 166.2 |
| | | 250mg | 843.5 | 124.9 | 877.0 | 165.1 |
| | | 500mg | 873.2 | 105.7 | 875.0 | 95.85 |
| Prolactin | 9 | Control | 340.4 | 187.1 | 216.7 | 64.13 |
| | | 125mg | 367.4 | 158.7 | 187.6 | 54.66 |
| | | 250mg | 325.9 | 124.2 | 178.0 | 54.19 |
| | | 500mg | 357.1 | 116.3 | 159.3 | 34.69 |

## Chapter 4

*Table 9.6 Mean pre-dose baseline, post dose scores and standard deviations for all behavioural outcomes*

| Measure | N | Treatment | Baseline | | Repetition 1 | | Repetition 2 | | Repetition 3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Serial 3 incorrect responses | 20 | Control | 1.95 | 12.27 | 2.25 | 3.34 | 2.70 | 3.53 | 3.40 | 4.11 |
| | | Juice | 2.8 | 17.39 | 2.05 | 3.02 | 3.55 | 3.38 | 4.10 | 3.60 |
| Serial 3 correct responses | 20 | Control | 44.2 | 51.11 | 44.65 | 11.82 | 45.50 | 11.96 | 44.90 | 10.40 |
| | | Juice | 42.85 | 76.74 | 44.05 | 16.72 | 43.75 | 15.70 | 42.05 | 13.92 |
| Serial 3 number of responses | 20 | Control | 46.15 | 54.74 | 46.90 | 11.79 | 48.20 | 12.36 | 48.30 | 10.39 |
| | | Juice | 45.65 | 69.13 | 46.10 | 14.99 | 47.30 | 15.07 | 46.15 | 13.79 |
| Serial 7 incorrect responses | 20 | Control | 2.95 | 12.09 | 2.95 | 3.05 | 3.85 | 3.00 | 2.74 | 3.44 |
| | | Juice | 2.8 | 12.78 | 2.75 | 3.30 | 2.75 | 2.65 | 2.94 | 2.75 |
| Serial 7 correct responses | 20 | Control | 24.25 | 49.01 | 27.05 | 27.05 | 26.80 | 10.81 | 10.79 | 10.89 |
| | | Juice | 25.25 | 54.02 | 28.00 | 28.30 | 28.00 | 11.91 | 10.88 | 11.74 |
| Serial 7 number of responses | 20 | Control | 27.2 | 45.06 | 30.00 | 30.10 | 30.65 | 9.30 | 9.59 | 9.50 |
| | | Juice | 28.05 | 48.87 | 30.75 | 31.60 | 30.75 | 10.92 | 9.76 | 10.65 |
| RVIP correct responses | 20 | Control | 53.5 | 62.59 | 53.25 | 52.00 | 53.63 | 23.55 | 21.97 | 25.91 |
| | | Juice | 55.79 | 57.76 | 56.25 | 51.63 | 52.63 | 22.75 | 23.89 | 23.01 |
| RVIP false alarms | 20 | Control | 0.85 | 26.59 | 0.70 | 1.15 | 1.00 | 0.92 | 1.50 | 1.41 |
| | | Juice | 0.84 | 35.04 | 1.10 | 0.65 | 1.35 | 1.25 | 1.18 | 1.46 |
| RVIP reaction time | 20 | Control | 482.88 | 54.45 | 483.24 | 478.14 | 477.06 | 57.99 | 49.35 | 64.43 |
| | | Juice | 482.53 | 55.75 | 479.83 | 497.88 | 447.42 | 51.76 | 88.73 | 115.29 |
| Fatigue | 20 | Control | 28.6 | 77.9 | 44.60 | 26.88 | 50.70 | 29.98 | 54.40 | 31.30 |
| | | Juice | 23.75 | *90.3* | 34.90 | 21.75 | 43.00 | 22.95 | 47.10 | 27.04 |

## Chapter Five

*Table 9.7 Mean pre-dose baseline, post dose scores and standard deviations for all haemodynamic parameters*

| Measure | Treatment | N | Baseline | | 15 minutes | | 30 minutes | | 45 minutes | | 1 hour | | 1.5 hours | | 2 hours | | 2.4 hours | | 3 hours | | 4 hours | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Heart rate (BPM) | Control | 8 | 60.50 | 11.22 | 61.38 | 11.73 | 65.25 | 20.89 | 65.50 | 10.04 | 65.50 | 13.03 | 62.88 | 11.32 | 61.00 | 14.40 | 56.00 | 13.54 | 58.13 | 14.86 | 58.63 | 9.44 |
| | Juice | | 63.63 | 8.52 | 63.63 | 8.52 | 60.50 | 7.15 | 62.63 | 12.99 | 64.25 | 5.80 | 65.38 | 8.62 | 63.75 | 10.71 | 57.88 | 12.37 | 59.25 | 9.13 | 56.13 | 8.64 |
| DVP - SI | Control | 8 | 5.71 | 1.08 | 6.34 | 6.34 | 6.34 | 6.34 | 6.34 | 6.34 | 6.51 | 1.06 | 6.07 | 1.20 | 6.20 | 1.18 | 6.16 | 1.24 | 6.15 | 1.24 | 5.94 | 1.20 |
| | Juice | | 6.63 | 0.97 | 6.79 | 0.97 | 6.37 | 0.95 | 6.93 | 1.74 | 6.57 | 1.10 | 6.55 | 1.20 | 6.53 | 1.38 | 6.24 | 1.02 | 6.46 | 1.16 | 6.17 | 1.10 |
| DVP - RI | Control | 8 | 61.88 | 16.34 | 69.00 | 19.14 | 62.00 | 16.38 | 65.63 | 18.69 | 67.63 | 16.62 | 56.00 | 10.46 | 61.13 | 12.84 | 67.00 | 12.65 | 67.38 | 8.23 | 60.13 | 9.95 |
| | Juice | | 69.13 | 12.98 | 69.13 | 12.98 | 68.13 | 14.86 | 61.75 | 10.69 | 64.75 | 5.70 | 64.25 | 10.82 | 63.25 | 21.09 | 66.25 | 14.47 | 67.50 | 18.16 | 64.63 | 16.06 |
| Glucose (mmol/L) | Control | 8 | 4.25 | 0.47 | 5.91 | 1.64 | 5.70 | 1.61 | 4.12 | 1.27 | 3.56 | 0.88 | 3.65 | 0.59 | 3.92 | 0.29 | 3.98 | 0.40 | 4.17 | 0.15 | 4.15 | 0.17 |
| | Juice | | 4.23 | 0.44 | 5.02 | 1.06 | 5.33 | 1.01 | 4.71 | 1.38 | 3.83 | 1.11 | 3.66 | 0.65 | 3.90 | 0.34 | 4.03 | 0.34 | 4.21 | 0.45 | 4.07 | 0.17 |
| Lactate (mmol/L) | Control | 8 | 0.72 | 0.24 | 1.12 | 0.61 | 1.52 | 0.51 | 1.39 | 0.46 | 1.21 | 0.35 | 0.80 | 0.33 | 0.68 | 0.19 | 0.69 | 0.20 | 0.78 | 0.18 | 0.69 | 0.24 |
| | Juice | | 0.93 | 0.37 | 0.98 | 0.38 | 1.25 | 0.48 | 1.52 | 0.54 | 1.46 | 0.32 | 0.98 | 0.22 | 0.88 | 0.21 | 0.69 | 0.34 | 0.79 | 0.17 | 0.62 | 0.44 |
| MAO-B (nmol $H^2O^2$) | Control | 8 | 1074 | 376.6 | 2367 | 3305 | 1013 | 277.5 | 956.1 | 367.0 | 1050 | 253.3 | 1259 | 573.6 | 1434 | 637.5 | 1458 | 686.7 | 1504 | 545.7 | 3563 | 5785 |
| | Juice | | 1526 | 1095 | 52.31 | 420.3 | 0.00 | 197.3 | 0.00 | 125.10 | 0.00 | 221.8 | 0.00 | 179.4 | 0.00 | 139.5 | 36.85 | 186.3 | 0.68 | 198.5 | 215.9 | 205.38 |
| Prolactin (mIU/L) | Control | 8 | 285.3 | 81.90 | 239.2 | 53.58 | 220.8 | 54.68 | 207.0 | 58.71 | 201.70 | 68.72 | 216.3 | 78.22 | 236.4 | 62.69 | 177.9 | 71.69 | 185.0 | 86.57 | 188.5 | 90.99 |
| | Juice | | 285.8 | 63.10 | 246.7 | 64.22 | 208.3 | 63.20 | 189.6 | 59.63 | 164.67 | 51.80 | 130.7 | 36.30 | 109.6 | 38.27 | 121.0 | 46.66 | 127.3 | 57.46 | 150.6 | 95.00 |

*Table 9.8 Mean pre-dose baseline, post dose scores and, standard deviations for MAO-B, prolactin glucose, lactate and DVP 24 hour parameters*

| Measure | Treatment | N | 24h | |
|---|---|---|---|---|
| | | | Mean | SD |
| MAO-B (nmol $H^2O^2$) | Control | 6 | 1739 | 681.0 |
| | Juice | | 1417 | 542.2 |
| Prolactin (mIU/L) | Control | 6 | 294.5 | 72.35 |
| | Juice | | 286.1 | 127.1 |
| Glucose (mmol/L) | Control | 8 | 4.50 | 0.35 |
| | Juice | | 4.34 | 0.60 |
| Lactate (mmol/L) | Control | 8 | 1.01 | 0.44 |
| | Juice | | 0.66 | 0.30 |
| Heart Rate (BPM) | Control | 8 | 63.50 | 12.75 |
| | Juice | | 57.63 | 12.78 |
| DVP -SI | Control | 8 | 6.31 | 0.98 |
| | juice | | 6.44 | 1.22 |
| DVP -RI | Control | 8 | 55.75 | 7.44 |
| | Juice | | 65.00 | 6.09 |

*Table 9.9 Mean pre-dose baseline, post dose scores and standard deviations for all behavioural parameters*

| Measure | N | Treatment | Baseline | | Repetition 1 | | Repetition 2 | | Repetition 3 | | Repetition 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Finger tap number of taps | 34 | Control | 500.4 | 63.1 | 495.1 | 57.97 | 490.0 | 107.6 | 476.9 | 54.19 | 484.7 | 60.59 |
| | | Delcyan™ | 508.8 | 51.19 | 490.11 | 61.51 | 501.9 | 128.1 | 478.4 | 55.03 | 496.9 | 59.68 |
| | | Juice | 523.1 | 101.9 | 501.0 | 92.60 | 502.9 | 95.58 | 484.5 | 52.91 | 488.2 | 74.84 |
| Immediate word recall correct | 34 | Control | 6.03 | 0.61 | 5.37 | 1.57 | 5.69 | 1.60 | 5.51 | 1.36 | 5.40 | 1.59 |
| | | Delcyan™ | 6.09 | 0.51 | 5.40 | 1.33 | 5.29 | 1.43 | 4.91 | 1.84 | 5.20 | 1.73 |
| | | Juice | 6.31 | 0.53 | 5.49 | 1.87 | 5.37 | 1.61 | 5.29 | 1.76 | 5.31 | 1.78 |
| Immediate word recall incorrect | 34 | Control | 0.71 | 0.3 | 0.63 | 0.69 | 0.43 | 0.74 | 0.74 | 1.15 | 0.77 | 0.91 |
| | | Delcyan™ | 0.77 | 0.35 | 0.57 | 0.88 | 0.83 | 0.89 | 1.06 | 1.45 | 1.00 | 1.35 |
| | | Juice | 0.49 | 0.25 | 0.86 | 1.00 | 1.14 | 1.26 | 0.74 | 1.04 | 0.74 | 1.01 |
| DSST correct | 34 | Control | 57.77 | 4.05 | 60.29 | 12.43 | 60.31 | 11.48 | 61.46 | 11.70 | 60.37 | 11.46 |
| | | Delcyan™ | 54.91 | 2.69 | 59.14 | 8.96 | 61.26 | 9.92 | 60.46 | 9.11 | 61.20 | 10.43 |
| | | Juice | 55.91 | 3.81 | 59.63 | 11.68 | 59.40 | 11.82 | 61.49 | 11.67 | 62.86 | 12.02 |
| DSST incorrect | 34 | Control | 0.14 | 0.11 | 0.06 | 0.24 | 0.11 | 0.32 | 0.11 | 0.32 | 0.06 | 0.24 |
| | | Delcyan™ | 0.11 | 0.1 | 0.11 | 0.32 | 0.11 | 0.40 | 0.00 | 0.00 | 0.09 | 0.28 |
| | | Juice | 0.11 | 0.13 | 0.11 | 0.32 | 0.14 | 0.43 | 0.11 | 0.53 | 0.06 | 0.24 |
| Numeric working memory % correct | 34 | Control | 96.36 | 3.9 | 95.93 | 3.50 | 96.60 | 3.50 | 95.99 | 3.58 | 95.56 | 4.50 |
| | | Delcyan™ | 95.99 | 3.26 | 96.30 | 2.48 | 96.26 | 2.44 | 95.83 | 3.60 | 95.96 | 3.46 |
| | | Juice | 96.13 | 3.48 | 96.57 | 3.65 | 96.23 | 3.44 | 95.99 | 4.03 | 96.10 | 4.87 |
| Numeric working memory RT (msec) | 34 | Control | 860.5 | 210 | 835.7 | 222.7 | 841.9 | 226.9 | 836.1 | 230.9 | 834.6 | 234.6 |
| | | Delcyan™ | 834.3 | 231 | 822.9 | 237.9 | 802.5 | 179.2 | 854.8 | 385.9 | 824.1 | 199.4 |
| | | Juice | 867.8 | 255 | 810.6 | 208.0 | 820.6 | 205.5 | 825.1 | 223.8 | 778.7 | 207.2 |
| Corsi blocks RT (msec) | 34 | Control | 6424 | 1775 | 5.87 | 0.94 | 5.74 | 0.84 | 5.86 | 0.82 | 5.77 | 0.77 |
| | | Delcyan™ | 6273 | 1468 | 5.89 | 0.74 | 5.92 | 0.91 | 5.87 | 0.84 | 5.57 | 0.94 |
| | | Juice | 6360 | 1384 | 5.87 | 0.95 | 6.03 | 0.73 | 5.66 | 1.33 | 5.81 | 0.99 |
| Corsi blocks span | 34 | Control | 5.87 | 0.93 | 6070 | 1640 | 5842 | 1482 | 6283 | 1622 | 5814 | 1466 |
| | | Delcyan™ | 5.89 | 0.73 | 6729 | 1583 | 6198 | 1279 | 6148 | 1606 | 5823 | 1599 |
| | | Juice | 5.87 | 0.94 | 6239 | 1744 | 6061 | 1375 | 6085 | 1561 | 5984 | 1467 |
| Peg and ball thinking time | 34 | Control | 3129 | 1824 | 2811 | 1160 | 2773 | 1115 | 2618 | 1201 | 2675 | 1032 |
| | | Delcyan™ | 2939 | 1214 | 2857 | 1086 | 2851 | 1154 | 2700 | 934 | 2763 | 1046 |
| | | Juice | 2934 | 1029 | 2796 | 1083 | 2801 | 894 | 2704 | 817 | 2666 | 759 |
| Peg and ball working time (msec) | 34 | Control | 9623 | 2660 | 9043 | 2420 | 8806 | 2084 | 8773 | 2281 | 8798 | 2157 |
| | | Delcyan™ | 9467 | 2091 | 8903 | 1644 | 8763 | 2088 | 8724 | 1764 | 8737 | 1941 |
| | | Juice | 9232 | 1952 | 8795 | 1708 | 8851 | 1188 | 8516 | 1236 | 8419 | 1473 |
| Peg and ball errors | 34 | Control | 3.88 | 4.29 | 4.00 | 2.91 | 2.97 | 3.11 | 3.68 | 3.55 | 3.97 | 4.58 |
| | | Delcyan™ | 4.35 | 3.89 | 3.09 | 3.58 | 2.71 | 2.47 | 4.06 | 3.98 | 3.32 | 3.21 |
| | | Juice | 3.18 | 3.2 | 3.00 | 2.83 | 3.79 | 3.84 | 2.97 | 3.39 | 3.38 | 1.84 |
| Digit vigilance % correct | 34 | Control | 95.29 | 4.7 | 92.76 | 9.51 | 93.33 | 10.47 | 90.69 | 10.44 | 93.79 | 8.34 |
| | | DelcyanTM | 93.33 | 16.1 | 94.25 | 7.91 | 94.83 | 5.75 | 89.42 | 16.62 | 93.56 | 8.50 |
| | | Juice | 95.75 | 5.37 | 94.02 | 9.61 | 93.79 | 10.46 | 93.33 | 8.40 | 95.52 | 7.42 |
| Digit vigilance RT (msec) | 34 | Control | 453.1 | 41.6 | 459.13 | 51.97 | 469.67 | 55.76 | 473.37 | 50.91 | 470.03 | 54.75 |
| | | Delcyan™ | 448.7 | 51.7 | 471.35 | 55.36 | 471.30 | 52.76 | 473.04 | 54.94 | 471.77 | 50.23 |
| | | Juice | 448.1 | 41.2 | 460.58 | 56.38 | 462.71 | 53.12 | 471.22 | 60.44 | 471.89 | 48.98 |
| Digit vigilance false alarms (number) | 29 | Control | 0.07 | 0.23 | 0.310 | 0.541 | 0.172 | 0.384 | 0.241 | 0.511 | 0.172 | 0.468 |
| | | Delcyan™ | 0.31 | 1.04 | 0.310 | 0.541 | 0.172 | 0.468 | 0.345 | 0.614 | 0.207 | 0.491 |
| | | Juice | 0.14 | 0.32 | 0.103 | 0.310 | 0.379 | 1.015 | 0.310 | 0.541 | 0.241 | 0.830 |
| Stroop accuracy (%) | 29 | Control | 98.38 | -2.35 | 98.24 | 2.24 | 98.77 | 1.66 | 97.84 | 2.61 | 98.19 | 2.44 |
| | | Delcyan™ | 98.38 | -1.99 | 98.73 | 1.48 | 98.77 | 1.66 | 98.38 | 1.99 | 98.38 | 1.61 |
| | | Juice | 98.48 | -1.62 | 98.43 | 1.92 | 98.38 | 2.58 | 98.33 | 2.17 | 98.14 | 2.31 |
| Stroop reaction time (msec) | 29 | Control | 810 | -177 | 791.2 | 170.1 | 791.3 | 161.2 | 781.8 | 142.8 | 777.0 | 162.7 |
| | | Delcyan™ | 788.2 | -131 | 758.8 | 115.3 | 770.1 | 129.5 | 787.1 | 247.3 | 799.7 | 270.2 |
| | | Juice | 799.4 | -137 | 763.8 | 129.6 | 764.9 | 125.4 | 785.3 | 151.3 | 736.2 | 116.5 |
| RVIP accuracy (%) | 29 | Control | 55.39 | 19.2 | 58.44 | 20.88 | 57.34 | 20.91 | 56.80 | 21.91 | 56.80 | 19.75 |
| | | Delcyan™ | 53.67 | 17.7 | 57.58 | 20.92 | 56.33 | 17.61 | 56.09 | 21.50 | 57.42 | 21.34 |
| | | Juice | 58.91 | 20.9 | 59.06 | 20.34 | 59.84 | 21.19 | 57.42 | 21.49 | 58.36 | 22.98 |
| RVIP false alarms (number) | 29 | Control | 1.31 | 1.2 | 1.656 | 1.619 | 1.563 | 1.645 | 1.344 | 1.537 | 1.125 | 1.212 |
| | | Delcyan™ | 1.13 | 1.2 | 1.469 | 1.565 | 1.188 | 1.091 | 1.156 | 1.081 | 0.938 | 1.105 |
| | | Juice | 1.59 | 1.8 | 1.594 | 1.757 | 1.000 | 1.164 | 1.438 | 1.458 | 1.375 | 1.519 |
| RVIP reaction time (msec) | 29 | Control | 501.5 | 49.9 | 505.2 | 56.9 | 506.8 | 60.7 | 497.9 | 57.8 | 504.8 | 53.8 |
| | | Delcyan™ | 507.2 | 46.8 | 507.3 | 50.5 | 511.9 | 53.6 | 506.9 | 55.3 | 512.7 | 67.0 |
| | | Juice | 513.3 | 50.8 | 504.7 | 57.4 | 505.8 | 52.7 | 506.9 | 51.2 | 494.1 | 54.5 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Delayed word recall % correct (number) | 35 | Control | 3.83 | 0.68 | 2.057 | 1.909 | 2.086 | 1.721 | 1.943 | 1.939 | 1.886 | 1.676 |
| | | Delcyan™ | 4.03 | 0.51 | 1.971 | 1.543 | 1.771 | 1.816 | 1.943 | 1.862 | 2.371 | 1.848 |
| | | Juice | 3.57 | 0.53 | 2.257 | 2.005 | 2.143 | 2.060 | 1.743 | 1.502 | 2.114 | 1.952 |
| Delayed word recall incorrect (number) | 35 | Control | 1.17 | 0.37 | 1.343 | 1.413 | 1.171 | 1.505 | 1.429 | 1.989 | 1.000 | 1.237 |
| | | Delcyan™ | 1.06 | 0.37 | 1.143 | 1.458 | 1.600 | 1.866 | 1.000 | 1.534 | 1.743 | 2.254 |
| | | Juice | 0.54 | 0.61 | 1.457 | 1.597 | 1.657 | 1.814 | 1.486 | 1.755 | 1.257 | 1.837 |
| Word recognition RT (msec) | 35 | Control | 972.9 | 218 | 1012.3 | 229.4 | 969.2 | 224.3 | 1006.7 | 298.4 | 955.7 | 239.2 |
| | | Delcyan™ | 933.3 | 189 | 980.3 | 228.3 | 967.6 | 208.2 | 962.3 | 252.4 | 963.2 | 254.4 |
| | | Juice | 992.1 | 245 | 974.5 | 227.4 | 969.0 | 209.1 | 945.1 | 212.2 | 905.4 | 168.0 |
| Word recognition % correct | 35 | Control | 75.81 | 9.51 | 72.19 | 8.85 | 73.05 | 9.48 | 69.14 | 8.64 | 73.24 | 10.95 |
| | | Delcyan™ | 77.71 | 8.61 | 73.14 | 9.11 | 68.38 | 10.71 | 70.48 | 8.86 | 70.76 | 9.50 |
| | | Juice | 77.24 | 9.03 | 72.95 | 10.38 | 72.57 | 9.67 | 70.86 | 11.12 | 70.57 | 12.38 |
| Picture recognition RT (msec) | 35 | Control | 872.9 | 155 | 882.5 | 156.2 | 914.7 | 216.1 | 921.5 | 215.7 | 887.4 | 209.7 |
| | | Delcyan™ | 830.9 | 120 | 857.4 | 132.3 | 878.4 | 169.6 | 914.3 | 188.4 | 872.6 | 155.0 |
| | | Juice | 857.7 | 221 | 898.4 | 180.4 | 849.3 | 148.9 | 872.8 | 174.9 | 866.8 | 168.1 |
| Picture recognition % correct | 35 | Control | 94.95 | 6.39 | 94.57 | 5.25 | 93.05 | 5.01 | 93.05 | 5.85 | 92.19 | 5.88 |
| | | Delcyan™ | 95.43 | 5.11 | 94.48 | 5.42 | 94.57 | 6.16 | 92.28 | 6.56 | 91.71 | 7.20 |
| | | Juice | 95.43 | 4.37 | 92.95 | 7.04 | 94.57 | 6.11 | 93.62 | 6.69 | 92.00 | 8.37 |
| Bond-Lader alert (mm) | 35 | Control | 65.83 | 13.8 | 62.09 | 14.47 | 59.97 | 16.29 | 56.87 | 18.58 | 58.38 | 15.95 |
| | | Delcyan™ | 65.34 | 13.3 | 65.96 | 13.80 | 65.77 | 13.13 | 64.81 | 13.77 | 64.51 | 13.59 |
| | | Juice | 66.47 | 14.6 | 64.00 | 14.50 | 60.74 | 16.09 | 57.84 | 16.96 | 60.07 | 17.01 |
| Bond-Lader content (mm) | 35 | Control | 71.03 | 13.7 | 71.69 | 11.88 | 71.32 | 13.28 | 72.64 | 12.94 | 72.14 | 11.97 |
| | | Delcyan™ | 71.77 | 10.9 | 71.03 | 12.56 | 70.71 | 11.56 | 70.41 | 12.79 | 72.19 | 11.49 |
| | | Juice | 73.08 | 11.5 | 72.69 | 9.84 | 72.55 | 11.34 | 73.30 | 10.97 | 73.86 | 10.74 |
| Bond-Lader calm (mm) | 35 | Control | 61.81 | 14.2 | 64.44 | 14.44 | 63.49 | 14.26 | 67.83 | 14.19 | 65.70 | 13.72 |
| | | Delcyan™ | 63.1 | 15.1 | 60.63 | 14.62 | 57.46 | 14.48 | 56.42 | 15.40 | 59.78 | 14.69 |
| | | Juice | 63.39 | 13.6 | 63.69 | 13.90 | 66.36 | 12.66 | 67.17 | 13.49 | 64.61 | 12.14 |
| VAS fatigue (mm) | 35 | Control | 37.23 | 18.2 | 42.46 | 16.09 | 44.57 | 18.19 | 52.23 | 19.43 | 47.51 | 16.93 |
| | | Delcyan™ | 39.69 | 16.4 | 42.57 | 18.73 | 48.03 | 16.46 | 50.06 | 17.12 | 49.49 | 17.20 |
| | | Juice | 34.46 | 17.1 | 38.74 | 15.67 | 43.40 | 21.19 | 50.49 | 20.70 | 46.83 | 18.52 |
| VAS mentally energised (mm) | 35 | Control | 56.91 | 15.3 | 54.51 | 13.92 | 52.34 | 16.08 | 47.60 | 18.94 | 49.69 | 15.93 |
| | | Delcyan™ | 54.63 | 16.7 | 50.83 | 18.70 | 47.57 | 18.15 | 46.97 | 16.44 | 51.89 | 17.45 |
| | | Juice | 58.23 | 18.9 | 51.14 | 19.55 | 47.34 | 16.85 | 46.37 | 20.86 | 46.40 | 18.16 |
| VAS physically energised (mm) | 35 | Control | 58.46 | 15.2 | 52.86 | 15.35 | 50.94 | 17.23 | 47.80 | 18.08 | 48.03 | 16.00 |
| | | Delcyan™ | 56.89 | 15.9 | 49.91 | 18.48 | 48.51 | 15.50 | 46.83 | 16.56 | 50.63 | 16.85 |
| | | Juice | 57.03 | 17.8 | 55.20 | 17.79 | 50.83 | 18.94 | 48.94 | 19.75 | 48.23 | 17.96 |

*Table 9.10 Mean pre-dose baseline, post dose scores and standard deviations for all blood parameters*

| Measure | N | Treatment | Baseline | | 60 Minutes | | 140 minutes | | 180 minutes | | 200 minutes | | 230 minutes | | 350 minutes | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Blood glucose (mmol/L) | 35 | Control | 4.79 | 0.08 | 5.088 | 0.806 | 4.272 | 0.398 | 4.388 | 0.651 | 5.489 | 0.957 | 7.704 | 1.001 | 5.582 | 0.642 |
| | | Delcyan™ | 4.8 | 0.09 | 5.324 | 1.110 | 4.313 | 0.656 | 4.436 | 0.779 | 5.615 | 1.049 | 7.701 | 1.206 | 5.551 | 0.826 |
| | | Juice | 4.78 | 0.12 | 5.579 | 0.891 | 4.143 | 0.484 | 4.241 | 0.390 | 5.272 | 1.151 | 7.634 | 1.257 | 5.540 | 0.900 |
| Blood lactate (mmol/L) | 35 | Control | 1.47 | 0.13 | 2.025 | 0.729 | 1.334 | 0.647 | 1.136 | 0.540 | 1.544 | 0.568 | 1.814 | 0.671 | 1.247 | 0.512 |
| | | Delcyan™ | 1.35 | 0.15 | 1.851 | 0.511 | 1.297 | 0.553 | 1.136 | 0.492 | 1.477 | 0.510 | 1.648 | 0.397 | 1.298 | 0.516 |
| | | Juice | 1.36 | 0.12 | 2.149 | 0.815 | 1.329 | 0.526 | 1.118 | 0.462 | 1.583 | 0.643 | 1.909 | 0.647 | 1.239 | 0.558 |
| MAO- activity (nmol $H^2O^2$) | 5 | Control | 1192 | 446 | 1119 | 922.8 | | | | | | | | | | |
| | | Delcyan™ | 1090 | 328 | 811.5 | 352.4 | | | | | | | | | | |
| | | Juice | 906 | 394 | 1135 | 937.4 | | | | | | | | | | |
| Prolactin (mIU/L) | 11 | Control | 270.2 | 30.9 | 333.1 | 42.6 | | | | | | | | | | |
| | | Delcyan™ | 287.2 | 55.5 | 293.2 | 58.1 | | | | | | | | | | |
| | | Juice | 232.4 | 17.4 | 270.1 | 22.9 | | | | | | | | | | |