A systematic evaluation of the psychological and behavioural effects of the combined consumption of glucose and caffeine and comparison to the effects produced by consuming either substance in isolation

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Abstract

Extensive research has found glucose and caffeine to have beneficial effects on cognition and mood. Broadly, glucose has been found to improve memory and caffeine to improve attention and alertness. Relatively little research has investigated the effects of their combined consumption, although to date, similar effects on cognitive performance and mood have been found. The aim of this thesis was to systematically evaluate the behavioural effects of combined consumption of these substances and compare them with the effects of consuming either substance in isolation. Moderating factors, such as cognitive effort, were considered along with the evaluation of neural and neuroendocrine responses.

The first study (chapter 2) found evidence of beneficial effects of caffeine, glucose and their combination on memory and mood, with individual effects varying across doses. However, concurrent measurement of the neuroendocrine response found no effects (chapter 3). Investigation into pre-retrieval administration of the substances memory performance (chapter 4) found no effects of any substance, in contrast to the beneficial effects found for pre-learning administration. A parallel assessment of glucose and caffeine on different attentional networks and systems (chapter 5) failed to find any effects on this aspect of cognitive performance. In chapter 6 the effects of the substances on participants who were in a sub-optimal state were examined. The findings were not able to show that effects of the substances can be more clearly elucidated when participants are not performing optimally. The final experimental study (chapter 7) investigated the effects of caffeine and glucose on neurocognitive processes, but no beneficial effects were found.

Overall, the findings suggest that the effects of caffeine, glucose and their combination are modulated by dose and domain.

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Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Ethical approval for the research presented in this thesis was sought and granted by Lancaster University Ethics Committee.

Name:

Signature:

Date:

Chapter 1

Introduction and Literature Review

1.1 General Introduction

Energy drinks are widely consumed beverages in Europe and North America (Woojae, 2003). They are marketed as providing a 'boost' in performance and mood when needed, helping to increase alertness, energy and decrease drowsiness (Smit & Rogers, 2002). Much work has been done on two of the main ingredients usually contained in these drinks, glucose and caffeine when they are consumed in isolation. There is ample evidence to suggest that when consumed in isolation both of these substances have an effect on cognition and mood (Addicott & Laurienti, 2009; Brice & Smith, 2002; Durlach, 1998; Foster, Lidder, & Sünram, 1998; Haskell, Kennedy, Wesnes, & Scholey, 2005; Lorist & Tops, 2003; Martin & Benton, 1999; Owens & Benton, 1994; Scholey, Harper, & Kennedy, 2001; Smit & Rogers, 2000; Sünram-Lea, Foster, Durlach, & Perez, 2001; Sünram-Lea, Dewhurst, & Foster, 2008; Sünram-Lea, Owen, Finnegan, & Hu, 2011). In terms of the aspects of cognition affected, generally speaking glucose has been found to have most robust beneficial effects on verbal episodic memory (Foster *et al.*, 1998; Meikle, Riby, & Stollery, 2005; Sünram-Lea *et al.*, 2001), and caffeine has been found to increase alertness, ameliorate fatigue and have a beneficial effect on attention (Smith, 2002). However relatively little work has been done on the effects of these substances when consumed in combination on cognition and mood.

This chapter is going to provide an overview of the literature on the effects of caffeine and glucose on cognition and mood. Firstly the effects of glucose and caffeine in isolation will be examined. Then current research on the effects of caffeine and glucose when administered in combination, including in the form of energy drinks, i.e. in conjunction with other active ingredients, will be discussed along with potential mechanisms of actions.

1.2 Glucose

This section will examine the effects of glucose on behavioural measures. The optimal dose will be considered along with factors which may moderate the effects of glucose in individuals. Finally potential mechanisms of action will be discussed.

1.2.1. Cognitive effects of glucose

Glucose is the brain's primary fuel. Since relatively little glucose can be stored, the brain is reliant on a continuous supply of glucose as its primary fuel, delivered via the bloodstream (Wenk, 1989). The effect of glucose on cognition has been extensively studied in an acute, short-term context in which a glucose load is administered and cognitive performance assessed shortly afterwards. Beneficial effects of glucose administration have been observed across different populations using this experimental paradigm. For example, glucose administration has been found to enhance cognitive function in young and aged animals and humans (Benton, Owens, & Parker, 1994; Donohoe & Benton, 2000; Foster *et al.*, 1998; Messier, 2004; Smith, Riby, Eekelen, & Foster, 2011; Sünram-Lea *et al.*, 2001; Sünram-Lea *et al.*, 2011), and clinical populations including Alzheimer's Disease and Schizophrenia (Fucetola, Newcomer, Craft, & Melson, 1999; Newcomer, Craft, Fucetola, Moldin, Selke, Paras, & Miller, 1999; Stone, Seidman, Wojcik, & Green, 2003;). In addition, in patients with Diabetes Mellitus improved glycaemic control has been found to lead to improvements in cognitive function (Meneilly, Cheung, Tessier, Yakura, & Tuokko, 1993; Naor, Steingruber, Westhoff, Schottenfeld-Naor, & Gries, 1997; Ryan, Freed, Rood, Cobitz, Waterhouse, & Strachan, 2006).

It has been found that memory in particular is sensitive to enhancement by glucose, specifically verbal declarative memory (Foster, *et al.*, 1998; Meikle, Riby, & Stollery, 2005; Sünram-Lea *et al.*, 2001). The evidence also suggests that the beneficial effects of glucose are enhanced when tasks have a high cognitive load, for example when they require an element of divided attention or are cognitively demanding (Foster, *et al.*, 1998; Kennedy & Scholey, 2000; Sünram-Lea, *et al.*, 2002b). This effect is found especially when looking at the performance of healthy young adults where it is generally considered that they are performing at an optimum level to begin with (Messier, 2004; Smith *et al.*, 2011). For example, using 25g glucose Kennedy & Scholey (2000); Scholey, Harper, and Kennedy, (2001); Sünram-Lea, Foster, Durlach, and Perez, (2002a); and Sünram-Lea, Foster, Durlach and Perez (2004) found significant effects on a difficult serial subtractions task (serial sevens). However glucose effects were not observed when using the less demanding serial threes task (Kennedy & Scholey, 2000; Scholey & Kennedy, 2004) with glucose loads of 25g and 37.5g respectively. Sünram-Lea *et al.*, (2002b) investigated the effect of glucose under conditions of increased cognitive load by utilising a secondary task to divide participants' attention. They found that compared to placebo, 25g glucose improved memory performance on a word recall task only when participants completed a secondary task during encoding , but not when participants encoded the list without a secondary task. They suggested that the additional cognitive task potentially 'depletes' episodic memory capacity and/or glucose–resources and is therefore critical to the observation of cognitive facilitation by glucose.

1.2.2 Mood effects of glucose

The effects of glucose on mood are equivocal, some studies have found it has no effect on mood (Sünram-Lea *et al.,* 2011), whilst others have found it to have effects (Benton & Owens, 1993; Owens, Parker & Benton, 1997). Benton and Owens (1993) conducted three experiments to examine the effect of glucose on mood. In the first they examined the short term effects of 50g glucose; in the second they examined the effects of sustained high blood glucose by administering 50g glucose initially, followed by two further 25g doses at 45 and 75 mins (both studies utilised a placebo control); finally they examined consumption of 50g on participants' negative responses to a frustrating situation. They found that both short term increases and sustained increases in blood glucose were associated with participants feeling less tense. Participants also exhibited less negative responses to a frustrating situation, as assessed by ethological descriptions of their behaviour, after consuming a glucose drink compared to placebo. Owens *et al.,* (1997) examined the effect of 50g glucose across three different cognitively demanding tasks (Stroop; Rapid Visual Information Processing and a difficult hand-eye coordination test), and found falling blood glucose levels were associated with participants rating themselves as feeling less energetic. Two underlying physiological processes have been postulated for these effects of glucose on mood (Benton, 2002). Firstly that in an attempt to normalise blood glucose levels the autonomic nervous system is activated and this is responsible for the increase in self-reported tension; secondly that the feeling of decreased energy may be due to neuroglycopenia, i.e. a shortage of glucose in the brain (Benton, 2002). Indeed the result seen in Owens *et al.,* (1997) may be due to the cognitively demanding tasks inducing localised neuroglycopenia (Benton, 2002). Sünram-Lea *et al.,* (2011) found no mood effects of 15g, 25g, 50g or 60g glucose compared to a placebo as assessed by the Bond-Lader visual analogue scales. However, when participants were divided into 3 different groupings based on an equal split of their BMI, they found that those with a high BMI

(25.60-31.80) rated themselves as significantly more alert following all of the active drinks compared with the low (17.8-21.90) and medium (22.70-24.90) BMI groups. These findings suggest that individual moderating factors may affect individuals' mood in response to glucose.

1.2.3 Dose

As with many substances affecting cognitive performance, research has shown that the dose-response for the enhancement of memory by glucose follows an inverted U shape (Gold, Vogt, & Hall, 1986, Sünram-Lea *et al.*, 2011). In human populations, glucose has been shown to be effective at doses ranging from 25g-75g (Messier, 2004). Sünram-Lea *et al.*, (2011) investigated the dose-response of glucose in memory facilitation and mood in healthy young adults. They found that whilst glucose improved performance on a range of memory tasks only the long-term memory task adhered to the previously observed the inverted U-shaped dose-response curve, whereby 25g was the optimal dose for improving performance. Improvements on the Serial 3's Subtraction task (a numeric working memory task) followed a cubic trend, with improvements following the lowest and highest doses and those on the Spatial Working Memory task following a quartic trend, where 25g lead to a significant improvement in performance, but there were also improvements in performance following the highest dose (60g). This suggests that the optimal dose is dependent upon the cognitive domain under investigation.

1.2.4 Moderating factors

It has also been shown that individual differences in age, Body Mass Index (BMI) and glucoregulation among participants are important moderators of optimal dose and more general susceptibility to glucose facilitation of cognitive performance (Donohoe & Benton, 1999a; Hall, Gonder-Frederick, Chewning, Silveira & Gold, 1989; Messier, Tsiakas, Gagnon, Deorochers, & Awad, 2003; Riby, Meikle, & Glover, 2004; Sünram-Lea *et al.*, 2011). With regards to age, in younger adults lower doses around 25g are generally more effective, whereas in older adults higher doses around 50-75g are more effective (Messier, 2004). However, there is likely to be an interaction with glucoregulation as this declines with age. Older participants were found to have improved episodic memory following 25g glucose (Riby, *et al.*, 2004). However, older adults with poorer glucose regulation have been found to perform worse on cognitive tasks following 50g glucose (Donohoe & Benton, 1999a; Messier, *et al.*, 2003). A high BMI is also associated with poorer glucoregulation and Sünram-Lea *et al.*, (2011) found that those with a high BMI (>25) showed impaired cognitive performance after 60g of glucose, compared to an improvement in performance following the same dose in those with low and medium BMI (<25). Whilst this research has found that a faster rate in decline in blood glucose levels i.e. a smaller area under the curve has been associated with better memory, Donohoe and Benton (2000) found that rather than improved memory being just the result of a more efficient glucoregulatory response, it was specifically related to participants' ability to reach baseline again following nadir. 'Nadir' is the point at which, following the ingestion of glucose and subsequent rise in blood glucose levels, blood glucose levels slightly dip below baseline levels before rising and returning to baseline again.

1.2.5 Summary

To summarise, glucose has been found to have its most robust effects at a dose of 25g on verbal declarative memory. There is evidence that the most facilitative dose is dependent on the cognitive domain, and the effects of glucose can only be evidenced when task demand is high. There are also several other moderating factors which augment the effect of glucose, for example; age, glucoregulation; BMI.

1.3 Glucose Mechanisms of Action

There are several proposed mechanisms of action for glucose. The precise mechanisms by which increased peripheral and/or central glucose availability affects cognitive processes are still unclear. Whilst there are two broad theoretical approaches to the potential mechanism: energetic demand and domain specific models, one does not necessarily exclude the other and they may both provide explanations depending on different parameters.

It is suggested that a central mechanism of action is responsible for glucose enhancing effects because it is able to cross the blood-brain barrier and it has been shown that central and systemic administration of glucose produce similar behavioural effects (Stefani, Nicholson, & Gold, 1999). Therefore when it is administered only centrally it does not pass through the peripheral systems. Glucose exerts robust effects on long-term memory tasks, in particular declarative memory. The hippocampus is the brain region most strongly implicated in long-term memory performance (Aggleton and Brown, 1999). Consequently, the hippocampus has been postulated to play a critical role as the glucose enhancement effect is most reliably found on the domain of episodic memory and the hippocampus is a key structure in episodic memory functioning (Shastri, 2002; Wincour, 1995). One study

in support of this used the remember-know-guess paradigm (Sünram-Lea *et al.*, 2008). Following the ingestion of a glucose drink participants recalled significantly more words as 'remembered' compared to placebo. No differences were found on the 'know' or 'guess' responses. This supports the theory as the hippocampus is thought to be preferentially involved in 'recollection' based recognition memory but not 'familiarity' (Aggleton & Brown, 2006; Rugg & Yonelinas, 2003; Sünram-Lea, *et al.*, 2008). The hippocampus is densely populated with insulin receptors compared to other brain regions (Lathe, 2001; Unger *et al.*, 1989), and it is suggested that this might be the one of the potential underlying mechanism responsible for the glucose enhancement effect on verbal declarative memory (Craft, Dagogo-Jack, Wiethop, Murphy, Nevis, Fleischman *et al.*, 1993). Indeed, both acute and chronic administration of intranasal insulin, a mechanism that enables the direct delivery of glucose to the central nervous system, has been shown to improve declarative memory without concomitant changes in plasma insulin and glucose levels (Benedict *et al.*, 2004, 2007; Reger *et al.*, 2008a,b). Messier (2004) however highlights that, as it is not possible to increase plasma glucose concentration in humans without a rise in blood insulin levels, it is not possible to be sure that insulin is the mediator between the ingestion of glucose and memory improvements. For example, Craft *et al.*, (1999) found that using a euglycemic clamp to raise blood insulin levels, whilst blood glucose levels remained constant, improved memory in patients with Alzheimer disease. However additional glucose had to be administered to prevent blood glucose levels from failing whilst insulin levels were increased and so rather than showing that insulin is the mechanism of action, it only suggests that raised blood glucose levels are not necessary for the memory enhancement effect (Messier, 2004). It remains difficult to tease apart the relative effects of glucose and insulin on cognitive function, and whether they can

have effects independently or whether they have an interrelated function (Smith *et al.*, 2011).

The availability of glucose as mere energy fuel, particularly in the brain may be behind the glucose enhancement effect (Scholey *et al.*, 2001). A greater reduction in blood glucose has been observed after performing more cognitively demanding tasks (Scholey *et al.*, 2001). This could explain why beneficial effects are seen after the ingestion of glucose prior to these types of tasks, as without the additional glucose load glucose supply may become depleted which in turn might have a detrimental impact on cognitive performance (Scholey *et al.*, 2001). However, the hippocampus in particular has evolved protection against temporary fluctuations in glucose supply by having higher stores of glycogen compared to other areas of the brain (Dalsgaard, Madsen, Secher, Laursen & Quistorff, 2006). It is also unlikely that glucose uptake needed during a cognitively demanding task would be greater than the overall glucose demand of the brain and the amount of glucose produced is carefully matched to the glucose used in order to maintain consistent blood glucose levels (Messier, 2004). Messier (2004) suggested instead that it may be autonomic changes, arising from increased stress and physiological arousal, and caused by the emotions arising from the task such as experiencing difficulty, that lead to variations in blood glucose in more demanding tasks. There is also evidence that the memory enhancement effect of glucose is seen even after the increase in blood glucose following administration of glucose has subsided (Sünram-Lea, Foster, Durlach & Perez, 2002a). Sünram-Lea *et al.*, (2002a) found enhanced recall on a memory task 24 hours after administration of a glucose drink, which demonstrates that the glucose memory enhancement effect does not depend on elevated plasma glucose levels *per se*. Therefore, other mechanisms which are facilitated by an increase in glucose are more likely to be the mechanism for the glucose enhancing effect rather than just the increase in glucose *per se*.

In addition, there is evidence indicating that glucose affects cognitive processes, in particular memory through an enhancement of brain acetylcholine synthesis and/or its release (see Messier 2004 for review). When glucose is metabolised via the Krebs cycle, it produces acetylcoenzyme A which is necessary, along with choline, for acetylcholine (ACh) synthesis (Messier, 2004). Kopf, Buchholzer, Hilgert, Löffelholz, & Klein, (2001) found that memory performance improved on a maze task when glucose and choline were administered individually and in combination. They suggested that increased hippocampal ACh synthesis lead to the memory improvement, and therefore the facilitation of memory by glucose could be mediated by increased ACh availability (Smith *et al.,* 2011). Raggozzino, Pal, Unick, Stefani and Gold (1996) found that when rats explored a four-arm maze, the increase in hippocampal ACh output was dose dependently increased by peripheral glucose injections. A 50% increase in ACh synthesis was seen during the exploration of the four-arm maze and this was further increased by another 50% when 250mg/kg peripheral glucose injection was administered. This was also the dose at which the animals exhibited better memory performance during the task. No effect on ACh synthesis was found when a higher dose of 1000mg/kg was administered. The pattern of ACh output increasing dose dependently following glucose injections up to a certain point, mirrors the dose-dependent effects of glucose seen on behavioural performance, whereby after a certain point administration of glucose does not result in further improvements (Sünram-Lea *et al.,* 2011).

It has also been suggested that glucose exerts its memory effects via potassium adenosine triphosphate (K_{ATP}) channel function. When glucose is metabolised it increases intra-neural ATP levels and causes a K_{ATP} channel blockade, which in turn causes the neuron to become depolarised and this mediates neurotransmitter release by increasing the probability if stimulus-evoked neurotransmision (Stefani & Gold 2001; Stefani *et al.,*

1999). Stefani *et al.*, (1999) compared the effects of glucose, glibenclamide (a K_{ATP} channel blocker) and saline on spatial working memory performance in rats. They found that relative to the saline placebo, task performance was improved following both the glucose and glibenclamide individually and when administered in combination at lower doses. They concluded that given the similarity of the effects on task performance these results support the theory that glucose exerts its effects by modulating K_{ATP} channel function (Stefani *et al.*, 1999). As with ACh, an important finding here is the dose-dependent effects of this mechanism, as the effects of glucose have been found to be dose-dependent (Messier, 2004). However, the effects of glucose on K_{ATP} channel function has not been examined directly, and therefore it is not possible to know if glucose and glibenclamide are exerting their effects via a common neurophysiological mechanism, or if each has a different underlying mechanism which are resulting in the same effects (Smith *et al.*, 2011).

Glucose may exert its cognitive effects on central mechanisms via peripheral mechanisms, specifically the liver and the vagus nerve have been implicated. The suggestion is that following high doses of glucose and fructose (>1000mg/kg), changes in the liver to the cell membrane transport are detected by the coeliac ganglion and transformed into neural signals and then carried to the brain by the vagus nerve (Messier & White, 1987, White, 1991). This is supported by the finding that coeliac ganglion lesions, which block most of the efferents of the liver, have been found to abolish the glucose memory effect (White, 1991). The main relay station for the vagal nerve fibres in the brain is the nucleous of the solitatory tract in the brainstem. The nucleus has projections into forebrain areas such as the amygdala and hippocampus and therefore the hippocampus could be involved with both central and peripheral actions of glucose on memory (White, 1991). Stimulation of the vagus nerve has also been found to modulate cognitive performance, both improving

(Clark, Naritoku, Smith, Browning & Jensen, 1999; Sackeim *et al.,* 2001) and impairing (Helmstaedter, Hoppe & Elger, 2001). Whilst these results should be interpreted with caution due the participants having either epilepsy or treatment resistent depression (Messier, 2004), they still provide evidence of a potential mechanism.

The evidence for one sole mechanism of action for glucose is not clear. Given the variation in the behavioural effects of glucose and the dose-dependent nature of these, it is likely that the faciliatative effect of glucose relies on a variety of underlying mechanism of action (Sünram-Lea & Owen, 2017). This may be further modulated by specific participant characteristics, for example insulin insensitivity.

1.4 Caffeine

The following section will examine the behavioural effects of caffeine. Dose and time dependent effects as well as other moderating factors will be discussed. The potential underlying mechanisms will then be discussed.

1.4.1 Cognitive effects of caffeine

In terms of its behavioural effects, caffeine has been found to reduce simple reaction time (Chubley, Bye, Henson Peck & Riddington, 1979; Smith, Thomas, Perry & Whitney, 1997; Smith, Maben, & Brockman, 1994); improve sustained attention (Smith, Kendrick, & Maben 1992; Brice & Smith, 2001b); improve concentration (Hindmarch, *et al.,* 2000); improve performance on delayed memory tasks (Kelemen & Creeley, 2001; Smith *et al.,* 1999); and improve encoding of new information (Smith, Clark, & Gallagher, 1999).

In a review Smith (2002) concluded that following caffeine consumption the strongest effects are found on attention. Brice and Smith (2001a) investigated the effect of caffeine on a sustained attention task and a driving simulator task as a more naturalistic measure of attention. They administered 3mg/kg of caffeine and found that performance was improved on both tasks with greater accuracy on the sustained attention task and fewer steering wheel movements in the driving simulation task, which evidenced improved sustained attention during this task. Performance on a choice reaction time task which required focused attention was also improved in two studies, one with caffeine at the level of 40mg (Smith *et al.*, 1999) and in the second with 1.5mg/kg and 3mg/kg of caffeine (approximately 105mg and 210mg respectively for a 70kg person) (Brice & Smith, 2001b). In the second study there was no difference between the 1.5mg/kg or 3mg/kg dose in terms of performance on this CRT task (Brice & Smith, 2001b).

Caffeine has also been found to have some beneficial effects on memory (Kelman & Creeley, 2001; Smith *et al.*, 1999b). Smith *et al.*, (1999b) found that whilst there was no beneficial effect of caffeine (40mg) on a free recall memory task, it did significantly speed up the response times in a delayed recognition task. Participants who received caffeine also completed significantly more trials in a semantic processing task, which consisted of sentence verification into 'true' or 'false' classifications, and measures speed of retrieval of information from general knowledge. However Kelman & Creeley (2001) suggested that caffeine's memory benefit might be due to state-dependent memory effects as most studies administer the encoding and recall parts of the memory following a single dose of caffeine, and therefore caffeine is present during both phases of the task. They administered 4mg/kg of caffeine on either day 1 (encoding), day 2 (recall) or on both days. They found that when caffeine was administered on both days, recall was significantly better than when it was only administered on one, which supports their theory that it is

state-dependent memory effects rather than caffeine *per se* that is having an affect (Kelman & Creeley, 2001).

1.4.2 Mood effects of caffeine

Caffeine has predominantly been found to increase alertness and reduce fatigue (Brice & Smith 2001a; Haskell *et al.,* 2005; Hindmarch, Rigney, Stanley, Quinlan, Rycroft & Lane, 2000; Kennedy, Galloway, Dickau & Hudson, 2008; Smith, Sturgess & Gallagher, 1999). Negative mood effects such as anxiety and tension have been found at higher doses above 500mg (Griffths, Juliano & Chausner, 2003; Sicard, Perault, Enslen, Chaufford, Vandel & Tachan, 1996). However doses of these amounts are unlikely to be commonly ingested (Smith, 2002).

Haskell *et al.,* (2005) found that following both 75mg and 150mg caffeine participants rated themselves as significantly more alert and significantly less mentally fatigued compared to placebo after they had completed a cognitively demanding battery. Doses as low as 12.5mg and up to 100mg were found to attenuate ratings of feeling bored following a similar cognitively demanding battery (Smit & Rogers, 2000). Additionally, the 100mg dose led to increased self-ratings of 'energetic arousal'. Conversely, some research has found no mood effects of 100mg and 200mg of caffeine (Svensson, Persson & Sjoberg, 1980; Swift & Tiplady, 1988). It has been proposed that mood effects follow from changes in cognitive performance and this may be an explanation for the lack of mood effects in some studies (Rusted, 1999).

1.4.3 Dose

Although previous research initially administered large doses of around 200-250mg to explore the effects of caffeine (Addicott & Laurienti, 2009), more recently research has focused on amounts of caffeine that may be consumed in one to two cups of coffee, which is approximately 75-150mg (Brice & Smith, 2002; Haskell, Kennedy, Wesnes, & Scholey, 2005). Research has found that following doses of this level, caffeine can increase alertness and reduce fatigue (Glade, 2010; Smith, 2002). For example, Haskell *et al.*, (2005) found that participants rated themselves as significantly more alert and less mentally fatigued after the completion of a battery of computerised cognitive tasks, following ingestion of both 75mg and 150mg of caffeine. Brice and Smith (2002) compared a more naturalistic pattern of caffeine consumption (65mg of caffeine consumed at four time-points); to a single large dose (200mg) to see if they had the same effects. The doses were chosen on the basis that there would be the same amount of caffeine in the system after 5 hrs from the first consumption of the 65mg dose. Both treatments led to improvements in attention; specifically better performance on reaction time tasks (faster simple reaction time and improved accuracy on a choice response task), improved accuracy on a vigilance task, faster self-paced responding a sustained response task, increased speed for encoding new information in a categoric search task and improved tracking accuracy on a dual tracking/detection task. These results suggest that results observed following administration of a large single dose, may actually be representative of more naturalistic consumption patterns (Brice and Smith, 2002).

Doses of caffeine, below those found in a typical cup of tea or coffee, have also been examined; Smit and Rogers (2000) examined the effects of 0, 12.5, 25, 50 and 100mg of caffeine. The participants completed a battery of tasks consisting of a simple reaction time

task, a Rapid Visual Information Processing (RVIP) task and a mood questionnaire. All doses led to improvements in performance on both cognitive tasks and attenuated mood in terms of preventing an increase in feeling 'bored' which increased towards the end of the testing session after placebo. The 100mg also increased 'energetic arousal'. The most notable element however is that there was a very flat dose response in terms of improvements on the performance tasks (Smit & Rogers, 2000), i.e. performance did not differ significantly following administration of the dosages ranging from 12.5mg dose to 100mg (Smit & Rogers, 2000).

1.4.4 Moderating factors

It has been suggested that caffeine is most effective when alertness levels are low (Smith, 2002). Lorist, Snel & Kok (1994) found that whilst administration of 200mg of caffeine, followed by a maintenance dose of 50mg caffeine, shortened participant's reaction time, larger improvements were shown in participants who were fatigued compared to those who were well rested. Similarly, Schweitzer, Muehlbach and Walsh (1992) found that caffeine (4mg/kg) administered prior to a single night shift improved alertness and attenuated the decline in performance and alertness due to circadian rhythms in the early hours of the morning when participants were engaged in a Simulated Assembly Line Task (SALT). Research has also shown that caffeine can attenuate the dip in performance that is associated with the post-lunch period (Smith, Rusted, Eaton-Williams, Savory & Leatherwood 1990). Smith *et al.,* (1990) examined the effects of caffeine both before and after lunch on a sustained attention task (Bakan vigilance task). Caffeine improved performance both before and after lunch, and removed the decline in performance that was seen after lunch following the decaffeinated drink.

Age appears to be another factor that has been shown to moderate the behavioural effects of caffeine (Hogervorst, Riedel, Schmitt, & Jolles, 1998; Lorist, Snel, Mulder, & Kok, 1995; Swift & Tiplady, 1988). Swift and Tiplady (1988) found that 200mg improved more aspects of cognitive performance in elderly participants (attention and reaction time) compared to younger adults. However, whilst the younger participants rated themselves as significantly more alert, interested, calmer, and steadier following caffeine consumption, the elderly participants reported no such effects. Moreover, Hogervorst *et al.,* (1998) found that 225mg caffeine improved performance on a word learning list in middle-aged participants (aged 46-54yrs), but not in younger (aged 26-34yrs) or older (aged 66-74yrs) participants. Lorist *et al.,* (1995) examined the effect of 250mg caffeine on reaction time and event-related potentials (ERPs) in young (18-23yrs) and elderly (60-72yrs) participants. Following the placebo the results showed that elderly participants were slower in their identification of relevant information and the evaluation of the stimuli. However caffeine improved performance and ERPs on both young and elderly participants. Caffeine also ameliorated the deficits in the P3b latency (an indication of stimulus evaluation time) associated with ageing.

One major controversy surrounding the behavioural effects of caffeine is whether caffeine consumption has any net benefits, or if the positive effects found are merely due to 'withdrawal reversal'; as much of the research uses participants who have abstained from caffeine prior to taking part in the research (James, 1994; James, 1998; James & Rogers, 2005; Rogers & Dernoncourt, 1998; Yeomans, Ripley, Davies, Rusted & Rogers, 2002). For example, James (1998) found no evidence of improvement in participants' performance after they had caffeine when they had consumed caffeine habitually prior to testing, but did find that performance on a short-term memory task was impaired when participants were withdrawn. Yeomans *et al.,* (2002) also tested the withdrawal reversal

hypothesis using a methodology where they pre-loaded participants with 0, 1 or 2mg/kg caffeine at breakfast, followed 60 minutes later by a second drink containing either 0 or 1mg/kg caffeine. They tested participants' performance before and after the drinks and found that both initial doses of caffeine at breakfast improved self-rated alertness, decreased reaction time and the 1mg/kg dose also increased accuracy on the Rapid Visual Information Processing (RVIP) task. However, whilst the 1mg/kg dose 60 minutes post breakfast resulted in increased self-rated alertness and decreased reaction time, the subsequent dose had no effect on performance or mood in participants who had already received caffeine at breakfast. The researchers interpreted these results as supporting the caffeine reversal hypothesis as once participants were no longer caffeine deprived there was no additional benefit of subsequent caffeine administration (Yeomans *et al.,* 2002). James and Rogers (2005) in their review of the literature suggest that the typical placebo-controlled studies that are widespread in this research, by their design, fail to discern whether there are net benefits of caffeine administration or if the effects are solely due to withdrawal reversal. They suggest that only long-term withdrawal studies are able to investigate this question clearly and that the evidence from these studies suggest that any beneficial effects of caffeine can be explained by the effects of withdrawal reversal. However, other researchers have argued that these effects cannot be explained by withdrawal reversal (Addicott &Laurienti, 2009; Childs & de Wit, 2006; Haskell *et al.,* 2005; Smith, 2002; Smith, Christopher & Sutherland, 2013). Some of the studies which support the withdrawal reversal hypothesis have been criticised for their methodology. For example, James (1998) did not take any baseline measures, and therefore pre-existing differences prior to treatment could not be accounted for (Smith, 2002). Moreover, studies investigating the effect of caffeine in low or non-consumers found that caffeine has a positive effect on cognitive performance and mood (Childs & de Wit, 2006; Haskell *et al.,*

2005; Smith *et al.*, 2013). Childs and de Wit (2006) used participants who consumed less than 300mg caffeine per week, and examined the effects of 0, 50, 150 and 450mg caffeine on cognitive performance and mood. They found that caffeine improved attention, increased feelings of arousal, positive mood and self-rated feelings of high as assessed by Visual Analogue Scales used to detect drug effects. However impaired performance was found on a working memory task (Childs & de Wit, 2006). Haskell *et al.*, (2005) compared habitual caffeine consumers (consumed tea/coffee more than 50mg caffeine/day), to non-habitual caffeine consumers (didn't consume tea/coffee and in total consumed less than 50mg caffeine/day). They found no baseline differences between groups and caffeine significantly improved a number of cognitive tasks including simple reaction time, digit vigilance reaction time, numeric working memory reaction time, and sentence verification accuracy irrespective of the caffeine consumption status. Moreover, in both groups, reduced fatigue and increased alertness was observed following caffeine consumption (Haskell *et al.*, 2005). Smith *et al.*, (2013) also compared the effects of 2mg/kg caffeine in overnight-withdrawn and non-consumers. They analysed the baseline scores prior to caffeine administration and found no significant effects of caffeine withdrawal. In both groups caffeine improved mood and cognitive performance relative to placebo. The effects between groups only differed on ratings of alertness and anxiety and fewer lapses of attention, where caffeine had a larger effect compared to non-consumers. Evidence demonstrating effects in non-habitual consumers suggest that the effects are unrelated to withdrawal reversal (Childs & de Wit, 2006; Haskell *et al.*, 2005; Smith, 2002, Smith *et al.*, 2013).

Whilst the issue of whether caffeine has any true net benefits rather than simply improves performance due to withdrawal reversal remains controversial; it has been shown that consumer status does alter caffeine's effects (Haskell & Kennedy, 2011; Rogers,

Heatherley, Mullings & Smith, 2013). Rogers *et al.*, (2013) compared the effects of overnight caffeine abstinence and subsequent caffeine administration in non to low (caffeine intake < 40mg per day) and medium to high (caffeine intake ≥ 40mg per day) caffeine consumers. They found that caffeine withdrawal was associated with negative effects at the earlier 10.30am testing session, these effects increased in the later afternoon testing sessions where participants reported themselves to be sleepier, less alert and had poorer performance on the cognitive tasks. In the medium-high participants who consumed caffeine in the morning, improvements in these outcome measures were seen. However, in non-low participants caffeine administration only decreased ratings of sleepiness. The authors suggest that the failure of caffeine to improve mental performance and increase mental alertness in these participants was due to caffeine increasing their ratings of anxiety/jitteriness. Caffeine did however improve the psychomotor performance of both groups (faster typing speed, simple and choice reaction time responses). The authors concluded that although caffeine has beneficial effects on performance and with regular consumption consumers become tolerant to its effects of increasing anxiety/jitteriness, this increase in tolerance also extends to its effects on sleepiness and therefore mental alertness and mental performance fail to be enhanced by subsequent caffeine administration. Differences in neurocognitive responses to caffeine have also been identified. Haskell and Kennedy (2011) examined the effects of 75mg caffeine at rest and during cognitive tasks on pre-frontal cerebral-haemodynamics using near infrared spectroscopy. They compared habitual consumers (≥ 3 cups of tea and/or coffee per day) to non-habitual consumers (no tea or coffee and ≤ 1 caffeinated soft drink per day). They found that whilst caffeine significantly decreased cerebral blood flow, there was a significant interaction with consumption status. There was an exaggerated effect in non-consumers and no significant effect in consumers. The authors suggest that this shows that modulation of cerebral blood

flow does occur with a typical single serving of caffeine, but that regular consumers develop a tolerance for this effect.

1.4.5 Caffeine Summary

To summarise, caffeine has been found to have its most robust effects on alertness and attention. This is particularly evident when sustained performance is required or the consumer is already in a fatigued state. There is some evidence that caffeine can improve memory performance, but overall the evidence remains equivocal. The beneficial effects of caffeine are seen at doses similar to a typical cup of tea or coffee (50-75mg), although doses as low as 12.5mg have been found to be equally effective. By contrast high doses, for example over 500mg have been found to lead to decrements in performance and increase anxiety. The effects of caffeine on an individual are also moderated by factors such as age and consumer status.

1.5 Caffeine mechanisms of action

After caffeine has been ingested it is rapidly absorbed by the gastrointestinal tract, with peak plasma levels occurring around 30-60 minutes post consumption, and the half-life being around 3-5 hours (Lorist & Tops, 2003). Whilst caffeine has been found to have a variety of different mechanism of action including activation of adenylate cyclase; mobilisation of calcium from the sarcoplasmic reticulum and inhibition of phosphodiesterase (Nehlig, Daval, & Debry 1992; Sawyok & Yaksh, 1993), the action of caffeine at levels that are achieved through usual human consumption are thought to be

mainly related to its actions in the antagonism of the adenosine receptors (Fredholm, Bättig, Holmén, Nehlig, & Zvartau, 1999). Adenosine triphosphate (ATP) is used for cellular energy and it is manufactured from glucose via the Krebs cycle. When ATP is broken-down cyclic adenosine monophosphate (cAMP) is formed and when cAMP is broken-down adenosine is formed (Fredholm *et al.*, 1999). Adenosine then builds up throughout the day whilst the person is awake and signals tiredness in preparation for sleep (Fredholm *et al.*, 1999). Caffeine is able to pass through the blood-brain barrier due to its hydrophobic properties (Nehlig, 2010). Its double-ringed molecular structure is similar to that of adenosine, and therefore caffeine is able to bind to the adenosine receptors in the brain acting as a competitive agonist (Poltev *et al.*, 2010). As adenosine is A central nervous system depressant, through its' antagonism of the adenosine receptors, caffeine removes the endogenous adenosinergic tonus and leads to an increase in neurotransmission (Nehlig, 1999; Nehlig *et al.*, 1992). In particular caffeine's effects are thought to be due to its competitive antagonistic actions at the A_1 and A_{2A} adenosine receptor subtypes (Garrett & Griffiths, 1997; Lorist & Tops 2003). Although present in almost all brain areas, adenosine A_1 receptors have the greatest concentration in the hippocampus, cerebral and cerebellar cortex and certain thalamic nuclei (Goodman & Snyder, 1982; Fastbom, Pazos & Palacios, 1987). Adenosine A_{2A} receptors are found to be concentrated in the dopamine-rich regions of the brain (Fredholm *et al.*, 1999), namely the striatum, nucleus accumbens and olfactory tubercle (Javis & Williams, 1989; Ongini & Fredholm, 1996). Moreover, adenosine receptors are found on many central neurons including noradrenergic, dopaminergic, cholinergic and glutaminergic systems (Daly and Fredholm, 1998). Consequently, through its action on adenosine receptors located in these pathways, caffeine exerts its effects on numerous neurotransmitter systems.

The neuronal effects of caffeine in inhibiting adenosine can directly explain the beneficial behavioural effects seen from caffeine consumption, specifically improvements in alertness and vigilance and a reduction in fatigue (Liberman *et al.,* 2002). Of particular interest to the observed behavioural effects appears to be an increase in dopaminergic activity (Garrett & Griffiths, 1997), which has been suggested to mediate caffeine's stimulating effects (Ferré, Fuxe, von Euler, Johansson, & Fredholm, 1992). It is interesting to note, however that baseline arousal appears to be an important moderating factor of its effect on different neurotransmitter systems (Smith, Brice, Nash, Rich and Nutt, 2003). For example, the behavioural effects of caffeine proposed to pertain to increased cholinergic activity (including faster encoding of information, improved vigilance performance) have been observed in both alert and fatigued participants , whereas those pertaining to noradrenergic effects (including faster simple reaction time and fewer 'attentional lapses') have mainly been observed in fatigued participants (Smith, Sutherland & Christopher, 2005). The beneficial effects of caffeine in low arousal states may therefore be because it counteracts the reductions in central noradrenaline turnover (Smith *et al.*, 2003).

1.6 Glucose and caffeine in combination

Whilst there is a paucity of studies which have examined the effects of glucose and caffeine consumed together on cognition and mood, those that have been conducted have included a wide range of behavioural and physiological measures. As well as cognitive performance and mood; sleep quality, manual dexterity, frontal functions and physiological parameters have been examined (Adan & Serra-Grabulosa, 2010; Alford, Cox, & Wescott, 2001; Barthel, Mechau, Wehr, Schnittker, Liesen & Weib, 2001; Jay,

Petrilli, Ferguson, Dawson, & Lamond, 2006). The research has often focused on their effects together in the form of 'energy drinks'. This section will examine the effects of these substances in combination on cognition, mood and physiological outcomes; methodological limitations and directions for future research will then be considered.

1.6.1 Memory

Individually both glucose and caffeine have been shown to improve memory function (Foster, *et al.,* 1998; Kelemen & Creeley, 2001; Meikle, Riby, & Stollery, 2005; Smith *et al.,* 1999; Sünram-Lea *et al.,* 2001). Beneficial effects have also been found on memory when the are consumed in combination (Adan & Serra-Grabulosa, 2010; Alford *et al.,* 2001; Sünram-Lea, Owen-Lynch, Robinson, Jones and Hu, 2012). Alford *et al.,* (2001) looked at the effects of 'Red Bull' energy drink, which contains 80mg caffeine and 5.25g glucose and found that it significantly improved immediate recall memory. Other researchers have found beneficial effects on both immediate and delayed memory (Adan & Serra-Grabulosa, 2010; Sünram-Lea, *et al.,* 2012). Adan and Serra-Grabulosa (2010) compared the effects of a combination of 75mg caffeine and 75g glucose consumed in a water vehicle, to each of the treatments in isolation consumed in a water vehicle and a plain water placebo. As part of their battery of tasks they assessed memory using the Auditory Verbal Learning Memory Test (immediate and consolidation memory). Immediate recall was improved following the caffeine and glucose combination compared to either treatment in isolation or placebo, with more words remembered in the last two immediate recall trials of the RAVLT. Delayed word recall was also improved following the caffeine and glucose combination compared to the placebo or glucose in isolation. However other research has only found effects on delayed memory performance (Scholey

& Kennedy, 2004; Sünram-Lea *et al.*, 2012). Sünram-Lea *et al.*, (2012) examined the effects of an energy drink in a stressful, fire-fighting training situation. They looked at two types of energy drinks, the first contained 50g glucose and 40mg caffeine and the second contained 10.25g fructose/glucose and 80mg caffeine and these were both compared to a matched placebo. Participants were all taking part in a fire-training course. The first energy drink (50g glucose/40mg caffeine) ameliorated the decline in performance due to stress (and physical exercise) in delayed recall. Scholey and Kennedy (2004) investigated the individual components of an energy drink, including flavouring levels of herbs. They used the Cognitive Drug Research (CDR) Battery to assess cognitive performance. Participants received either a placebo (which was just the vehicle containing mainly water with artificial sweeteners and flavourings to make it matched to the active treatment); or the vehicle plus 75mg caffeine; or the vehicle plus 37.5g glucose; or the vehicle plus flavouring level of herbs (Ginseng 12.5mg, Ginkgo biloba extract 2.004mg); or the complete energy drink which contained 75mg caffeine, 37.5g glucose and the flavouring levels of herbs. Participants received all of the drinks at separate visits administered in a randomised balanced order. The complete energy drink led to significant improvements on what they termed '*secondary memory*' (which combines the percentage accuracy scores of the delayed word recognition, delayed picture recognition, immediate word recall and delayed word recall tasks) (Scholey & Kennedy, 2004). When the constituents of the energy drink were administered alone, only caffeine showed a trend for improved performance on *'secondary memory'*. In contrast Smit & Rogers,(2002) found no effect on memory performance. They compared the effects of two energy drinks to equivalent volume of bottled spring water placebos and 'nothing' which was a short break. Energy drink A was 150ml and Energy drink B was 250ml. Both had an equivalent caffeine content of 75mg, and they both contained glucose and were iso-caloric, but no further

details were given as to the exact amount of glucose. They also contained different variations of vitamins and drink A also contained ferrous gluconate. They found no effects on either immediate or delayed memory recall by any of the treatments.

With regards to working memory, there is little evidence to support any benefits of caffeine and glucose when consumed together. Whilst Scholey and Kennedy (2004) found some evidence to suggest a beneficial effect on working memory as there was a trend towards an increase in total responses on serial subtraction tasks, other researchers have found no effects. Adan and Serra-Grabulosa (2010) assessed working memory using the backward Digit Span of WAIS and found no treatment effects. Urquiza and Vieyra (2015) examined the effects of caffeine and sugar (glucose and fructose) on working memory using the N-Back task (1 back/2 back/3 back). They compared three treatments: Decaffeinated coffee with sugar (15g), caffeinated coffee with no sugar (~125mg caffeine), and caffeinated coffee with sugar. They found no effects from sugar alone and no evidence of a synergistic effect between caffeine and sugar. They did find significant improvements following the caffeine treatment and the combination treatment on the 2-back task and following caffeine on the 3-back task. In addition, there was some evidence to suggest that the benefits were increased in non-habitual caffeine consumers when they consumed caffeine and sugar.

Overall whilst there is some evidence to suggest that glucose and caffeine in combination are beneficial for verbal episodic memory it is not unequivocal. One of the reasons for this may be due to the dosages investigated. Whilst in the single dose literature 25g glucose has been found to be most effective in enhancing verbal episodic memory (Sünram-Lea *et al.*, 2012), this dose has not been investigated in the combined literature. It may be that as with the single literature there is an optimum dose to achieve beneficial effects.

1.6.2 Attention

Attention is another factor which has been investigated as the consumption of caffeine alone has been found to improve attention (Brice & Smith 2001a; Hindmarch, *et al.*, 2000; Kennedy, *et al.,* 2008; Smith, *et al.*,, 1999). There is some evidence of beneficial effects of caffeine and glucose when consumed together (Adan & Serra-Grabulosa, 2010; Gershon, Shiner and Ronen, 2009; Howard & Marczinski, 2010; Kennedy & Scholey, 2004; Mets, Ketzer, Blom, van Gerven, van Willigenberg, Olivier and Verster, 2010; Mucignat-Caretta, 1998; Scholey & Kennedy 2004; Smit & Rogers, 2002).

Howard & Marczinski (2010) found that 'Red Bull' decreased reaction times on a cued go-no-go task. They used three different doses, 1.8ml/kg, 3.6ml/kg and 5.4ml/kg and found that although all the doses elicited improvements, compared to the placebo and a no drink condition; it was actually the lowest dose which had the greatest effect. This dose was approximately equivalent to half of a 250ml can for an average 70kg individual and was equivalent to approximately 45.6mg caffeine and approximately 2.5g glucose. Kennedy & Scholey (2004) carried out two studies looking at different energy drinks. In the first study there were two active energy drinks, one contained 68g glucose and 38mg caffeine and the other contained 68g glucose and 46mg caffeine. In the second study the energy drink contained 60g glucose and 33mg caffeine. Both studies used a matched placebo for comparison. They examined the effects on the Cognitive Demand Battery (CDB) which comprised of two minutes of Serial 3 subtractions, two minutes of Serial 7 subtractions and five minutes of the Rapid Visual Information Processing (RVIP) task, and a mental fatigue visual analogue scale. Completion of these tasks took approximately 10 minutes and they were repeated 6 times in total post-dose. All three active treatments improved accuracy on the RVIP task. In study one this was evident from 35-39 minutes after

treatment and in the second study from 45-49 minutes after treatment. Smit & Rogers (2002), investigated attention using the Simple Reaction Time (SRT) and Rapid Visual Information Processing (RVIP) tasks and found that both energy drinks improved performance on SRT, whereas only drink A showed a significant improvement on the RVIP task compared to 'nothing'. Both drinks contained 75mg caffeine, the amount of glucose was unspecified, but they were iso-caloric, however drink A was a smaller quantity. Adan & Serra-Grabulosa (2010) assessed attention using the California Computerised Assessment Package (reaction time, sustained attention, and visual scanning speed). They found that reaction time was faster in the simple reaction time task following 75mg caffeine, 75g glucose and, both the caffeine and glucose in combination compared to placebo. Scholey and Kennedy (2004) in their examination of the effects of a whole energy drink compared to its components found that the complete energy drink (containing 75mg caffeine and 37.5g glucose) led to significant improvements on what they described as the '*speed of attention*' factor (which combines the reaction time results for simple reaction time, choice reaction time and digit vigilance) compared to placebo. This effect was not found following either caffeine or glucose alone, however there was a trend towards improved '*accuracy of attention*' (which combines the percentage accuracy scores for choice reaction time and digit vigilance), following caffeine on its own.

The effects of these substances have also been investigated on driving performance which requires the maintenance of attention over a prolonged period (Gershon *et al.*, 2009; Mets *et al.*, 2010). Gershon *et al.,* (2009) compared the use of what they termed a 'Functional Energy Drink' (FED) to a manual-dexterity/mastication activity (MD/MT task) (which was shelling and eating sunflower seeds) as strategies for preventing fatigue whilst driving. The FED used, whilst not explicitly stated as 'Red Bull', was described as a commercially available Energy Drink containing the exact same ingredients as 'Red Bull'. The dose used

in this study was 2 x 250ml cans which contained 160mg caffeine and 10g glucose in total, consumed 20 minutes before the 2 hour morning driving task commenced. This was followed by a second 2 hour evening driving task on the same day for each condition. Driving performance, as measured by speed, steering and lane deviations, and performance on a peripheral target detection task was significantly better following the FED compared to the MD/MT task and control condition. Although it should be noted that the authors point to the problem that the MD/MT task participants had to use their right hand to pick up the sunflower seeds and this might have impacted on their ability to perform the other tasks (Gershon *et al.,* 2009). Mets *et al.,* (2010) were interested in studying the effects of a FED on fatigue during a prolonged driving task. They had participants drive for 2 hours, stop for a 15 minute break, and then drive for another 2 hours. During the 15 minute break participants consumed either a 'Red Bull' FED or a placebo, which was Red Bull without the active ingredients (caffeine (75mg), taurine, glucuronolactone, B vitamins (niacin, pantothenic acid, B6, B12 and inositol), but still containing the glucose (5.25g) and saccharose. They also compared this to a condition whereby the participants drove for 4 hours without taking a break. They found no significant differences after the first 2 hours of driving between conditions. After ingestion of the 'Red Bull' FED there was a significant reduction during the 3rd and 4th hours in the Standard Deviation of Lateral Position (SDLP) i.e. the weaving of the car. During the 3rd hour the 'Red Bull' FED also reduced the standard deviation of speed and improved the subjective driving quality (Mets *et al.,* 2010). This research shows that when consumed together caffeine and glucose can improve attention.

However other research has not found beneficial effects of caffeine and glucose on attention (Anderson & Horne, 2006; Jay *et al.,* 2006; Mucignat-Caretta, 1998; Sünram-Lea *et al.,* 2012). Mucignat-Caretta (1998) examined the effects of 'Red Bull' compared to a

matched placebo on 12 participants' (6 male and 6 female) performance on a simple reaction time and a Go-no-go reaction time task. They found that 'Red Bull' had no effect on simple reaction time and only significantly improved performance in females on a Go-no-go reaction time task. The author suggests that this may be related to caffeine acting differently on different cognitive strategies adopted by the males and females to complete the task. Specifically, that as the data showed males to be responding consistently faster than females, caffeine was able to modulate the performance of the females compared to the males as the males had already reached their maximum performance and so no further improvement was possible (Mucignat-Caretta, 1998). Jay *et al.,* (2006) investigated a 'Functional Energy Drink (FED) that although not stated to be 'Red Bull', had almost identical ingredients. They looked at the impact of the FED on subsequent quality of sleep. Participants were administered either two FED's, one at each of two separate time points, or they did not consume anything. The FED's were 250ml each and contained 1000mg taurine, 600mg glucoronolactone, 80mg caffeine, 5.25g glucose, 21.5g sucrose, B vitamins and flavours. Participants had an extended period of wakefulness (24.5hrs) during which the two FED's were administered or not, followed by a recovery sleep which was followed in turn by post sleep assessments. Sleepiness was assessed using a 10 minute Psychomotor Vigilance Task (PVT) during the post sleep assessment. The important measure is 'lapses' where the response is longer that 500ms after the stimulus has appeared. The PVT consists of watching a computer screen and pressing a response button when a digital millisecond clock appears on the screen. They found no effects of the FED on performance on the PVT. Anderson and Horne (2006) investigated the effects of an energy drink containing 42g glucose and 30 mg caffeine on reaction time and subjective sleepiness ratings. Participants were sleep restricted to 5 hours the night before, and completed the Psychomotor Vigilance Test (PVT) for 90 minutes. They found that participants had

slower reaction times and more 'lapses' in concentration during the final 30 minutes of the PVT following the energy drink. Sünram-Lea *et al.,* (2012) used a Letter Cancellation Task and a Letter-digit Substitution Task to measure attention. However they found no effects of either energy drink (50g glucose & 40mg Caffeine / 10.25g fructose/glucose and 80mg caffeine) on attention.

The effects of caffeine and glucose in combination on attention are therefore not conclusive. However as such wide ranging doses have been examined, and often administered in conjunction with other potentially active ingredients, it could be that as has been found in the single dose literature there is an optimal dose or dose range for the effects and this would go some way towards explaining the equivocal results.

1.6.3 Executive Function

A small amount of research has examined the effects of glucose and caffeine on executive function; however the majority have found no effects (Adan & Serra-Grabulosa, 2010; Sünram-Lea *et al.,* 2012). Adan & Serra-Grabulosa (2010) assessed executive function using the Wisconsin Card Sort task, but they found no effects of 75mg of caffeine or 75g glucose or their combination on the task. Sünram-Lea *et al.,* (2012) included a Grammatical Reasoning Task in their study which is an executive function task, but they did not find any effects of either of the two energy drinks (50g glucose and 40mg caffeine; 10.25g fructose/glucose and 80mg caffeine).

However, Scholey, Savage, O'Neill, Owen, Stough, Priestley and Wetherell (2014) assessed the effects of 25g glucose, 60g glucose and a combination of 60g glucose and 40mg caffeine on participants' multi-tasking performance compared to placebo.

Participants completed a multi-tasking framework, consisting of the simultaneous completion of mathematical processing, Stroop, memory search and tracker tasks, at baseline and then 30 minutes post dose. Overall they found that the group who had received the 60g glucose and 40mg caffeine combination scored significantly higher total scores on the multi-tasking framework when compared to placebo or the 60g glucose group. There was also a trend for a treatment effect on the speed of response on the Stroop task, they found that the 60g glucose/40mg caffeine combination group performed significantly faster than the placebo and 60g glucose groups. The Stroop task measures selective attention and response inhibition, and therefore assesses executive function as participants have to inhibit their response to the meaning of the word and respond instead to its physical properties (Scholey *et al.,* 2014). The authors suggest that the improvement in executive functioning following the combination dose is due to the ability to allocate more attentional resources to the demands of the multi-tasking framework. However the authors also acknowledge that due to the design of the study i.e. caffeine was only administered in combination with glucose and not alone, that these effects may solely be attributable to caffeine and not due to its combined effects with glucose (Scholey *et al.*, 2014).

The findings here suggest that there may be some beneficial effects of caffeine and glucose in combination on executive functioning, but that these may be mediated by cognitive load. The multi-tasking nature of the tasks in the Scholey *et al.*, (2014) study can arguably be said to increase the cognitive load of the task, compared to tasks used in the other studies and therefore this additional cognitive load may be necessary to see the beneficial effects of consuming caffeine and glucose in combination.

1.6.4 Mood

In terms of mood, the effects of consuming glucose and caffeine in combination have been found to be mainly positive (Gershon, *et al.*, 2009; Howard & Marczinski, 2010; Kennedy & Scholey, 2004; Mets *et al.*, 2010; Smit & Rogers, 2002; Sünram-Lea *et al.*, 2012). Howard & Marczinski (2010) found that following three doses of 'Red Bull' participants' rating of stimulation were increased (as measured by the Biphasic Alcohol Effects Scale (BAES) where participants rate their feelings of stimulation and sedation after drink consumption), and their ratings of mental fatigue decreased. As with the cognitive effects they found that the mood effects were greatest following the lowest dose (1.8ml/kg) compared to a placebo and a no drink condition. This equated to approximately 45.6mg caffeine and 2.5g glucose for an average 70kg individual. Kennedy & Scholey (2004) found that during the completion of an extended cognitively demanding task, participants' ratings of mental fatigue were improved following two of the three energy drinks examined, containing 68g glucose/46mg caffeine and 60g glucose/33mg caffeine, but not following the 68g glucose/38mg caffeine combination. Smit & Rogers (2002) assessed 12 visual analogue scales; revitalised, energetic, awake, alert, clearheaded, overall mood, relaxed, thirsty, tense, fatigued, bored and tired, in their study. They conducted a Principle Components Analysis on the mood data and identified three main dimensions; 'Energetic arousal'; 'Tense arousal', and 'Thirst'. Following both of the energy drinks (Drink A 150ml, Drink B 250ml; both 75mg caffeine and iso-caloric), 'Energetic Arousal' was increased and ratings of boredom were reduced. After drink B participants rated themselves as significantly less 'Tense' compared to after 'nothing'. 'Overall mood' was also better after drink B. Mets *et al.*, (2010) found in their driving study that ingestion of 'Red Bull' (5.25g glucose/80mg caffeine) reduced subjective sleepiness significantly in the 3rd and 4th hour and during the 3rd hour it also reduced the mental effort required to

perform the task compared to placebo. Sünram-Lea *et al.,* (2012) used the Bond-Lader, the State-Trait Anxiety Inventory (STAI) and Stress Arousal Checklist to measure mood changes in participants across the day during a stressful fire-fighting training situation. The first energy drink, which contained 50g glucose and 40mg caffeine, led to a reduction in anxiety and significantly reduced self-reported levels of stress following the search and rescue, but the second energy drink containing 10.25g fructose/glucose and 80mg caffeine had no effect. Arousal, alertness, contentedness and calmness were not affected by any of the drinks.

Some studies however have found evidence of negative effects on mood (Anderson & Horne, 2006; Gershon *et al.,* 2009). Gershon *et al.,* (2009) found the participants rated themselves as significantly less fatigued in the morning driving session following the FED, which contained 160mg caffeine and 10g glucose, and the manual-dexterity/mastication activity (MD/MT task). However in the afternoon session there was a slight 'rebound' effect of the FED and the participants reported themselves as more fatigued than compared to the control condition. Anderson & Horne (2006) found that an energy drink containing 42g glucose and 30mg caffeine did not counteract sleepiness as measured by the Karolinska Sleepiness Scale (KSS) in participants who had restricted sleep (5hrs) the night before compared to a taste-matched placebo.

Whilst the evidence for mood effects is not conclusive, the evidence to date suggests largely beneficial effects of caffeine and glucose when consumed together. Overall the pattern of results is similar to that found when either substance is consumed alone. Many of the studies described above have found that participants report feeling less sleepy, less mentally fatigued, increased energy, less anxious and less tense after consumption of caffeine and glucose (Gershon *et al.,* 2009; Howard & Marczinski, 2010; Kennedy & Scholey, 2004; Mets *et al.,* 2010; Smit & Rogers, 2002; Sünram-Lea *et al.,* 2012).

Caffeine alone has been found to increase alertness and reduce fatigue (Glade, 2010; Smith, 2002); while glucose has been associated with feeling less tense (Benton & Owens, 1993) and falling blood glucose has been associated with less energy (Owens, Parker & Benton, 1997).

1.6.5 Physiological

The effects of caffeine and glucose in combination have also been examined across a wide range of neurocognitive and other physiological outcome measures (Barthel *et al.*, 2001; Gershon *et al.*, 2009; Jay *et al.*, 2006; Rao, Henglong & Nobre, 2005; Reyner & Horne, 2002; Serra-Grabulosa, Adan, Falcón & Bargallo, 2010; Specterman *et al.*, 2005). Rao *et al.*, (2005) examined Event-related potentials (ERP's) during a sustained visual selection attention task, following administration of an energy drink containing 60g glucose and 40mg caffeine, compared to a colour and taste matched placebo. They found that as well as improving performance in terms of speed and accuracy on the sustained visual selection attention task, the ERP readings following the energy drink showed earlier visual cortical processing and later components related to decision-making and responses were also enhanced. Serra-Grabulosa *et al.*, (2010) investigated the effects of caffeine and glucose alone and in combination on sustained attention using functional magnetic resonance imaging (fMRI). Participants consumed either a placebo of 150ml of water, the water plus 75g glucose, the water plus 75mg caffeine or the water plus 75g glucose and 75mg caffeine. They used a continuous performance test (CPT-IP) to measure sustained attention. Although there were no differences between the groups on the performance of the task, the participants who received the glucose-caffeine combination treatment had significantly lower activation in the bilateral parietal and the left prefrontal cortex. As

these areas are both thought to be related to attention and memory processes the authors interpreted it as showing that the efficiency of the attentional resources was increased following the glucose-caffeine combination, leading to lower activation (Serra-Grabulosa *et al.,* 2010). Specterman *et al.,* (2005) examined the effects of an energy drink containing 68g glucose and 46mg caffeine; 68g glucose in carbonated water; 46mg caffeine in carbonated water, compared to a carbonated water placebo, on Motor-evoked potentials (MEPs). These are an index of corticospinal excitability and therefore can be used to investigate the effect of the energy drink and its' components on voluntary control pathways and this may have implications on performance (Specterman *et al.,* 2005). They used Electromyographic (EMG) recordings to monitor the response elicited by Transcranial Magnetic Stimulation (TMS) and Maximal Electrical Stimulation. They found that the MEPs rose after consumption of the energy drink, and the larger MEPs occurred when blood glucose concentrations were at their highest. However the individual effects of caffeine and glucose added together were much greater than the effects seen when they were administered together in the energy drink (Specterman *et al.,* 2005). The authors suggest that the maximal threshold of excitability of the synapses might be reached by either of the active ingredients alone and therefore there can be no additional effect when they are consumed in combination. Sünram-Lea *et al.,* (2012) found no effects of either of their treatment drinks (50g glucose and 40mg caffeine; 10.25g fructose/glucose and 80mg caffeine) compared to a matched placebo on cortisol measures taken during a stressful, fire-fighting training situation. Gershon *et al.,* (2009) as described above, also measured heart rate variability was used as an objective, physiological measure of fatigue and found variability did increase across the drives in both morning and evening, showing that participants were fatiguing during the task. However the variability was significantly reduced following the FED and MD/MT task.

Barthel *et al.,* (2001) used 'Red Bull' to examine the effects of taurine, caffeine and physical stress on the readiness potential or Bereitschaftpotentiale (BP's), preceding voluntary self-paced pedalling movements. They compared a 'Verum' test drink which was original 'Red Bull' (80mg caffeine and 5.25g glucose per 250ml); a 'Control' test drink which was 'Red Bull' without taurine or glucuronolactone; and a 'Placebo' test drink which was 'Red Bull' without taurine, glucuronolactone, or caffeine, but with glucose and saccharose. The drinks were all 500ml and therefore contained twice the amount of ingredients found in the standard 250ml 'Red Bull' serving, both the 'Verum' and the 'Control' test drinks contained 160mg caffeine. Participants cycled with increasing intensity during the testing sessions. They found that whilst BP's increased at a lower workload following the 'Control' caffeine condition, the 'Verum' condition which included taurine and caffeine prevented this 'over-shoot'. Participants also felt better at rest and after exercise following the 'Verum' condition.

Jay *et al.,* (2006) looked at what they termed a 'Functional Energy Drink' (FED) that although not stated to be 'Red Bull', had almost identical ingredients. They examined the impact of the FED on subsequent quality of sleep. Participants were administered either two FED's, one at each of two separate time points, or they did not consume anything. The FED's were 250ml each and contained 1000mg taurine, 600mg glucoronolactone, 80mg caffeine, 5.25g glucose, 21.5g sucrose, B vitamins and flavours. Participants had an extended period of wakefulness (24.5hrs) during which the two FED's were administered or not, followed by a recovery sleep which was followed in turn by post sleep assessments. EEG was used to measure quality of sleep during the recovery sleep and sleepiness was assessed using a 10 minute Psychomotor Vigilance Task (PVT) during the post sleep assessment. Following the administration of the FED's sleep onset latency was unaffected and participants still achieved the same amount of slow wave sleep. There were no effects

on the PVT task. Horne and Reyner (2001) found that 2 x 250ml cans Red Bull (10.5g glucose and 80mg caffeine) improved performance on a driving simulator task where 'lane drifting' was used as a measure of sleepiness impairing performance and the participants were sleep restricted to 5 hours sleep (by delaying their bedtime) prior to participating. In a further study Reyner and Horne (2002) also used EEG to measure objective sleepiness parameters. They found that following a single serving of Red Bull (containing 5.25g glucose and 80mg caffeine) that there was a reduction in 'lane drifting' and subjective sleepiness in the first 90 minutes of a 2 hour drive There was a trend for the alpha (α) and theta (θ) power to be reduced during the middle hour of the drive, indicating that the participants were less sleepy, following the energy drink.

These studies suggest that caffeine and glucose consumed together can modulate various physiological parameters and improve behavioural performance. However, effects on physiological parameters can also occur in the absence of any behavioural improvements, and therefore suggests that they can improve physiological processes so that the same behavioural effects can be achieved through less resource.

Table 1.1 Summary of previous findings for combined glucose and caffeine administration on cognitive domains

Domain	Studies	Evidence For Benefits	Studies	Evidence Against Benefits	Summary of Evidence
Immediate Memory	Adan & Serra-Grabulosa (2010) Alford *et al* (2001)	Verbal declarative memory – Improved Immediate free recall. Verbal declarative memory – Improved Immediate free recall.	Smit & Rogers (2002)	Verbal declarative memory – No effect on Immediate free recall.	Limited evidence for memory effects, however most robust effects seen on verbal declarative memory tasks.
Delayed Memory	Adan & Serra-Grabulosa (2010) Sünram-Lea *et al* (2012) Scholey & Kennedy 2004	Verbal declarative memory – Improved Delayed free recall. Verbal declarative memory – Improved Delayed free recall. Secondary memory factor improved (delayed word recognition, delayed picture recognition, immediate word recall and delayed word recall)	Smit & Rogers (2002)	Verbal declarative memory – No effect on delayed free recall	
Working Memory			Adan & Serra-Grabulosa (2010)	No effect on WAIS (Digit span backwards)	No evidence to support a beneficial effect.

			Scholey & Kennedy (2004) Urquiza & Vieyra (2015)	No effect on Working memory factor (numerical & spatial tasks) No effects on 1,2 & 3-Back task	
Attention	Adan & Serra-Grabulosa (2010) Gershon *et al* (2009) Howard & Marczinski (2010) Kennedy & Scholey (2004) Mets *et al* (2010) Mucignat-Caretta (1998) Scholey & Kennedy (2004) Smit & Rogers (2004)	Improved performance on sequential reaction time tasks. Improved vehicle control measures (lane drifting, speed). Improved performance behavioural control task. Improved accuracy on RVIP. Improved vehicle control measures (lane drifting, speed). Improved go-no-go task performance. Improvement on speed of attention factor (combines RT – SRT, CRT & digit vigilance). Improved SRT & RVIP	Anderson & Horne (2002) Jay *et al* (2006) Mucignat-Caretta (1998) Sünram-Lea *et al* (2012)	PVT performance reduced. No effect on PVT performance. No effect on SRT. No effects on a letter cancelation task.	Moderate evidence to support an effect on attention. In particular evidence to suggest this more effective when the task is more cognitively demanding.

		performance.			
Executive Function	Scholey *et al* (2014)	Improved performance on multitasking.	Adan & Serra-Grabulosa (2010) Sünram-Lea *et al* (2012)	No effect on the Wisconsin Card Sort Task. No effects on a grammatical reasoning task.	The evidence for beneficial effects is weak. The only supporting study included a moderating factors of increased mental effort.
Improvement in Mood	Howard & Marczinski (2010) Kennedy & Scholey (2004) Smit & Rogers (2002) Mets *et al* (2010) Sünram-Lea *et al* (2012)	Decreased Mental Fatigue. Decreased Mental Fatigue during demanding task. Increase Energetic Arousal. Reduced sleepiness. Reduced stress & anxiety.	Gershon *et al* (2009) Anderson & Horne (2006)	Reduced feelings of fatigue, but later a re-bound effect to feel more tired. Increased sleepiness.	The evidence for mood effects is moderate, but more specifically decreasing mental fatigue.
Neuro-cognitive Effects	Rao *et al* (2005) Serra-Grabulosa *et al* (2010) Specterman *et al* (2005) Barthel *et al* (2001)	ERPs showed enhanced processing. fMRI showed activation the same, but performance increased, suggesting utilising resource more effectively. Improved motor-evoked potentials.			Moderate evidence for effects on Neuro-cognitive processes. With a suggestion that it makes processes more efficient.

	Reyner & Horne (2006)	BP's (readiness potentials) enhanced. Less sleepiness as measured by EEG.			
Hormonal Effects			Sünram-Lea *et al* (2012)	No effects on cortisol response to a stressful task.	Weak, not enough evidence to draw a conclusion.
Physiological Effects	Gershon *et al* (2009)	Heart-rate variability (as a proxy measure for fatigue) was reduced.			Weak, not enough evidence to draw a conclusion.
Sleep	Jay *et al* (2006)	No evidence of disruption of subsequent sleep.			Weak, not enough evidence to draw a conclusion.

1.6.6 Methodological Limitations

Overall there are no clear patterns to the results found after the consumption of glucose and caffeine, and this, at least in part can be attributed to methodological limitations. The focus of much of the 'Red Bull' research is the effects of glucuronolactone, rather than glucose. Glucuronolactone is produced in the liver by the metabolism of glucose, and is used to build connective tissue. For example, Seidl, Peyrl, Nicham, & Hauser (2000) examined the effects of capsules that contained 80mg caffeine, 1g taurine and 600mg glucoronolatone (CTG capsules) compared to placebo capsules (containing wheat-bran). In both conditions participants also consumed 250ml of water with the capsules. They recorded event-related potentials whilst participants were performing the d2 test which measures attention capacity (in a stressful situation); the P300 event-related potential can be used as a marker for attention (Seidl, Hauser, Bernert, Marx, Freilinger & Lubec, 1997). They also used the Basler Befindlichkeits Skala to measure changes in the actual status of mood or subjective feelings of well-being. They found that CTG capsules improved reaction time and also preserved the P300 latencies which showed significant delay following placebo. Indicating that attention was improved following the CTG capsules (Seidl *et al.*, 2000). The results of the d2-test confirmed the ERP-results, with the CTG capsules improving psychomotor speed and improving overall concentration (Seidl *et al.*, 2000). They also found that mood declined following the placebo treatment whereas the active treatment prevented this decline (Seidl *et al.*, 2000). Whilst glucoronolatone was not the only potential ingredient in this study treatment, the results here suggest that it can have both physiological and behavioural effects or modulate the effects of other active ingredients.

This focus on glucoronolatone also has implications on the findings in terms of the effect of caffeine and glucose as in some studies the placebo drink contains glucose.. In Barthel *et al.,*'s study (2001) the placebo they used contained 10.5g of glucose as they only took out the taurine, glucuronolactone and caffeine. This was also the case in Howard and Marczinski' s study (2010) where the placebo still contained glucose. For their average 78kg individual this was 29.3g of glucose. This means that it is difficult to know exactly what effects glucose may be having in combination with these other substances. Although it must be noted that, Warburton *et al.,* (2001) carried out two studies investigating the effects of 'Red Bull' on cognition and mood using a sugar free placebo drink (study 1) and a placebo drink which contained a relatively small amount of glucose (6.5 g; study 2). In both studies they used 'Red Bull' [taurine (1g), glucuronolactone (600mg), caffeine (80mg), glucose (5.25mg), sucrose (21.5mg), inositol (50mg), niacin (20mg), vitamin B6 (5mg), vitamin B5 (5mg), vitamin B2 (1.5mg), vitamin B12 (0.005mg)] . They assessed RVIP, verbal reasoning, verbal memory, spatial memory and mood and both studies showed an almost identical pattern of results. On the RVIP task the active treatment increased accuracy and decreased reaction time. On the verbal reasoning task the active treatment improved reaction time, but did not improve accuracy. On the verbal memory task there was no improvement in words remembered or errors made for either immediate or delayed recall. There were no improvements of accuracy or reaction time on the spatial memory task. The active treatment improved self-reported ratings of alertness, clear-headed, attentive and quick-witted (study 2 only). This would suggest that glucose at the levels found in 'Red Bull' (approximately 5.25g per 250ml), is not exerting any significant effect in these studies. This could be because the levels are much lower than those typically found to enhance cognitive performance e.g. 25-50g (Foster, *et al.,* 1998; Kennedy & Scholey, 2000; Meikle, Riby, & Stollery, 2005; Sünram-Lea *et al.,* 2001;

Sünram-Lea, *et al.,* 2002). Also (although it is not always detailed clearly in the studies), 'Red Bull' contains other ingredients such as B vitamins that may remain in the placebo (as only glucuronolactone, taurine and caffeine are removed). These ingredients may have effects on cognition and mood that are not taken into account as previous research has shown they also have their own effects on cognition and mood (Bryan, Calvaresi, & Hughes, 2002). Therefore it is possible that these additional ingredients are affecting the results of the studies, and therefore this warrants further investigation.

Another factor which could influence the results of the studies is the properties of the treatment drinks. For example, Smit, Cotton, Hughes, and Rogers, (2004) conducted three studies to examine the effects of carbonation in energy drinks. For all three studies they looked at the effects on SRT, RVIP, immediate and delayed word recall, letter search and mood questionnaires. In the first study they compared the effects of a full energy drink containing 1000mg taurine, 75mg caffeine and 37.5g carbohydrate to an energy drink placebo and still water. The energy drink had immediate effects on the reaction time of the SRT and these were sustained for at least half an hour. The accuracy performance on the RVIP task was also improved immediately and this was sustained following the full energy drink. The full energy drink also had a positive impact with participants reporting increases in Energetic Arousal and its components. Smit *et al.,* (2004) suggest that the effect can be seen as preventing a decline in Energetic Arousal that occurs after the placebo treatment. This was noticeable from 30-60 minutes and sustained until 90 minutes post treatment. Participants also scored significantly higher on Hedonic Tone. Following the full energy drink participants also reported themselves as significantly more 'jittery' and 'tense', however the scores for all the treatments were relatively low on these dimensions. In the second study the researchers compared a full energy drink, this time containing 75mg caffeine and 37.5g carbohydrate, with a no caffeine energy drink (37.5g

CHO), and a no carbohydrate energy drink (75mg caffeine), an energy drink placebo and a none carbonated energy drink (75mg caffeine, 37.5g CHO). They found improvements in reaction time after the no carbohydrate (caffeine containing) treatment and mood was also modulated by caffeine with increases in Energetic Arousal in comparison to the other treatments. There was a lack of any effects due to carbohydrate content. The caffeine appears to have ameliorated the decline in performance and mood which was evident following the non-caffeine placebos. In the third study they compared a full energy drink containing 62.5mg caffeine and 37.5g carbohydrate, to an energy drink without carbohydrate (62.5mg caffeine) and an non-carbonated energy drink (62.5mg caffeine and 37.5g carbohydrate). They found there was a significant effect of the full energy drink on the Letter Search task. This was due to impairment in participants' reaction time on the final and most difficult block in the last session. They also found however that the full energy drink reduced 'jitteriness' at 50 minutes and 'tension' at 73 minutes post treatment. There was also a trend for the carbonised energy drink to decrease scores on the RVIP task 45 minutes post treatment. However carbonation led to a significant immediate increase in assertive ratings and this tailed off by 50 minutes. There was also a trend for participants to feel more 'cheerful', 'clearheaded' and less 'fatigued' after the full energy drink towards the end of the session compared to the non-carbonated equivalent. Feeling 'tense' decreased over time following the carbonated energy drink compared to non-carbonated. Feeling 'sluggish' also decreased significantly immediately following the carbonated drink compared to the non-carbonated. Although 'stomach bloated' was increased immediately following the carbonated treatment, this only lasted until 50 minutes post treatment. This series of studies demonstrates that the properties of the treatment drink can lead to differing performance even when the active ingredients are the same. Therefore

differences in the treatment vehicles could add to the equivocal results that have been found in the research to date.

When examining the doses of glucose and caffeine used in this research, it is difficult to get a clear picture as to which are the most effective doses. This is due to such varying doses being used in the research. Much of the research has used caffeine doses of around 75mg, which is approximately the same as one cup of coffee (Smith, 2002). This dose has been shown to be effective in modulating cognitive performance and mood in the caffeine literature as discussed previously. Many of the effects on cognition seen after an energy drink treatment are related to effects that caffeine has, for example, improvements in attention and increases in alertness (Smith, 2002). There are few effects on aspects of cognition that are generally found to be enhanced by glucose (Smith *et al.*, 2011), as discussed previously. This may be related to the levels of glucose administered in the energy drinks, 25g glucose has been suggested to be the optimal (Messier, 2004), however the doses administered can be more than twice this much (Adan & Serra-Grabulosa, 2010; Anderson & Horne, 2006; Kennedy & Scholey, 2004; Smit *et al.*, 2006). One explanation for the lack of effects could be that the levels of glucose being administered are too high and they are taking participants beyond the optimum level for glucose facilitation, as would be expected from the inverted U-shaped dose response (Parsons & Gold, 1992). Another possibility is the tasks used in these studies. Previous glucose research has found that the enhancement in verbal episodic memory is only reliably found when dual-tasks procedures are employed (Foster, *et al.*, 1998; Kennedy & Scholey, 2000; Sünram-Lea, *et al.*, 2002). The energy drink research has so far failed to use this method when administering memory tasks. The dual-tasking procedure is necessary to increase cognitive demand for participants who otherwise would be performing at their optimum and

therefore not be as susceptible to the beneficial effects of glucose (Sünram-Lea, *et al.*, 2002).

There are several other methodological issues which make it harder to draw any firm conclusions from the research. For example the choice of placebo used could have an effect (Smit *et al.*, 2006). Several studies have used water or a non-matched drink as their placebo (Adan & Serra-Grabulosa, 2010; Alford *et al.*, 2001; Serra-Grabulosa *et al.*, 2010; Smit & Rogers, 2002). Smit *et al.*, (2006) explored the role of familiarity/expectation effects of energy drinks. They looked at a well-known brand, Lucozade Energy (54g glucose, 30mg caffeine) and compared it to a placebo which was matched to this original drink, a Novel Full Energy Drink (54g glucose, 30mg caffeine) and a placebo that was matched to the novel drink. Participants attended an initial study day where they completed the baseline tasks and then received a small taste of each drink and ranked them according to their preference. After which they consumed their randomised treatment and completed the tasks. Over the next three weeks they attended the laboratory a further 7 times and at each of these visits they took a sip of their allocated treatment. At a final visit participants again completed the baseline tasks and ranked all of the drinks according to their preference, from their most favourite to their least. They then consumed their treatment drink and completed the tasks. The tasks they used were the SRT, RVIP, Letter Search, Serial 7's, Mood Questionnaires (Profile of Mood States (POMS); Activation-Deactivation Checklist (AD ACL)) and VAS's. On the first study day the two full energy drinks and the original placebo maintained performance on SRT and RVIP compared to deterioration in performance following the novel placebo. The strongest effects were found during the most demanding parts of the tasks for the SRT and RVIP. In contrast significant effects were found on the easiest, least memory taxing part of the Letter Search task. Ratings of 'cheerful' were increased immediately post-treatment for all drinks, especially

the full original drink. These findings on the first study day show that both the full energy drinks and the original matched placebo can lead to improvements in performance associated with energy drinks and so suggests that some of these may be due to expectancy effects. By the second study day participants had become familiar with their treatment drinks. It was found that compared to the placebo drinks both the full drinks led to an improvement and maintenance of alerting and energising moods. The full drinks also improved and maintained performance on the RVIP task. This suggests that after repeated exposure participants learn the effects of the drinks, i.e. that they do not experience any enhancement following the placebo drinks. This study highlights how important it is to have sensory matched drinks in order to avoid expectancy effects on the data (Smit *et al.,* 2006).

Another aspect which has not been systematically explored is the optimal delay between drink administration and cognitive testing. Both plasma caffeine levels and blood glucose levels reach their peak at around 30 minutes after ingestion (Donohoe & Benton, 2000; Lorist & Tops, 2003). Whilst much of the research has begun the cognitive testing at 30 minutes post-dose (e.g. Adan & Serra-Grabulosa, 2010; Alford, *et al.,* 2001; Horne & Reyner, 2001; Howard & Marczinski, 2010; Mucignat-Caretta, 1998; Rao, *et al.,* 2005; Scholey & Kennedy, 2004; Serra-Grabulosa, *et al.,* 2010), other research has used different post-dose time points; for example 5 minutes (Smit, *et al.*, 2006), 10 minutes (Anderson & Horne, 2006; Kennedy & Scholey, 2006), up to 60 mins (Seidl, *et al.,* 2000) and 75 minutes (Sünram-Lea, *et al.,* 2011). Gershon, *et al.,* (2009) assessed driving performance for 2 hours in the morning following an energy drink and then again for 2 hours in the evening. As discussed previously, they found a 'rebound' effect in the evening driving session with participants rating themselves as significantly more fatigued in the evening

session following consumption of the energy drink in the morning. These results illustrate that the time course of the effects following the consumption of energy drinks needs further investigation.

The characteristics of the participants have also not been well controlled for. For example, the studies have used a wide range of ages from 18 to 56 years and so age might be a confounding factor. For example, glucoregulation can decline during ageing and affects the way that glucose is processed (Convit, 2005). Consequently, age might be an important moderating factor (Smith *et al.*, 2011). The same applies to Body Mass Index (BMI), as it can have an effect on glucose regulation (Sünram-Lea *et al.*, 2011). In many of the studies BMI is simply not reported and where it is, it ranges from normal weight participants to one study where the range goes up to as high as 43.3 Kg/m^2 which is classified as morbidly obese (Smit *et al.*, 2004). The variations of BMI and its potential impact on glucoregulation has not been taken into account by the researchers and therefore it has unknown implications on the findings. By comparison, research into the effects of glucose alone has found BMI to affect glucoregulation and the dose-response profile (Sünram-Lea *et al.*, 2011). For example Sünram-Lea *et al.*, (2011) found that participants who had a low to medium BMI benefited from administration of higher glucose loads, whereas those in the high BMI group showed decrements in performance following high glucose loads. Habitual caffeine consumption of participants has also often not been taken into account. Whilst some studies have only included low to moderate caffeine consumers (Adan & Serra-Grabulosa, 2010; Anderson & Horne, 2006; Alford *et al.*, 2001; Horne & Reyner, 2001; Jay *et al.*, 2006; Mucignat-Caretta, 1998; Reyner & Horne, 2002), others included a wide range of consumers e.g. 0-533.2mg daily (Smit *et al.*, 2004), 46-705mg daily (Smit *et al.*, 2006). It has been shown that caffeine has differing effects in those who consume little to no caffeine compared to those that

consume caffeine daily (Haskell *et al.,* 2005; Kennedy & Haskell, 2011). Therefore the caffeine consumption of participants should be taken into account and controlled for when conducting this research.

Overall there are a number of methodological limitations and confounding factors that have not been controlled for and which might explain the equivocal findings in the literature.

1.7 Potential Mechanisms of Action for Combined Glucose and Caffeine Administration

Apart from the mechanisms of action that glucose and caffeine exert in isolation, there are a number of potential mechanisms that might explain the effects of combined caffeine and glucose administration on cognitive performance. For example, caffeine has been found to increase glucose uptake and/or release (Graham, Sathasivam, Rowland, Marko, Greer, & Battram, 2001; Greer, Hudson, Ross, & Graham, 2001; Keijzers, De Galan, Tack, & Smits, 2002; Lee, Hudson, Kilpatrick, Graham, & Ross, 2005; Petrie *et al.,* 2004; Pizziol *et al.,* 1998; Thong *et al.,* 2002). Pizziol *et al.,* (1998) administered 200 mg of caffeine or a placebo prior to an Oral Glucose Tolerance Test (OGTT) and found that following caffeine participants had a greater blood glucose response and this was independent of insulin. The authors suggest that this may be because of caffeine-induced catecholamine release. However other research has found that following ingestion of caffeine, the insulin response is increased without a corresponding decrease in the glucose tolerance response, suggesting that caffeine's effect of blood glucose is mediated by its effects on insulin response, whereby it reduces the glucose tolerance (Graham *et al.,* 2001; Lee *et al.,* 2005;

Petrie *et al.,* 2004). For example, Graham *et al.,* (2001) administered either 5mg/kg caffeine or placebo prior to an OGTT on different days. The participants then consumed 75g of dextrose 1 hour later. Following the caffeine treatment insulin levels were increased for a prolonged period in comparison to the placebo condition, with an increased Area Under the Curve (AUC) for the caffeine condition. The authors suggest that caffeine affects insulin's ability to clear the glucose load, i.e. that it induces temporary insulin insensitivity (Graham, *et al.,* 2001). Similar results were found in obese men before and after a weight loss intervention (Petrie *et al.,* 2004). Caffeine (5mg/kg) or a placebo was consumed 1 hour prior to an OGTT, and insulin response during the OGTT was greater following the treatment both before and after weight loss, however glycaemic response remained the same both before and after weight loss and following caffeine or placebo (Petrie, *et al.,* 2004).

Moreover, intestinal glucose absorption is affected by caffeine (Van Nieuwenhoven, Brummer, & Brouns, 2000). Van Nieuwenhoven *et al.,* (2000) found that when a carbohydrate electrolyte solution was co-administered with caffeine (~120mg), glucose absorption was significantly increased (compared to after administration of the carbohydrate electrolyte solution alone). The authors suggest that because the glucose uptake is an energy requiring process, the energy for this could be provided by caffeine. Caffeine might enhance sodium-glucose-linked transporter protein activity which in turn leads to increased jejunal (intestinal) glucose uptake (Van Nieuwenhoven *et al.,* 2000). This effect on glucose absorption might explain why some research has found the increase in glucose uptake caused by caffeine to be independent of insulin (Graham *et al.,* 2001; Pizziol *et al.,* 1998; Van Nieuwenhoven *et al.,* 2000).

Another mechanism proposed for caffeine's effects on glucose uptake is via increase in adrenaline release. Adrenaline has antagonistic effects on insulin's actions, including

those of glucose disposal (Laurent, Petersen, Russell, Cline & Shulman, 1998), and adrenaline levels have been found to be elevated during OGTT's following caffeine administration (Battram, Bugaresti, Gusba, & Graham, 2007; Greer, Hudson, Ross & Graham, 2001; Keijzers *et al.*, 2002). However, Battram *et al.*, (2005) compared caffeine (5mg/kg), a placebo and either a placebo plus a low-dose adrenaline infusion, or a placebo plus a high-dose adrenaline infusion on glucose infusion rates via a isoglycaemic-hyperinsulinaemic clamp (which assesses glucose disposal by determining during a constant insulin infusion the amount of glucose needed to maintain the normal concentration of glucose in the blood). Caffeine and the low-dose adrenaline infusion resulted in the same adrenaline concentrations, but caused different decreases in glucose infusion rates, 13% and 5% respectively. The authors concluded that this suggests the involvement of other mechanisms (Battram *et al.*, 2005).

Caffeine and glucose in combination may have beneficial effects on cognitive function due to their respective effects on the cholinergic system. Several authors have suggested that the memory enhancing effects of glucose is because it is a substrate for acetylcholine synthesis (Sünram-Lea *et al.*, 2002b; Wenck, 1989), whilst caffeine's antagonism of the adenosine receptors leads to increased cholinergic activity which provides a pathway for psychostimulant effects of caffeine (Carter *et al.*, 1995). This may provide a mechanism in which caffeine and glucose could work in synergy to enhance cognitive performance above that achieved from either one independently (Scholey & Kennedy, 2004).

However, as discussed above, there are other physiological mechanisms by which caffeine and glucose could interact and exert their effects. It is unlikely that the complex physiological and cognitive effects of caffeine and glucose will be the result of a single mechanism of action and it is likely to be a combination of neurotransmitter, neurohormonal and metabolic mechanisms (Scholey & Kennedy, 2004). The complexity

of these interactions could go some way towards explaining the equivocal effects in the literature, particularly with the addition of different doses and even participant characteristics e.g. sleep deprivation.

1.8 Current Programme of Research

It is clear from the literature reviewed in this chapter that further research into the effects of caffeine and glucose in combination is warranted. The aim of the studies described in this thesis was to systematically evaluate the behavioural effects of combined consumption of glucose and caffeine and to compare these with the effects produced by consuming either substance in isolation. This includes parametric investigations into the dose-response effects of combined administration, investigation into the cognitive domains susceptible to combined consumption effects, as well as investigations into potential moderating factors, such as age, time of day, cognitive effort. Moreover, in some of the studies reported in this thesis, we used a convergent operations approach that combined psychological and behavioural assessment (cognitive testing and assessment of mood) with evaluation of neural mechanisms (for example brain imaging using event-related potentials) and biochemical mechanisms (most notably effects on corticosteroids and adrenaline) mechanisms. Using this approach our objective was to inform our theoretical understanding of the cognitive and physiological mechanisms involved as well as delineating the specific effects and identifying moderating factors.

Chapter 2

Dose response investigation into the effects of low doses of glucose and caffeine on cognitive performance, mood and hormonal stress response in healthy volunteers.

2.1 Introduction

Extensive research has examined the effects of caffeine and glucose independently on cognitive performance and mood and found both substances to have beneficial effects on various aspects of cognition (for reviews see Messier, 2004; Smith, 2002). In terms of their cognitive effect profile, glucose has been found to have most robust effects on verbal episodic memory (Foster, Lidder & Sünram, 1998; Messier, 2004; Smith, Riby, Eekelen, & Foster, 2011), whereas caffeine appears to have most beneficial effects on vigilance tasks and reaction time tasks that require a sustained response (Smith, 2002). More specifically, Foster *et al.*, (1998) found that 25g glucose significantly improved performance on tests of long-term verbal episodic memory; however it did not have any facilitation effect on short-term memory tasks or on long-term non-memory tasks. They concluded that glucose may specifically enhance long-term memory through either enhanced retention or retrieval. In comparison, caffeine appears to have most beneficial effects on attention and vigilance (Smith, 2002). Brice and Smith (2002) found that both a single large dose of 200mg and smaller doses of 65mg consumed at four time-points led to improvements in attention compared to a placebo. Specifically, they found improved performance on reaction time tasks, a vigilance task, a sustained response task and a dual tracking/detection task. In addition, response moderators have been identified for both substances.

There is evidence to suggest that the glucose facilitation effect is enhanced when tasks require an element of divided attention or they require high cognitive demand, particularly in healthy young adults where they are already considered to be performing optimally (Sünram-Lea, Foster, Durlach & Perez, 2002). Sünram-Lea *et al.*, (2002) found that following a dose of 25g glucose, the enhancement on memory was only seen when

participants performed a secondary hand-movement task whilst encoding the word list they would later have to recall. When the participants did not complete the secondary task, or when the target words were intermixed with non-target words, distinguished by the speaker's gender, there was no enhancing effect of glucose on memory. Therefore, it has been argued that in order to demonstrate the effect of glucose on memory performance, episodic memory capacity and/or the availability of glucose in the brain must be depleted. Kennedy and Scholey (2000) found a similar effect for working memory, whereby the glucose facilitation effect was only seen in the more difficult Serial 7s task compared to the easier Serial 3s task. This was again following 25g glucose. Scholey, Harper and Kennedy (2001) found again that glucose preferentially enhanced performance on the more difficult Serial 7s task. In addition, a significant reduction in peripheral blood glucose levels was observed irrespective of drink condition performing the more difficult Serial 7s task. Both these studies suggest that glucose preferentially enhances tasks which have a high cognitive load, and that glucose may increase neural energy expenditure (Kennedy & Scholey, 2000; Scholey *et al.*, 2001).

For caffeine, effects appear to be most pronounced when alertness levels are low and when performance demands are high (Lieberman, 1992). For example, caffeine has been found to improve performance on a demanding driving simulation task (Horne & Reyner, 1996; Reyner & Horne, 1997), but not on, for example, a simpler Serial 3s subtraction task (Kennedy & Scholey, 2000). Horne and Reyner (1996) compared the effect of a 15-minute nap, 150mg caffeine and a coffee placebo administered during a 30 min break between two 1 hr car simulator tasks. The caffeine and nap conditions both significantly reduced driving impairments, subjective sleepiness and drowsiness as indicated by electroencephalography (EEG) measurement. However, caffeine gave more consistent effects as not all participants were able to nap in the allocated time and therefore caffeine would be a more practical

measure to reduce sleepiness (Horne & Reyner, 1996). Reyner and Horne (1997) found similar effects in 12 sleep restricted (5hrs) individuals. They compared a nap, 200mg caffeine and a placebo, taken during a 30 min break prior to a 2hr drive in a simulator. They found that caffeine significantly reduced driving incidents and subjective and EEG measures of sleepiness, as measured by lane drifting in a car simulator, self-ratings on the Karolinska Sleepiness Scale and, alpha and theta brain waves.

Both glucose and especially caffeine administered in isolation have also been found to have effects on mood (Brice & Smith, 2002; Heatherley, Haywood, Seers & Rogers, 2005; Quinlan, Lane, & Aspinall, 1997; Reay, Kennedy & Scholey, 2006). Reay *et al.,* (2006) found that 120 mins after consuming 25g glucose participants reported decreased fatigue on a visual analogue scale (VAS). However, other studies found no effect of glucose on mood when the Profile of Mood States (POMS) was used (Scholey & Fowles, 2002; Winder & Borill, 1998). For caffeine, increased levels of alertness and reduction in fatigue have been observed at doses typically found in a cup of tea or coffee (50-75mg) (Smith, 2002). Moreover, improved 'energy' has been reported following 75mg caffeine (Heatherley *et al.,* 2005) and 100mg of caffeine was found to decrease anxiety 30 mins after consumption (Quinlan *et al.,* 1997). In addition, Brice and Smith (2002) found that caffeine increased alertness and anxiety, and this was independent of whether it was administered as on single dose of 200mg or as four separate doses of 65mg.

However, while the behavioural and physiological effects of glucose, and caffeine consumed in isolation are reasonably well documented, there has been relatively little research into their effects when taken in combination. The data available suggests that when administered in combination; glucose and caffeine can improve certain aspects of cognition and mood (Adan & Serra-Grabulosa, 2010; Alford, Cox & Wescott, 2001; Antei *et al.*, 2011; Barthel *et al.,* 2001; Gendal *et al.,* 2009; Gershon *et al.,* 2009; Mets *et al.,*

2010; Rao, Henglong & Nobre, 2005; Scholey *et al.*, 2014). For example, improvements have been observed on performance on sustained attention tasks including the Rapid Visual Information Processing (RVIP) task (Adan & Serra-Grabulosa, 2010; Kennedy & Scholey, 2004; Scholey & Kennedy, 2004; Smit, Cotton, Hughes & Rogers, 2004), memory tasks (Adan & Serra-Grabulosa, 2010; Scholey & Kennedy, 2004), as well as increased subjective feelings of arousal (Smit & Rogers, 2002).

More specifically, Adan and Serra-Grabulosa (2010) compared a combination dose of 75mg caffeine and 75g glucose to each active ingredient individually and placebo. They found that compared to the effects of glucose and caffeine on their own, the combination dose improved attention and encoding and consolidation of verbal material. They concluded that the combination of glucose and caffeine has synergistic effects over and above the individual effects of each substance. Kennedy and Scholey (2004) also reported such potential synergistic effects. In one study participants received an 'energy drink' containing 68g glucose and 38mg caffeine; a second 'energy drink' containing 68g glucose and 46mg caffeine; and a placebo. In the second study they received an 'energy drink' containing 60g glucose and 33mg caffeine and a placebo. The results demonstrated improved performance on sustained attention task following administration of the three active treatment compared to placebo. The tasks were performed repetitively as part of an extended 60 minute cognitive battery and when administered in combination, glucose and caffeine were able to ameliorate the performance deficits that arose during this extended period of cognitive demand. Scholey and Kennedy (2004) also compared the components of an 'energy drink' (37.5g glucose, 75mg caffeine, ginseng and ginkgo biloba at flavouring levels) to the whole drink and placebo. They found that the whole drink significantly improved participants' performance on 'secondary memory' factor (which combines the percentage accuracy scores of the delayed word recognition, delayed picture

recognition, immediate word recall and delayed word recall tasks) and 'speed of attention' factor (which combines the reaction time results for simple reaction time, choice reaction time and digit vigilance) as derived from tasks on the Cognitive Drug Research assessment battery. In a similar vein, Smit, *et al.*, (2006) found that on their second study day, after manipulating their participants' exposure and familiarity with two energy drinks and their placebos, that both energy drinks containing 54g glucose and 30mg caffeine improved and maintained sustained attention compared to their placebos. Sünram-Lea *et al.*, (2011) examined the effects of two 'energy drinks' containing glucose and caffeine, administered prior to a search and rescue fire-fighting training task on subsequent cognition, mood and various physiological response. One 'energy drink' contained 50g glucose and 40mg caffeine, the other one contained 10.25g fructose/glucose and 80mg caffeine and these were compared to placebo. They found that following the drink containing 50g of glucose and 40mg of caffeine prevented a stress related decrease in memory performance. Information processing performance was also improved by both 'energy drinks' compared to placebo.

Combined administration of glucose and caffeine also affects mood. In the above study Sünram-Lea *et al.*, (2011) found that the 'energy drink' containing 50g glucose and 40mg caffeine led to reduced anxiety and significantly reduced self-reported levels of stress. However, no effects of either drinks were found on arousal, alertness, contentedness and calmness. Kennedy and Scholey (2004), found that drinks which contained 68g glucose/46mg caffeine and 60g glucose/33mg caffeine both improved participants' self-assessed ratings of mental fatigue during a long, cognitively demanding task. Yet again no effects on mood were observed following an 'energy drink', containing 37.5g glucose and 75mg caffeine or its constituents.

However, whilst the research described here has shown that glucose and caffeine when administered together have some effects on cognitive function and mood, the results overall are equivocal. For example, Anderson and Horne (2006) found that compared to placebo, an 'energy drink' containing 42g glucose and 30mg caffeine, did not counteract sleepiness. Participants had slower reaction times and more lapses during a sustained vigilance task. Much of the research so far has shown effects on attention (Adan & Serra-Grabulosa, 2010; Kennedy & Scholey, 2004; Scholey & Kennedy, 2004; Smit *et al.*, 2006), which could potentially be attributed to the caffeine content, as this is one of the effects most commonly seen after administration of caffeine (Smith, 2002). The research may have failed to find robust memory effects of glucose and caffeine in combination as the dosages used were much higher than the 25g glucose which has been shown to have the most robust memory effects when glucose is administered in isolation. Research investigating the combined effects of glucose and caffeine has also not utilised a dual-tasking procedure at the stage of encoding, which may be critical in being able to demonstrate the memory enhancing effect of glucose (Sünram-Lea, Foster, Durlach & Perez, 2002). Also, the dose response profile for combined administration still needs to be established. In particular, there is a need to investigate the efficacy of lower dosage combinations and delineation of effects of component parts compared to combined administration.

Consequently, the aim of the study was to investigate the dose-response profile of glucose and caffeine alone and in combination on glycaemic response, cognitive performance, mood and hormonal response by implementing a parametric approach administering 0, 15, and 25g of glucose and 0, 20 and 40mg of caffeine (alone and in combination) in order to compare the effects of different dosage combinations of caffeine and glucose. Given the weight of previous evidence for a beneficial effect of glucose and caffeine on attention,

this was chosen as the primary outcome measure, as measured by the accuracy on the Digit Vigilance task. A broad range of cognitive tasks were also utilised to allow comprehensive assessment of potential effects across various other cognitive domains (i.e. episodic and working memory). With regards to verbal episodic memory, a dual-tasking paradigm was also implemented, since this has shown to be critical in demonstrating effects of glucose administration on memory performance (Sünram-Lea, Foster, Durlach & Perez, 2002). Mood was assessed using a variety of subjective measures to allow broad evaluation of effects and identification of test and questionnaire that are most sensitive to energy drink effects.

In addition, we investigated the effects of glucose and caffeine (combined and in isolation) on hypothalamic-anterior pituitary-adrenocortical axis (HPA axis) and sympatho-adrenomedullary axis (SAM axis) response. Activation of the HPA axis is associated with the release of glucocorticoids from the adrenal cortex (cortisol in humans) and activation of the SAM axis results an increase in endogenous adrenergic activity, resulting in increased catecholamine activity (adrenaline and noradrenaline). Both caffeine and glucose administration have been shown to affect cortisol and/or catecholamine release (for example Bergendahl , Iranmanesh, Evans & Veldhuis, 2010; Bergendahl, Vance, Iranmanesh, Thorner & Veldhuis, 1996; Vance and Thorner, 1989, Robinson, Sünram-Lea, Leach & Owen-Lynch, 2004; James, 2004; Lovallo, Whitsett, al ‘Absi, Sing, Vincent & Wilson, 2005, Graham, Rush, van Soeren, 1994). However, to date there is a lack of research investigating the combined effects of glucose and caffeine on hormonal response. Investigation of the effects of combined glucose and caffeine administration on these physiological parameters is important as these might –at least in part- mediate the behavioural effects. The results of the hormonal responses and their discussion will be presented in Chapter 3.

2.2 Method and Materials

2.2.1 Participants

Sixty-four healthy young adults aged 18-35yrs were recruited for the study. There were recruited via the Online Research Participation System (SONA) at Lancaster University. A sample size of 32 participants in each study was deemed to be sufficient as this was comparable to other studies utilising a similar design who had found beneficial effects of caffeine and glucose on attention when co-administered (Kennedy & Scholey, 2004; Scholey & Kennedy, 2004; Smit & Rogers, 2002). All were frequent caffeine consumers, consuming a minimum of 120mg caffeine per day. Participants were excluded if they; had a diagnosis of Diabetes Mellitus; had any intolerance or allergic reaction to substances that contain phenylalanine and/or caffeine; were non-native English speakers; had a history of neurological or psychiatric illness (excluding depression or anxiety); had a current diagnosis of neurological or psychiatric illness (including depression or anxiety); were currently taking medication or nutritional supplements (excluding contraceptive pill); were pregnant, seeking to become pregnant or breastfeeding; had a history of or currently abused drugs or alcohol; smoked. Eligibility was confirmed via a Clinical Records Form (CRF) after the participants had given their signed informed consent to take part.

2.2.2. Design

A double-blind placebo controlled, balanced mixed design was used. With participants randomly allocated to two different dosing regimens (‘moderate’ versus ‘low’), each comprised of three different treatment combinations (glucose, caffeine and a glucose

caffeine combination) and a matching inert placebo. There was a 7 day (+/-2) washout period between treatments. Assessments of cognition, mood, fatigue and hormonal response were completed pre-treatment (baseline) and 20 minutes after (post-dose).

2.2.3 Treatments

Drinks were supplied by GlaxoSmithKline Laboratories in 380ml lightly carbonated taste matched solutions. The 'low' dose regime consisted of a glucose drink (containing 15g glucose, 0mg caffeine); a caffeine drink (containing 0g glucose, 20mg caffeine); and a combined drink (containing 15g glucose and 20mg caffeine). The 'moderate' dose regime consisted of glucose (25g glucose, 0mg caffeine); caffeine (0g glucose, 40mg caffeine); caffeine and glucose (25g glucose, 40mg caffeine). Both regimes also utilised a taste matched placebo (0g glucose, 0mg caffeine).

Participants were instructed to consume one drink per test session within 5 minutes. Post-dose cognitive testing started 20 minutes after the drink administration. A 20-minute delay was chosen as peak plasma caffeine concentration is reached between 15 to 120 min after oral ingestion and brain levels remain stable for at least one hour following peak plasma levels (Nehlig, 1999; Nehlig & Boyet, 2000). Also this time frame was similar to the procedure of previous glucose studies (Foster, Lidder & Sünram, 1998) in order to ensure successful transfer of plasma glucose to the brain.

2.2.4 Procedures

Initial screening was done during sign-up using the Lancaster recruitment on-line system (SONA). At the first visit for screening and training, all participants completed the voluntary written informed consent prior to any study procedures being performed. The participant was screened by the researcher and the outcome of the screening activities was recorded in the CRF. Personal demographic information (such as age, education height and weight) was also collected at this visit. They also completed a caffeine consumption questionnaire. Training on the cognitive tasks was then completed. No drinks were administered during the practice sessions and performance data from these sessions was not included in the analysis.

Participants then attended the laboratory on a further 4 occasions to complete the testing sessions. Testing was carried out between 8.30am and 12 noon and participants were asked to fast for 12hrs prior to the session (i.e. no food or drink except water) and to abstain from alcohol for 24 hours prior to testing. Due to the cortisol awakening response participants were also asked to wake up no earlier than 6.30am and no later than 8am. There was a 7(+/-2) day washout period between active days of the study. Consequently, participants were required to attend a weekly morning session over a period of approximately five to six weeks. Participants were randomised on arrival at the lab for their first study day. All active study days followed the same procedure.

At the beginning of the study day, a small baseline sample of blood was taken, and further blood glucose measurements were taken 15 and 50 minutes after drink consumption. Immediately after the baseline blood sample two saliva samples were also taken using a Salivette (Sarstedt Ltd.). The first for the measurement of alpha amylase, and the second for cortisol. Further saliva samples were taken 45 minutes post-drink for alpha-amylase and 55 minutes post-drink for cortisol. The first blood and saliva samples were followed by pre-drink baseline evaluation of mood and cognition, using the cognitive test battery.

This was followed by administration of the day's treatment (following a double-blind procedure). The post-drink cognitive test session commenced 20 minutes after drink consumption. Each test session comprised of completion of the cognitive test battery (cognitive performance), the Bond-Lader visual analogue scales, the Mood, Alertness and Physical Symptoms (MAPS) Questionnaire, the Stress Arousal Checklist, and the Activation-Deactivation adjective checklist (mood measures) and all participants received a debriefing sheet at the final day of testing.

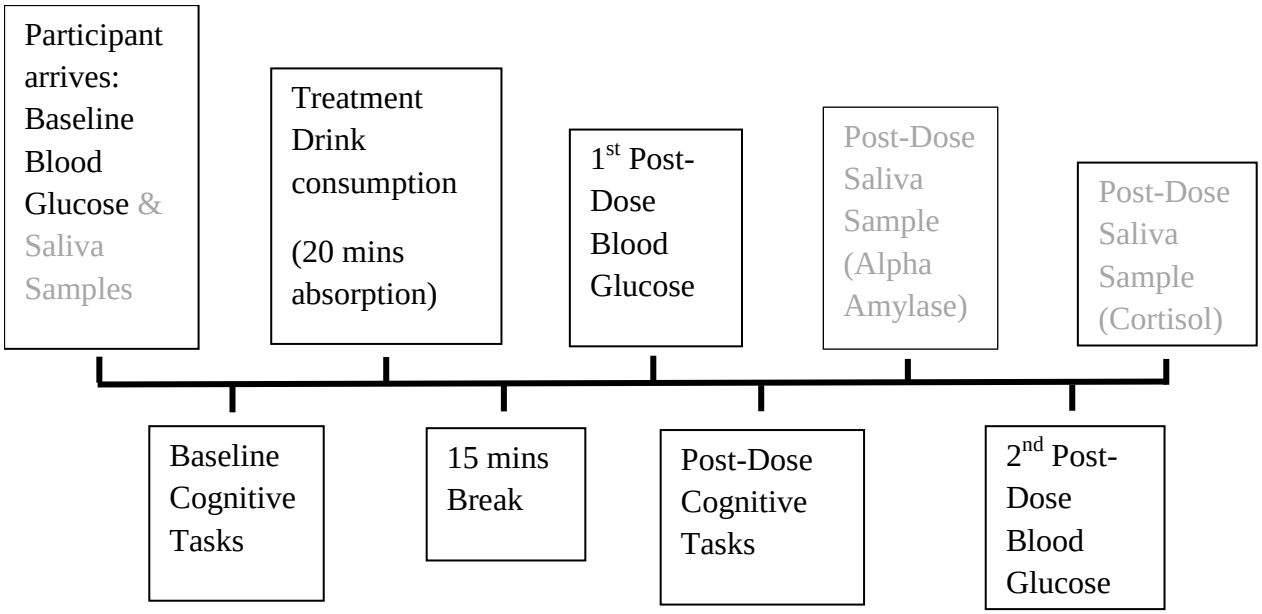

Figure 2.1 Schematic of the study day procedure

2.2.5 Assessments

Cognitive Tasks

Computerized assessment was used to evaluate cognitive performance. All tasks were delivered within the Computerized Mental Performance Assessment System (COMPASS), a purpose designed software application for the flexible delivery of randomly generated parallel versions of standard and novel cognitive assessment tasks. With the exception of

the paper and pencil tasks (word recall); all responses were made using the computer keyboard and mouse. In this case the assessment comprised a selection of standard psychometric tasks with stimuli chosen to possess good face validity in an 'everyday' context. The elements of the cognitive assessment are described below.

Word presentation

A list of 20 words matched for frequency, concreteness and imagery was presented on the monitor at the rate of 1 every 2 seconds for participants to remember. During encoding, participants were required to perform two complex hand-movement sequences (Sünram-Lea *et al.*, 2001). Each sequence was performed using both hands and contains three movements: fist – chop – slap and back-slap – chop – fist. Participants were told to alternate the sequence every fifth word and they were not informed when to change, only that they had to keep track of this themselves. Hand-movements were performed continually during word presentation.

Immediate word recall

Immediately after the words were presented participants were given 60-seconds to write down as many words as they could from the list they have just seen. Participants' responses were marked according to total number of errors and number of words recalled correctly.

Picture presentation

20 pictures were individually displayed in the centre of the screen at a rate of 1 every 3 seconds. Each picture was displayed for 1 second. Participants were required to remember the pictures.

Simple reaction time

The word ‘yes’ was presented repeatedly in the centre of the screen with inter-trial intervals varying randomly between 1 and 3.5 seconds. Participants were required to respond by pressing the space bar on their keyboard as quickly as possible, whenever the word appeared. Reaction times were recorded in milliseconds.

Digit vigilance

A single target digit was randomly selected and continuously displayed on the right side of the screen. In the centre a series of rapidly changing digits was displayed. Participants were required to press the space bar as quickly as possible, whenever the digit in the centre matches the target digit. Reaction times (milliseconds), percentage accuracy and number of false alarms were recorded.

Choice reaction time

The target words ‘yes’ and ‘no’ were repeatedly, randomly displayed individually in the centre of the screen. The inter-trial intervals varied randomly between 1-second and 3.5 seconds. Participants were instructed to respond by pressing the ‘m’ key for ‘yes’ and the ‘z’ key for ‘no’ on the keyboard as quickly and accurately as possible. Reaction times (milliseconds) and percentage accuracy were recorded.

Computerised Serial Sevens Task

This task evaluates working-memory performance (Hayman, 1942). Participants were required to compute a running subtraction of 7, starting from a randomly generated number. Participants were given 2 minutes to complete this task. Number of responses, number of correct responses and number of incorrect responses were recorded.

Computerized Corsi Block-Tapping Task

This task assessed the visual memory span (Milner, 1971). Illuminated squares appeared on the screen. The Squares flashed after each other in a tempo of one per second. Then the participant had to use the mouse to click on the buttons in the same order as they appeared on the screen. Outcome measures for this task were span and reaction time (milliseconds).

Computerized Serial Threes Task

This task evaluates working-memory performance (Hayman, 1942). Participants were required to compute a running subtraction of 3, starting from a randomly generated number. Participants were given 2 minutes to complete this task. Number of correct responses and number of incorrect responses were analysed.

Delayed word recall

Participants were given 60-seconds to write as many words as they could remember from the list they have seen at the beginning of the battery. Participant's responses were analysed according to total number of errors and number of words recalled correctly.

Delayed word recognition

The 20 original words and 20 distractor words were presented individually in a randomised order. Participants were asked to indicate whether each word had been in the original list or not by using the 'm' key for 'yes' or the 'z' key for 'no' on their keyboard. Outcome measures included in the analysis were percentage accuracy and reaction times for both distractors and targets. were recorded in addition to overall reaction times.

Picture recognition

The 20 original pictures and 20 distractor pictures were presented, individually in a randomised order. Participants were asked to indicate whether each word had been in the

original list or not by responding with the ‘m’ key for ‘yes’ or the ‘z’ key for ‘no’ on their keyboard. Percentage accuracy and reaction times for both distractors and targets were analysed.

Subjective Mood

The Bond-Lader visual analogue scales (VAS; Bond & Lader, 1974)

Visual Analogue Scales were presented on the screen immediately after the cognitive tests. Participants used the mouse to position an arrow at the point on the scale that represented their feelings at that moment. The 16 scales were combined as recommended by Bond and Lader (1974) to form three mood factors: ‘alertness’, ‘calmness’ and ‘contentment’.

Mood, alertness and physical symptoms questionnaire (MAPS) (Rogers *et al.*, 2008)

This computerised questionnaire consisted of seven unipolar and four bipolar visual analogue scales adapted from a similar questionnaire used in previous research on caffeine (e.g. Rogers *et al.*, 2005). ‘Headache’, ‘heart pound’, jittery/shaky’, ‘light-headed/feeling faint/dizzy’, ‘hands trembling’, ‘scared’ and ‘feeling hot/sweating (not due to heat)’ were rated on unipolar scales labelled ‘I don’t have this feeling at all’ (left-hand end=0) and ‘I have this feeling strongly (right-hand end=100). The bipolar scales were Relaxed (labelled ‘anxious/tense/nervous/on edge’=0 and ‘relaxed/calm’=100), Clearheaded (labelled ‘muzzy/dazed’=0, and ‘clearheaded’=100), Happy (labelled ‘sad/gloomy/miserable’=0 and ‘happy/cheerful/light-hearted’=100), and Alert (labelled ‘drowsy/sluggish/tired/fatigued’=0 and ‘alert/energetic/lively’=100). Instructions asked participants to rate ‘how you feel RIGHT NOW’. Scores for each item were obtained.

The Stress Arousal Checklist (SACL; Mackay *et al.*, 1978)

The SACL was used to measure stress and arousal levels. It consists of twenty-five adjectives which describe feelings and moods and participants were instructed to indicate on a four point scale how accurately each adjective matches their current state. The choices for the four-point scale were: 'Definitely Feel', 'Slightly Feel', 'Cannot Decide' and 'Definitely Do Not Feel'. The adjectives belong to distinct categories: stressors or arousers both of which could be either positive (e.g. nervous, stimulated) or negative (e.g. peaceful, sluggish) respectively. The classification was used to calculate the overall Stress and Arousal scores. The responses for each adjective are first scored as follows. If 'Definitely feel' or 'Slightly feel' are chosen for an adjective classified as positive, then the score is 1; if 'Cannot decide' or 'Definitely not' are chosen for an adjective classified as negative then the score is 1; in all other situations the score is 0. The overall STRESS score is obtained by summing over the scores for the adjectives classified under 'STRESS'. The overall Arousal score is obtained by summing over the scores for the adjectives classified under 'AROUSAL'.

The Activation-Deactivation adjective checklist (short form; AD ACL; Thayer 1989)

The AD ACL is a paper-pencil multidimensional self-rating test constructed and extensively validated for rapid assessments of activation or arousal states. The two core dimensions, energetic arousal (including tiredness) and tense arousal (including calmness) have been replicated repeatedly. Participants were instructed to use the four-point rating scale next to each word to describe their feelings at that moment. The choices for the four-point scale are: 'Definitely Feel', 'Slightly Feel', Cannot Decide' and 'Definitely Do Not Feel'. The 20 scales were then combined as recommended to form subscale adjectives: 'energetic', 'tired', 'tension' and 'calmness'. The scoring for the AD ACL was done by assigning 4, 3, 2, and 1 respectively to the 'Definitely Feel', 'Slightly Feel', Cannot Decide' and 'Definitely Do Not Feel' scale points, and summing or averaging the five

scores for each subscale. In order of appearance, the subscale adjectives are as follows: Energy (active, energetic, vigorous, lively, full-of-pep); Tired (sleepy, tired, drowsy, wide-awake, wakeful); Tension (jittery, intense, fearful, clutched-up, tense); Calmness (placid, calm, at-rest, still, quiet). Scoring for "wakeful" and "wide-awake" was reversed for the 'Tired' subscale. Furthermore, the two bipolar activation dimensions were evaluated: Energetic Arousal (EA); combining the two opposite poles, Energetic and Tired. The tiredness scores were reversed (but not wakeful and wide-awake) before adding the 10 scores. Tense Arousal (TA); combining the two opposite poles, Tension and Calmness. Calmness scores were reversed before summing the ten scores.

Blood glucose measurement

Blood glucose readings were obtained using the ExacTech® blood glucose monitoring equipment (supplied by MediSense Britain Ltd, 16/17 The Courtyard, Gorsey Lane, Coleshill, Birmingham B46 1JA), following the recommended procedure: The researcher swabbed the finger of the volunteer with a Sterets Isopropyl Alcohol BP Pre-injection swab (Seton Healthcare Group, Oldham, UK) and allowed the skin to air dry. The skin was punctured using an automatic lancing devise and a drop of blood was collected onto the analytical test strip. The volunteer applying a tissue blotted any excess blood. Ambidex-HG powder free polymer bonded latex examination gloves were worn by the experimenter at all times during the procedure. Each lancet and cap was only used once and then disposed of into a sharps container. Swabs, test strips, tissues and gloves were placed in a clinical waste sack.

Performance characteristics of ExacTech® blood glucose monitoring equipment:

Performance has been evaluated in clinical, laboratory and patient studies.

1) Precision:

Experienced operators performed testing in the laboratory. At each glucose level, readings were carried out using electrodes selected from a single box of G2 Sensor Electrodes.

Within Run	N	Mean mmol/l	SD mmol/l	CV (%)
Blood (Low)	20	2.94	0.19	6.5
Blood (Med Low)	20	5.28	0.23	4.3
Blood (Med High)	20	11.50	0.51	4.4
Blood (High)	20	21.17	1.17	5.5

2) Accuracy:

Accuracy was assessed by comparison with the YSI model 23AM Glucose Analyzer as used in clinical laboratories. The following data were obtained using fresh capillary blood:

n = 144

y =1.059(x) – 0.42 mmol/l

r = 0.979

Cortisol and Alpha Amylase Measurement

Assessment of salivary cortisol levels is the classic measurement of response to stress, associated with activation of the HPA axis. Salivary alpha-amylase is a measure of adrenergic activity (SAM axis). Collection of saliva, in preference to blood sampling, provided a non-invasive mechanism to collect physiological samples from subjects.

Saliva samples were collected using the salivette saliva sampling device (Sarstedt LTD, Leicester, UK). These consist of a small test tube fitted with an inner receptacle containing a sterile cotton wool bud. For the measurement of alpha-amylase, participants were instructed to give un-stimulated saliva samples by placing a Salivette in the top right hand corner of their mouth for a timed two-minute period. For the measurement of cortisol, participants were asked to lightly chew on the Salivette for one-minute. Ambidex-HG powder free polymer bonded latex examination gloves were worn by the experimenter at all times during saliva sampling. Excess saliva was removed using Sterets Isopropyl Alcohol BP Pre-injection swabs (Seton Healthcare Group, Oldham, UK). All saliva contaminated waste was placed in a yellow bio hazard bag and disposed of via Lancaster University's Biology Autoclave system. Samples were stored at -40oC until analysis. Saliva was recovered by centrifugation and salivary volume determined by weighing. This allows for the calculation of the saliva flow rate. Cortisol concentration (nmol/l), alpha amylase (μ/mL) concentration in saliva was determined by commercially available kits (Salimetrics, USA).

The results of these analyses will be reported in Chapter 3.

2.2.6 Statistical Analysis

The Intent To Treat (ITT) population was used for the efficacy analysis. The primary efficacy variable was Digit Vigilance accuracy. The secondary outcome variables were other measures of attention (Digit Vigilance speed and false alarms; Simple Reaction Time; Choice Reaction Time accuracy and speed), memory (Immediate Word Recall correct and errors; Delayed Word Recall correct and errors; Word Recognition accuracy, speed, target and distractor reaction time; Picture Recognition accuracy, speed, target and distractor reaction time), working memory (Serial 3s correct and errors; Serial 7s correct and errors; Corsi Block span and reaction time), mood measures (Bond-Lader; MAPS; SACL; ADACL). All cognitive and mood outcome measures were transformed into change from baseline scores. Using IBM SPSS Statistics Version 22 each dosing regimen (low and moderate) treatments were performed on the change from baseline scores using a linear mixed model. Treatment and period were added as fixed effects and subject as random effect to the model. Baseline average for each individual task was added as a covariate to the model. The Estimates of Fixed Effects were used to compare each active treatment with placebo.

For glycaemic treatment, comparisons were performed on the blood glucose levels using a linear mixed model. The model included a random effect for subject and fixed effects for period, treatment and time. The Estimates of Fixed Effects were used to compare each active treatment with placebo.

For the exploratory outcome measure, hormonal responses (cortisol and alpha amylase), measures were transformed into change from baseline scores and analysed using a linear mixed model. Treatment and period were added as fixed effects and subject as random effect to the model. The model also included baseline measures as a covariate.

2.3. Results

Dosing Group	N	Age (years)	Gender	BMI (kg/m^2)	Years in Education	Caffeine Consumption (mg)
Low	31	20.94 (2.22)	13 Males / 18 Females	22.10 (2.93)	16.29 (1.76)	231.02 (72.72)
Moderate	30	19.70 (1.32)	15 Males / 15 Females	23.89 (4.90)	15.33 (1.09)	235.41 (11.68)

Table 2.1. Participant Demographics (Means and Standard Deviations)

2.3.1 Glycaemic Response

There was a significant main effect of the low dose treatments on blood glucose, F (2.01, 60.19) = 29.81, $p < .001$. There was a significant effect of time, F (2, 113.94) = 26.44, $p < .001$ and treatment x time interaction F (6, 68.07) = 24.98, $p < .001$ were observed. Comparisons showed that baseline blood glucose levels did not differ, however after administration of glucose containing drinks, higher blood glucose levels were observed at both post dose measures compared to placebo and the caffeine only drink (all $p < .001$) (see Figure 2.2 for glycaemic response as a function of treatment and time).

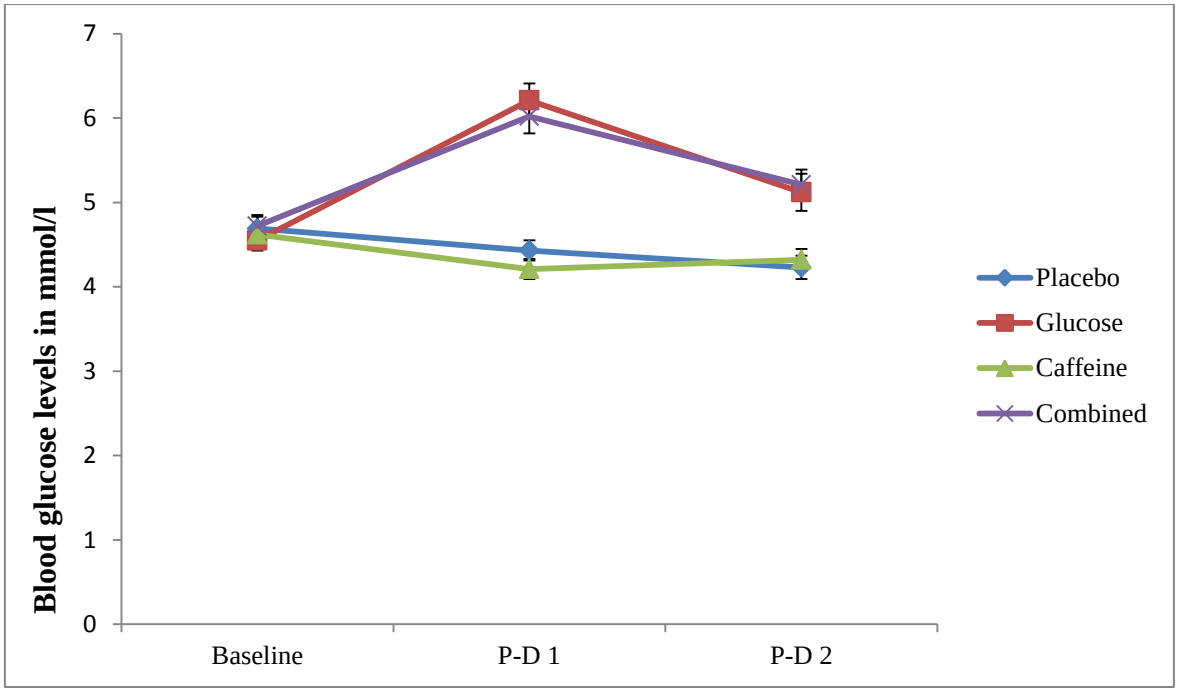

Figure 2.2 Glycaemic response to low dose treatments as a function of drink and time

Table 2.2 Glycaemic response to low dose treatments (means and standard deviations)

Time Point	Treatment			
	Placebo	Low Glucose (15g)	Low Caffeine (20mg)	Low Caffeine (20mg)/Low Glucose (15g)
Baseline	4.69 (0.14)	4.55 (0.12)	4.62 (0.11)	4.72 (0.13)
1st Post Dose	4.43 (0.12)	6.21 (0.20)	4.21 (0.12)	6.02 (0.20)
2nd Post Dose	4.23 (0.14)	5.12 (0.22)	4.32 (0.13)	5.21 (0.18)

The same picture emerged for the moderate treatment group, with significant effects of treatment, F (3, 92.45) = 99.76, p < .001), time (2, 115.53) = 37.76, p < .001, and a significant interaction between both factors, F (6, 80.98) = 40.10, p < .001. Again no significant differences at baseline were observed, but as expected glucose containing

drinks resulted in significantly higher glycaemic response at both post dose measures compared to placebo and the caffeine only drink (all $p < .001$) (see Figure 2.3 for glycaemic response as a function of treatment and time).

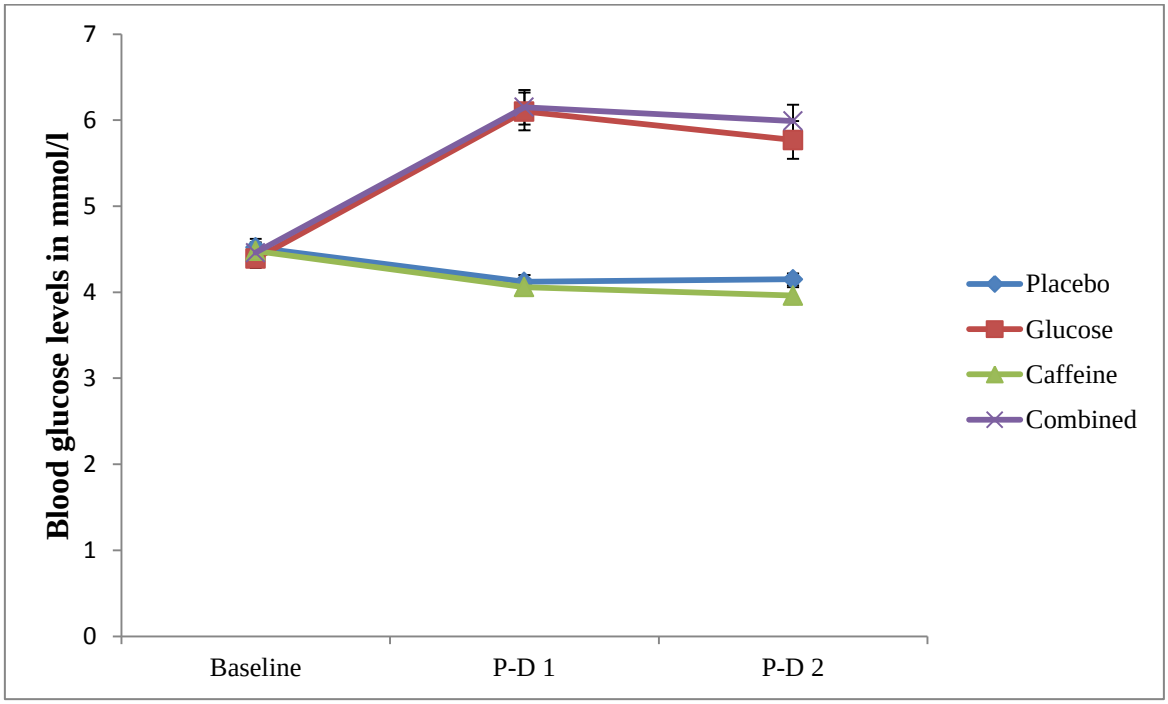

Figure 2.3 Glycaemic response to moderate dose treatment as a function of drink and time

Table 2.3 Glycaemic response to moderate dose treatment (means and standard deviations)

	Treatment			
Time Point	Placebo	Moderate Glucose (25g)	Moderate Caffeine (40mg)	Moderate Caffeine (40mg)/Low Glucose (25g)
Baseline	4.52 (0.10)	4.39 (0.11)	4.48 (0.10)	4.46 (0.07)
1st Post Dose	4.12 (0.08)	6.10 (0.22)	4.06 (0.07)	6.15 (0.20)
2nd Post Dose	4.15 (0.07)	5.77 (0.22)	3.96 (0.10)	5.99 (0.19)

2.3.2 Cognitive Performance

Performance data from tasks that were completed incorrectly were removed. For the low dose treatments, the data for one participant on the Serial 3s task on the day they consumed the low glucose/caffeine combination treatment drink was excluded as they completed the task incorrectly at baseline (all their responses were scored as errors). For the moderate dose treatments one participant's scores on the Serial 3s task on the day they consumed the low glucose/caffeine combination treatment drink were also excluded as they completed the task incorrectly at the post dose testing session. Two participant's scores on the Serial 7s task on the day they received the low glucose/caffeine combination treatment drink were excluded as one completed the task incorrectly at baseline and one incorrectly at post dose.

2.3.2.1 Primary Outcome:

Digit Vigilance % Correct

There was no significant main effect of the 'low dose' treatments on the percentage accuracy of the Digit Vigilance task, F (3, 39.34) = 0.04, p = .99, no main effect of period, F (3, 34.85) = 0.50, p = .69; or the interaction, F (9, 30.73) = 1.13, p = .37. There were no significant differences observed following administration of treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on the percentage accuracy of the Digit Vigilance task, F (3, 33.96) = 1.51, p = .23, or period, F (3, 37.62) =

1.83, p = .16; or their interaction, F (9, 23.73) = 0.57, p = .81. There were no significant differences observed following administration of treatment drinks compared to placebo.

2.3.2.2 Secondary Outcomes: Attention

Simple Reaction Time

There was no significant main effect of the 'low dose' treatments on the Simple Reaction Time task, F (3, 17.10) = 0.42, p = .74. There was no main effect of period, F (3, 12.56) = 0.60, p = .63; or the interaction, F (9, 20.52) = 1.27, p = .31. There were no significant differences observed following any of the active treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on simple reaction time, F (3, 23.28) = .73, p = .54; or period, F (3, 30.67) = 0.03, p = .99; or treatment x period interaction, F (9, 22.46) = 1.41, p = .24. No significant benefits from any of the active drinks were observed compared to placebo.

Choice Reaction Time % Correct

There was no significant main effect of the 'low dose' treatments on the percentage accuracy of the Choice Reaction Time task, F (3, 30.57) = 0.63, p = .60. There was no main effect of period, F (3, 29.47) = 0.97, p = .42; or the interaction effect between treatment and period, F (9, 24.27) = 1.43, p = .23. There were no significant differences observed following administration of treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on the percentage accuracy of the Choice Reaction Time task, F (3, 24.28) = 1.99, p = .14. There was no

main effect of visit, F (3, 26.10) = 2.67, p = .07; or interaction, F (9, 22.64) = .65, p = 74. Again, no significant benefits from any of the active drinks were observed compared to placebo.

Choice Reaction Time Reaction Time

There was no significant main effect of the 'low dose' treatments on the reaction time of the Choice Reaction Time task, F (3, 14.18) = 0.49, p = .69. There was no main effect of period, F (3, 12.86) = 1.52, p = .26; or the interaction, F (9, 17.80) = 0.61, p = .78. There were no significant differences observed following administration of treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on the reaction time of the Choice Reaction Time task, F (3, 22.00) = 1.09, p = .37; or main effect of period F (3, 22.03) = 0.14, p = .94; or the interaction, F (9, 22.06) = 1.32, p = .28. Again, there were no significant differences observed following administration of treatment drinks compare to placebo.

Digit Vigilance Reaction Time

The main effect of the 'low dose' treatments on the reaction time responses of the Digit Vigilance task failed to reach significance, F (3, 34.88) = 2.45, p = .08. There was no main effect of period, F (3, 31.99) = 0.66, p = .58; or interaction, F (9, 28.17) = 0.76, p = .66.Comparison with placebo showed there were no significant differences compared to following the administration of treatment drinks.

There was no significant main effect of the 'moderate dose' treatments on the reaction time responses of the Digit Vigilance task, F (3, 41.54) = 0.87, p = .47; or of period, F (3, 39.60) = 0.62, p = .61; or the interaction, F (9, 26.99) = 0.29, p = .97. There were no significant differences observed following administration of treatment drinks compared to placebo.

Digit Vigilance False Alarms

There was no significant main effect of the 'low dose' treatments on the false alarm responses on the Digit Vigilance task, F (3, 37.10) = 1.39, p = .26. There was no main effect of period, F (3, 46.52) = 1.01, p = .40; or the interaction effect between treatment and period, F (9, 27.47) = 0.53, p = .84. There were no significant differences observed following administration of treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on the false alarm responses on the Digit Vigilance task, F (3, 22.97) = 1.17, p = .34; period, F (3, 25.15) = 0.81, p = .50; or treatment x period, F (9, 17.50) = 2.04, p = .10. There were no significant differences observed following administration of treatment drinks compared to placebo.

Table 2.4 Measures of attention and vigilance, means and standard errors

Treatment		Combined	Glucose	Caffeine	Placebo
Low[1]	SRT	23.5	5.5	1.7	13.3
		(22.4)	(4.2)	(17.4)	(8.8)
	CRT %	1.32	0.50	-0.08	0.80
	Correct	(0.70)	(0.53)	(0.78)	(0.82)
	CRT RT	-20.2	0.8	7.1	3.9
		(20.1)	(6.4)	(15.7)	(5.5)
	Digit	0.10	-0.44	-0.53	-0.63
	Vigilance %	(1.65)	(0.98)	(1.20)	(1.36)
	Correct				
	Digit	10.1	16.1	2.6	12.9
	Vigilance	(3.9)	(4.2)	(3.7)	(3.3)
	RT				
	Digit	0.35	-0.06	0.11	0.13
	Vigilance	(0.15)	(0.13)	(0.17)	(0.13)
	False alarms				
Moderate[2]	SRT	7.9	14.1	6.2	14.0
		(4.9)	(4.5)	(4.6)	(5.8)
	CRT %	-0.84	-0.05	0.59	1.39
	Correct	(0.66)	(0.63)	(0.67)	(0.85)
	CRT RT	2.4	9.2	10.0	13.7
		(4.2)	(5.0)	(7.0)	(5.9)
	Digit	-1.39	-0.35	1.07	-1.43
	Vigilance %	(1.14)	(1.02)	(0.83)	(1.19)
	Correct				
	Digit	8.9	13.6	5.1	12.4
	Vigilance	(5.3)	(4.1)	(4.0)	(4.1)
	RT				
	Digit	-0.10	0.02	-0.14	0.21
	Vigilance	(0.11)	(0.14)	(0.11)	(0.17)
	False alarms				

[1] Change from baseline; covariates appearing in the model for low dose treatments are evaluated at the following average baseline values: Simple Reaction Time = 314.7; CRT % Correct = 95.16; CRT RT = 413.9; Digit Vigilance % Correct = 93.0; Digit Vigilance RT = 455.9; Digit Vigilance False Alarms = 0.29.

[2] Change from baseline; covariates appearing in the model for moderate dose treatments are evaluated at the following average baseline values: Simple Reaction Time = 296.4; CRT % Correct = 94.43; CRT RT = 389.7; Digit Vigilance % Correct = 94.50; Digit Vigilance RT = 457.0; Digit Vigilance False Alarms = 0.30.

2.3.2.3 Secondary Outcomes: Memory

Immediate Word Recall Correct

No significant main effect of the 'low dose' treatments, F (3, 43.02) = 0.03, p = .99 was observed. There was no main effect of period, F (3, 39.24) = 0.69, p = .56; or interaction effect between treatment and period, F (9, 24.42) = 0.58, p = .80.

For 'moderate dose' regimen, no significant effect of treatment was observed, F (3, 47.65) = 1.59, p = .21. There was no main effect of period, F (3, 101.29) = 2.49, p = .07; or significant interaction between treatment and period, F (9, 53.87) = 1.11, p = .37.

In addition, no significant differences were observed following administration of any of the active treatment drinks compared to placebo.

Immediate Word Recall Errors

There was no significant main effect of the 'low dose' treatments on number of errors in the Immediate Word Recall task, F (3, 38.33) = 1.43, p = .25. There was no main effect of period, F (3, 43.30) = 0.58, p = .63 and no treatment x period interaction, F (9, 26.23) =

0.44, p = .91. There were no significant differences observed following administration of treatment drinks compared to placebo.

For the ‘moderate dose’ treatments again no treatment effect was observed on number of errors, F (3, 34.60) = 0.44, p = .73. There was no significant main effect of period, F (3, 26.03) = 2.03, p = .14; or interaction between treatment and period, F (9, 30.16) = 0.24, p = .99. There were no significant differences observed following administration of treatment drinks compared to placebo.

Delayed Word Recall Correct

No significant main effect of the ‘low dose’ treatments, F (3, 39.72) = 0.32, p = .81, and no effect of period, F (3, 37.50) = 0.81, p = .50; or interaction between both factors, F (9, 22.76) = 1.45, p = .23.

A similar picture emerged for the ‘moderate dose’ regimen with no effect of treatment, F (3, 46.61) = 0.58, p = .63, period, F (3, 32.11) = 2.58, p = .07; or interaction, F (9, 49.00) = 1.21, p = .31.

Moreover, no differences were observed following administration of active drinks compared to placebo under both treatment regimens.

Delayed Word Recall Errors

There was no significant main effect of the ‘low dose’ treatments, F (327.22) = 1.71, p = .19, period, F (3, 30.17) = 0.56, p = .64 or treatment x period interaction, F (9, 26.32) = 1.56, p = .18.

For 'moderate dose' regimen, no effects of treatment, F (3, 34.60) = 0.44, p = .73, period, F (3, 26.03) = 2.03, p = .14; or interaction effect between treatment and period, F (9, 30.16) = 0.24, p = .99 was observed.

For both treatment regimens ('low' and 'moderate') no significant performance differences were observed following administration of treatment drinks compared to placebo (for performance on free recall task see table 2.4).

Table 2.5 Free Recall Performance, means and standard errors

Treatment		Combined	Glucose	Caffeine	Placebo
Low[1]	IFR correct	-.037	-0.53	-0.45	-0.47
		(0.33)	(0.46)	(0.46)	(0.39)
	IFR error	-0.06	0.12	0.03	-0.39
		(0.20)	(0.17)	(0.14)	(0.20)
	DFR correct	-1.45	-1.15	-.85	-1.36
		(0.40)	(0.50)	(0.52)	(0.42)
	DFR error	.30	.00	-.28	-.07
		(0.23)	(0.18)	(0.17)	(0.31)
Moderate[2]	IFR correct	-1.19	-0.74	-0.74	0.12
		(0.41)	(0.39)	(0.38)	(0.45)
	IFR error	-0.25	0.12	-0.07	0.03
		(0.29)	(0.23)	(0.11)	(0.21)
	DFR correct	-1.82	-1.82	-1.39	-1.90
		(0.59)	(0.43)	(0.31)	(0.29)
	DFR error	0.12	-0.05	0.03	-0.27
		(0.21)	(0.26)	(0.16)	(0.21)

[1] Change from baseline; covariates appearing in the model for low dose treatments are evaluated at the following average baseline values: IFR correct = 28.45; IFR Incorrect = 0.80.; DFR correct = 5.50; DFR error= 0 .80.

[2]Change from baseline; covariates appearing in the model are evaluated at the following values: IFR correct = 26.45, IFR Incorrect = 0.69; DFR correct= 4.72; DRF error = 1.08.

*Significant compared to placebo at p<0.05

Word Recognition Accuracy

There was no significant main effect of the 'low dose' treatments, F (3, 33.48) = 0.36, p = .79, and no main effect of period, F (3, 39.48) = 0.79, p = .51; or interaction, F (9, 25.22) = 0.79, p = .63. Compared to placebo none of the active drinks resulted in superior performance.

The same picture emerged for the 'moderate' treatment regime, with no effect of treatment, F (3, 18.36) = 0.66, p = .59, period, F (3, 27.29) = 1.48, p = .24; or interaction, F (9, 27.29) = 1.69, p = .14. Comparison of active drinks with placebo showed an advantage of the combined glucose and caffeine drink compared to placebo (p = .03).

Word Recognition Speed

There was no significant main effect of the 'low dose' treatments on the reaction time for the correct responses on the Word Recognition task, F (3, 39.68) = 0.17, p = .92. There was no main effect of period, F (3, 40.65) = 1.74, p = .18; or interaction between treatment and period, F (9, 33.95) = 0.91, p = .53. There were no significant differences observed following administration of treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on the reaction time for correct responses on the Word Recognition task, F (3, 18.36) = 0.66, p = .59, period, F (3, 27.29) = 1.48, p = .24; or interaction, F (9, 27.29) = 1.69, p = .14. Comparison of active drinks with placebo showed an advantage of the combined drink compared to placebo (p = .03).

Word Recognition Target Reaction Time

The effect of the 'low dose' treatments on the reaction time for targets failed to reach significance, F (3, 35.03) = 1.81, p =.16. There was no main effect of period, F (3, 39.88) = .65, p = .59; or interaction between treatment and period, F (9, 28.48) = .65, p = .75. However, comparison with placebo showed that after consumption of the glucose drink, participants reacted significantly faster to target words compared to placebo (p = .04).

There was no significant main effect of the 'moderate dose' treatments on the reaction time for targets on the Word Recognition task, F (3, 26.59) = 1.43, p = .26. There was no main effect of period, F (3, 25.17) = 3.32, p = .14; or the interaction effect between treatment and period, F (9, 29.22) = 1.02, p = .45. Moreover, no significant differences were observed following administration of treatment drinks compared to placebo.

Word Recognition Distractor Reaction Time

There was no effect of the 'low dose' treatments on the reaction time for distractors, F (3, 39.84) = 0.69, p = .56. There was no main effect of period, F (3, 41.70) = 0.89, p = .45; or interaction between treatment and period, F (9, 27.10) = 1.35, p = .26. No significant differences following administration of active treatment drinks compared to placebo were observed.

There was no significant main effect of the 'moderate dose' treatments on the reaction time for distractors on the Word Recognition task, F (3, 40.74) = 1.66, p = .19. There was no main effect of period, F (3, 39.12) = 0.30, p = .82; or treatment x period interaction, F (9, 26.26) = 1.06, p = .43. There were no significant differences observed following

administration of treatment drinks compared to placebo. (See Table 2.5. for the Word Recognition Performance, means and standard errors).

Table 2.6 Word Recognition Performance, means and standard errors

Treatment		Combined	Glucose	Caffeine	Placebo
Low[1]	Accuracy	-3.10	-4.31	-2.43	-2.90
		(1.58)	(2.15)	(1.56)	(1.62)
	Speed	-22.7	-30.5	-9.8	5.4
		(22.0)	(20.7)	(35.7)	(30.0)
	Target	-22.5	-74.2 *	-24.3	8.7
	Reaction	(26.1)	(21.8)	(30.8)	(31.9)
	Time				
	Distractor	-34.3	-18.9	15.2	13.2
	Reaction	(26.4)	(27.4)	(34.1)	(30.7)
	Time				
Moderate[2]	Accuracy	-0.21 *	-0.93	-1.66	-.97
		(1.39)	(1.43)	(1.86)	(1.33)
	Speed	-62.1 *	-27.4	-13.6	-28.1
		(30.1)	(13.9)	(20.6)	(27.2)
	Target	84.2	40.1	8.6	35.9
	Reaction	(43.8)	(17.8)	(26.5)	(27.1)
	Time				
	Distractor	-68.8	-11.4	-10.5	-56.60
	Reaction	(23.6)	(21.0)	(25.3)	(25.5)

Time

[1] Change from baseline; covariates appearing in the model for low dose treatments are evaluated at the following average baseline values: Word Recognition Accuracy = 73.86; Word Recognition Speed = 837.4; Word Recognition Target Reaction Time = 850.0; Word Recognition Distractor Reaction Time = 870.2.

[2]Change from baseline; covariates appearing in the model for moderate dose treatments are evaluated at the following values: Word Recognition Accuracy = 67.67; Word Recognition Speed = 814.5; Word Recognition Target Reaction Time = 831.5; Word Recognition Distractor Reaction Time = 842.7.

*Significant compared to placebo at $p < .05$

Picture Recognition Accuracy

There was no significant main effect of the 'low dose' treatments on the correct responses for the Picture Recognition task, F (3, 38.41) = 1.91, p = .15. There was a significant main effect of period, F (3, 35.49) = 3.37, p = .04, participants performed worse on study day 4 (period 4). There was no significant interaction between treatment and period, F (9, 26.42) = 0.80, p = .620. Compared to placebo, performance decrements were significantly reduced after the caffeine drink compared to placebo (p = .04).

There was no significant main effect of the 'moderate dose' treatments on the correct responses for the Picture Recognition task, F (3, 26.20) = 1.42, p = .26. There was no main effect of period, F (3, 34.14) = 0.98, p = .41, and no interaction effect between treatment and period, F (9, 26.77) = 0.94, p = .51. As observed for the 'low dose' group, decrements in performance on the task were significantly less following the caffeine treatment drink compared to placebo drink, (p = .02).

Picture Recognition Speed

There was no significant main effect of the 'low dose' treatments on the correct response reaction time for the Picture Recognition task, F (3, 25.46) = .91, p = .45. There was no main effect of period, F (3, 30.29) = .14, p = .94, and no interaction effect between treatment and period, F (9, 24.17) = 0.94, p = .51. There were no significant differences observed following administration of treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on the correct response reaction time for the Picture Recognition task, F (3, 32.55) = 0.55, p = .65. There was no main effect of period, F (3, 32.45) = 0.15, p = .93; or their interaction, F (9, 29.64) = 0.83, p = .60. There were no significant differences compared to placebo were observed following administration of active treatment drinks.

Picture Recognition Target Reaction Time

There was no significant main effect of the 'low dose' treatments on the target response reaction time for the Picture Recognition task, F (3, 20.02) = 0.82, p = .50. There was no main effect of period, F (3, 18.87) = .72, p = .55; or treatment x period interaction, F (9, 20.23) = 1.94, p = .10. Significant differences following administration of active treatment drinks compared to placebo were not identified.

There was no significant main effect of the 'moderate dose' treatments on the target response reaction time for the Picture Recognition task, F (3, 23.01) = 0.51, p = .68. There was no main effect of period, F (3, 33.41) = 0.08, p = .97; or interaction between treatment and period, F (9, 29.62) = 0.84, p = .59. There were no significant differences compared to placebo observed following administration of active treatment drinks.

Picture Recognition Distractor Reaction Time

There was no significant main effect of the 'low dose' treatments on the distractor response reaction time for the Picture Recognition task, F (3, 28.01) = 0.82, p = .50. There was no main effect of period, F (3, 32.30) = 0.58, p = .64; or interaction between treatment and period, F (9, 21.27) = 0.59, p = .80.

There was no significant main effect of the 'moderate dose' treatments on the distractor response reaction time for the Picture Recognition task, F (3, 37.25) = 0.74 p = .54. There was no main effect of period, F (3, 31.58) = 0.54, p = .66; or the interaction effect between treatment and period, F (9, 42.98) = 1.01, p = .45. There were no significant differences observed following administration of treatment drinks compared to placebo. (See Table 2.6 for the Picture Recognition Performance, means and standard errors).

Table 2.7 Picture Recognition Performance, means and standard errors

Treatment		Combined	Glucose	Caffeine	Placebo
Low[1]	Accuracy	-3.75	-4.80	-1.45 *	-4.86
		(1.25)	(1.64)	(1.03)	(1.26)
	Speed	-17.4	33.6	8.9	10.8
		(27.3)	(17.1)	(18.3)	(23.1)
	Target	-65.6	36.3	18.7	26.8
	Reaction	(64.3)	(18.8)	(19.7)	(22.0)
	Time				
	Distractor	20.8	46.3	0.7	-3.3
	Reaction	(23.0)	(22.7)	(25.0)	(32.5)
	Time				
Moderate[2]	Accuracy	-3.93	-3.54	-2.58*	-5.91
		(1.03)	(1.50)	(.97)	(1.47)
	Speed	-32.4	4.0	-11.8	-4.7
		(22.4)	(20.4)	(12.2)	(13.5)
	Target	25.9	13.3	11.1	-8.9
	Reaction	(23.3)	(23.2)	(21.3)	(17.9)
	Time				
	Distractor	35.2	5.3	17.5	3.2
	Reaction	(24.8)	(26.8)	(17.6)	(16.1)
	Time				

[1] Change from baseline; covariates appearing in the model for low dose treatments are evaluated at the following average baseline values: Picture Recognition Accuracy = 93.31; Picture Recognition Speed = 772.6; Picture Recognition Target Reaction Time = 768.0; Picture Recognition Distractor Reaction Time = 792.6.

[2] Change from baseline; covariates appearing in the model for moderate dose treatments are evaluated at the following average baseline values: Picture Recognition Accuracy = 91.54; Picture Recognition Speed = 780.9, Picture Recognition Target Reaction Time = 778.3; Picture Recognition Distractor Reaction Time = 802.0.

*Significant compared to placebo at $p < .05$

Working Memory

Serial 3's Correct

There was no significant main effect of the 'low dose' treatments on the correct responses on the Serial 3 task, F (3, 32.19) = 0.70, p = .56. There was no main effect of period, F (3, 37.41) = 0.07, p = .98; or interaction between treatment and period, F (9, 35.06) = 1.19, p = .33. There were no significant differences observed following administration of treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on the correct responses on the Serial 3 task, F (3, 35.99) = 1.06, p = .38. There was no main effect of period, F (3, 33.59) = 1.40, p = .26; or treatment x period interaction, F (9, 29.08) = 0.20, p =. 99. There were no significant differences observed following administration of treatment drinks compared to placebo,

Serial 3's Errors

There was no significant main effect of the 'low dose' treatments on the erroneous responses on the Serial 3 task, F (3, 37.06) = 0.85, p = .48. There was no main effect of period, F (3, 43.41) = 0.51, p = .68; or interaction between treatment and period, F (9,

30.69) = 1.22, p = .32. There were no significant differences observed following administration of treatment drinks compared to placebo.

For the 'moderate dose' treatments no significant effect of treatment, F (3, 37.53) = 0.59, p = .62, period, F (3, 35.69) = 0.70, p = .56; or interaction between treatment and period, F (9, 34.47) = 1.03, p = .44 were observed for number of errors. In addition, there were no significant differences following administration of treatment drinks compared to placebo.

Serial 7's Correct

There was no significant main effect of the 'low dose' treatments on the correct responses on the Serial 7 task, F (3, 48.03) = 0.74, p = .54. There was no main effect of period, F (3, 43.52) = 0.85, p = .48; or interaction, F (9, 26.50) = 1.49, p = .20. There were no significant differences observed following administration of treatment drinks compared to placebo.

For the 'moderate dose' group, no significant effect of treatment, F (3, 26.05) = 0.184, p = .91, period, F (3, 31.90) = 0.85, p = .48; or treatment x period interaction, F (9, 21.12) = 0.91, p = .53 were observed on the correct responses on the Serial 7 task. Moreover, no significant differences following administration of treatment drinks compared to placebo were evident.

Serial 7's Errors

There was no significant main effect of the 'low dose' treatments on the error responses on the Serial 7 task, F (3, 28.13) = 0.85, p = .48. There was no main effect of period, F (3,

40.46) = 0.48, p = .70; or interaction between treatment and period, F (9, 44.83) = 0.28, p = .98. There were no significant differences observed following administration of active treatment drinks compared to placebo.

The main effect of the 'moderate dose' treatments on the error responses on the Serial 7 task failed to reach significance, F (3, 29.85) = 2.54, p = .09. There was no main effect of period, F (3, 28.23) = 0.92, p = .45; or treatment x period interaction, F (9, 22.02) = 0.66, p = .74. There were no significant differences following administration of active treatment drinks compared to placebo.

Corsi Block Span Score

There was no significant main effect of the 'low dose' treatments on the span score on the Corsi Block task, F (3, 34.38) = 0.31, p = .82. There was no main effect of period, F (3, 32.27) = 0.01, p = .99; or interaction between treatment and period, F (9, 29.15) = 1.65, p = .15.

For 'moderate dose' no effect of treatment, F (3, 35.52) = 0.08, p = .97, period, F (3, 32.10) = 0.85, p = .48, or interaction between those factors, F (9, 28.50) =0. 95, p =.50 was observed.

Moreover, there were no significant performance differences following administration of any of the active treatment drinks in either of the dosing regimens compared to placebo.

Corsi Block Reaction Time

There was no significant main effect of the 'low dose' treatments on the reaction time score on the Corsi Block task, F (3, 32.55) = 1.30, p = .29. There was no main effect of period, F (3, 37.44) = 0.61, p = .62; but a significant period x treatment interaction, F (9, 24.84) = 2.59, p = .03, with performance following the combined drink on visit 4 better than combined drink on visit 3, p = .04. There were no significant differences observed following administration of active treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on the reaction time score on the Corsi Block task, F (3, 32.788) = 0.09, p = .97. The main effect of period did not reach significance, F (3, 31.64) = 2.26, p = .10, and the interaction between treatment and period was not significant, F (9, 25.76) = 0.72, p = .70. There were no significant differences observed following administration of treatment drinks compared to placebo. (See Table 2.7 for the Working Memory Performance, means and standard errors).

Table 2.8 Working Memory Performance, means and standard errors

Treatment		Combined	Glucose	Caffeine	Placebo
Low[1]	Serial 3's	4.33	2.27	1.53	2.08
	correct	(1.64)	(1.36)	(1.15)	(1.12)
	Serial 3's	0.61	1.19	0.42	(-.03)
	error	(0.43)	(0.61)	(0.40)	0.50
	Serial 7's	1.50	-0.48	0.65	1.33
	correct	(0.98)	(1.09)	(0.73)	(1.01)
	Serial 7s	-0.38	0.99	0.67	0.86
	error	(0.71)	(0.55)	(0.33)	(0.89)
	Corsi Block	-0.19	0.05	(-0.05)	-0.16
	span	(0.21)	(0.19)	(0.16)	(0.24)
	Corsi Block	726.8	-150.5	-118.3	-477.8
	RT	(556.5)	(270.3)	(323.2)	(271.0)

Moderate[2]	Serial 3's	3.00	3.20	1.94	0.99
	correct	(1.35)	(1.45)	(1.23)	(0.85)
	Serial 3's	1.14	0.36	0.45	0.16
	error	(0.61)	(0.70)	(0.86)	(0.42)
	Serial 7's	1.40	0.60	0.73	1.32
	correct	(0.93)	(0.97)	(0.88)	(1.04)
	Serial 7s	0.54	0.48	0.80	-0.86
	error	(0.55)	(0.61)	(0.59)	(0.47)
	Corsi Block	0.03	-0.06	-0.05	-0.06
	span	(0.16)	(0.23)	(0.12)	(0.14)
	Corsi Block	-338.8	-308.8	-490.5	-358.5
	RT	(290.2)	(326.5)	(259.8)	(171.3)

[1] Change from baseline; covariates appearing in the model for low dose treatments are evaluated at the following average baseline values: Serial 3s Correct = 44.18; Serial 3s Errors = 2.90.; Serial 7s Correct = 27.32; Serial 7s Errors = 2.97; Corsi Span Score = 6.21; Corsi Block RT = 5524.5.

[2] Change from baseline; covariates appearing in the model for moderate dose treatments are evaluated at the following average baseline values: Serial 3s Correct = 44.71; Serial 3s Errors = 3.04; Serial 7s Correct = 27.06; Serial 7s Errors = 3.21; Corsi Span Score = 6.18; Corsi Block RT = 5333.2.

*Significant compared to placebo at $p < .05$

2.3.2.4 Secondary Outcomes: Mood Results

Bond-Lader Visual Analogue Scales

Alert

There was no significant main effect of the 'low dose' treatments on self-ratings on the Alert scale of the Bond-Lader Visual Analogue Scales, F (3, 36.87) = 1.93, p = .14. There was a significant main effect of period, F (3, 77.09) = 2.92, p = .04, with participants reporting to be less alert at period 1 compared to their other visits (p = .06). However,

there was no significant interaction between treatment and period, F (9, 61.08) = 1.06, p = .40. Moreover, no significant differences were observed following administration of active treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on self-rated alertness, F (3, 59.57) = 1.95, p = .13; or period, F (3, 84.56) = 1.05, p = .37. There was a significant treatment x period interaction, F (9, 64.11) = 2.53, p = .02. Participants rated themselves as significantly more alert on study day 4 (period 4) following the combined drink, compared to on study day 1 (period 1) (p = .004) and study day 3 (period 3) (p = .002). Participants also rated themselves as significantly more alert on study day 4 (period 4) following the caffeine drink compared to on study day 1 (period 1), p = .007. Comparisons of the active drinks to placebo revealed that participants rated themselves as significantly more alert following the caffeine treatment drink (M = 4.11) and the combination treatment (M = 6.55), compared to placebo (M = 0.38), p = .04 and p = .001, respectively.

Content

There was no significant main effect of the 'low dose' treatments on the self-ratings on the Content scale of the Bond-Lader Visual Analogue Scales, F (3, 48.76) = 0.14, p = .94; or of period, F (3, 105.26) = 1.04, p = .38; or treatment by period interaction, F (9, 52.15) = 0.88, p = .55.

For the 'moderate dose' group, no significant effect of treatment, F (3, 57.05) = 0.27, p = .84; period, F (3, 92.55) = 0.67, p = .58; or treatment by period interaction, F (9, 59.15) = 1.09, p = .39 was observed for level of contentedness

In addition, no significant differences were observed following administration of active treatment drinks compared to placebo for either of the dosing regimens.

Calm

There was no significant main effect of the 'low dose' treatments on the self-ratings on the Calm scale of the Bond-Lader Visual Analogue Scales, F (3, 46.27) = 0.58, p = .63; period, F (3, 70.67) = 0.96, p = .42; or their interaction, F (9, 58.61) = 0.94, p = .50.

There was no significant main effect of the 'moderate dose' treatments , F (3, 49.94) = 0.62, p = .60; period, F (3, 101.48) = 1.97, p = .12; or treatment by period interaction, F (9, 62.10) = 1.43, p = .20 on this measure. No significant differences in level of self-reported calmness were observed following administration of active treatment drinks compared to placebo for either dosing regimens (see table 2.9 for Bond-Lader Mood ratings, means and standard errors).

Table 2.9 Bond-Lader Mood ratings, means and standard errors

Treatment		Combined	Glucose	Caffeine	Placebo
Low[1]	Alert	2.2	4.6	8.0	4.5
		(19.8)	(19.8)	(19.8)	(19.8)
	Content	1.5	0.8	2.2	1.5
		(1.7)	(1.5)	(1.7)	(1.7)
	Calm	-5.2	-5.4	-6.4	-3.0
		(4.2)	(4.3)	(4.5)	(4.1)
Moderate[2]	Alert	6.6 *	2.1	4.1 *	0.4
		(1.8)	(3.1)	(2.3)	(1.9)
	Content	-1.5	0.9	-0.9	-1.2
		(1.6)	(2.3)	(1.8)	(1.4)
	Calm	-11.4	-9.6	-8.0	-6.7

	(2.7)	(2.7)	(2.5)	(2.4)

[1] Change from baseline; covariates appearing in the model for low dose treatments are evaluated at the following average baseline values: Bond Lader Alert = 46.2; Bond Lader Content = 55.9; Bond Lader Calm = 60.7.

[2]Change from baseline; covariates appearing in the model are evaluated at the following values: Bond Lader Alert = 47.3; Bond Lader Content = 55.7; Bond Lader Calm = 63.0.

*Significant compared to placebo at $p < .05$

Mood, Alertness and Physical Symptoms Questionnaire (MAPS)

Headache

There was no significant main effect of the 'low dose' treatments on the self-ratings of headache on the MAPS, F (3, 47.33) = 0.58, p = .63; or period, F (3, 87.34) = 1.27, p = .29; or their interaction, F (9, 47.67) = 1.17, p = .33. There were no significant differences observed following administration of treatment drinks compared to placebo.

The main effect of the 'moderate dose' treatments on the self-ratings of headache on the MAPS did not reach significance, F (3, 47.18) = 2.27, p = .09. There was no main effect of period, F (3, 98.03) = 0.99, p = .40; or interaction between treatment and period, F (9, 48.98) = 0.85, p = .57. There were no significant differences observed following administration of active treatment drinks compared to placebo.

Heart Pound

There was no significant main effect of the 'low dose' treatments on the self-ratings of heart pounding on the MAPS, F (3, 52.80) = 0.31, p = .82; or period, F (3, 104.79) = 2.42, p = .07; or their interaction, F (9, 53.78) = 0.98, p = .47.

There was no significant main effect of the 'moderate dose' treatments on the self-ratings of heart pounding on the MAPS, F (3, 50.24) = 0.05, p = .99; or period, F (3, 101.34) = 0.87, p = .46; or their interaction, F (9, 58.65) = 1.14, p = .35.

There were no significant differences observed following administration of active treatment drinks compared to placebo for either of the dosing regimens.

Jittery/Shaky

There was no significant main effect of the 'low dose' treatments on the self-ratings of jitter/shaky on the MAPS, F (3, 57.68) = 0.36, p = .78; period, F (3, 94.43) = 1.24, p = .30; or interaction, F (9, 58.34) = 0.75, p = .66.

There was no significant main effect of the 'moderate dose' treatments on the self-ratings of jitter/shaky on the MAPS, F (3, 50.61) = 0.45, p = .72; or period, F (3, 99.58) = 0.99, p = .40; or their interaction, F (9, 53.40) = 0.80, p = .62.

There were no significant differences observed following administration of active treatment drinks compared to placebo for either of the dosing regimens.

Light-headed/feeling faint/dizzy

There was no significant main effect of the 'low dose' treatments on the self-ratings of light-headed/feeling faint/dizzy on the MAPS, F (3, 46.75) = 0.60, p = .62; or period, F (3,

93.98) = 0.25, p = .86; or their interaction, F (9, 48.33) = 0.76, p = .66. There were no significant differences observed following administration of treatment drinks compared to placebo.

There was a significant main effect of the 'moderate dose' treatments on the self-ratings of light-headed/feeling faint/dizzy on the MAPS, F (3, 48.14) = 4.20, p = .01. There was no main effect of period, F (3, 100.64) = 0.08, p = .97; or their interaction, F (9, 53.45) = 1.47, p = .18. There were no significant comparisons with placebo.

Hands-trembling

There was no significant main effect of the 'low dose' treatments on the self-ratings of hands-trembling on the MAPS, F (3, 98.88) = 0.38, p = .47; or period, F (3, 98.88) = 0.38, p = .77; or their interaction, F (9, 52.92) = 1.15, p = .35. There were no significant differences observed following administration of treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on the self-ratings of hands-trembling on the MAPS, F (3, 50.05) = 0.85, p = .48; or period, F (3, 97.91) = 0.25, p = .86; or their interaction, F (9, 54.14) = 0.72, p = .69. There were also no significant differences observed following administration of treatment drinks compared to placebo.

Scared

There was no significant main effect of the ‘low dose’ treatments on the self-ratings of scared on the MAPS, F (3, 59.88) = 0.35, p = .79; or period, F (3, 93.73) = 0.68, p = .57; or their interaction, F (9, 62.24) = 0.86, p = .56.

There was no significant main effect of the ‘moderate dose’ treatments on the self-ratings of scared on the MAPS, F (3, 44.80) = 0.42, p = .74; or period, F (3, 85.67) = 0.16, p = .92; or their interaction, F (9, 45.87) = 1.14, p = .36.

There were no significant differences observed following administration of treatment drinks compared to placebo for either of the dosing regimens.

Feeling hot/sweating (not due to heat)

There was no significant main effect of the ‘low dose’ treatments on the self-ratings of feeling hot/sweating on the MAPS, F (3, 54.61) = 1.30, p = .29; or period, F (3, 97.25) = 0.54, p = .66; or their interaction, F (9, 55.29) = 0.83, p = .59.

There was no significant main effect of the ‘moderate dose’ treatments on the self-ratings of feeling hot/sweating on the MAPS, F (3, 38.81) = 0.30, p = .82; or period, F (3, 72.03) = 0.09, p = .97; or their interaction, F (9, 55.24) = 0.79, p = .68.

There were no significant differences observed following administration of treatment drinks compared to placebo for either of the dosing regimens.

Relaxed

There was no significant main effect of the ‘low dose’ treatments on the self-ratings of relaxed on the MAPS, F (3, 54.51) = 0.54, p = .66. There was a significant main effect of

period, F (3, 96.58) = 3.26, p = .03, participants rated themselves as least relaxed at their first visit (period 1), p = .02. There was no significant interaction between treatment and period, F (9, 61.82) = 0.33, p = .96.

There was no significant main effect of the 'moderate dose' treatments on the self-ratings of relaxed on the MAPS, F (3, 53.04) = 0.62, p = .61; or period, F (3, 96.97) = 2.59, p = .06. There was no significant interaction between treatment and period, F (9, 55.93) = 0.28, p = .98.

There were no significant differences observed following administration of treatment drinks compared to placebo for either of the dosing regimens.

Clearheaded

There was no significant main effect of low dose' treatments on the self-ratings of clearheaded on the MAPS, F (3, 53.75) = 1.24, p = .31. There was a significant main effect of period, F (3, 101.35) = 3.63, p = .02, participants rated themselves as least clearheaded at their first and last visit (period 1 and 4) although these differences were not significant, p = .06 and p = .09 respectively. There was no significant interaction effect between treatment and period, F (9, 55.22) = 1.02, p = .43.

There was no significant main effect of the 'moderate dose' treatments on the self-ratings of clearheaded on the MAPS, F (3, 52.66) = 0.73, p = .54; or period, F (3, 98.79) = 0.72, p = .54; or treatment x period, F (9, 56.19) = 1.84, p = .08.

There were no significant differences observed following administration of treatment drinks compared to placebo for either of the dosing regimens.

Happy

There was no significant main effect of the 'low dose' treatments on the self-ratings of feeling happy on the MAPS, F (3, 50.98) = 0.87, p = .46; or period, F (3, 106.48) = 1.58, p = .20; or their interaction, F (9, 58.84) = 1.03, p = .43.

There was no significant main effect of the 'moderate dose' treatments on the self-ratings of happy on the MAPS, F (3, 58.12) = 0.14, p = .93; or period, F (3, 90.28) = 0.53, p = .60; or their interaction, F (9, 59.94) = 0.84, p = .58.

There were no significant differences observed following administration of treatment drinks compared to placebo for either of the dosing regimens.

Alert

There was no significant main effect of the 'low dose' treatments on the self-ratings of alert on the MAPS, F (3, 52.99) = 0.60, p = .62; or period, F (3, 103.97) = 2.58, p = .06; or their interaction, F (9, 56.82) = 1.17, p = .33.

There was no significant main effect of the 'moderate dose' treatments on the self-ratings of alert on the MAPS, F (3, 53.68) = 1.20, p = .32; or period, F (3, 96.65) = 1.15, p = .33; or their interaction, F (9, 56.93) = 1.85, p = .08.

There were no significant differences observed following administration of treatment drinks compared to placebo for either of the dosing regimens. (See Table 2.10 for Mood, Alertness and Physical Symptoms Questionnaire, means and standard errors).

Table 2.10 Mood, Alertness and Physical Symptoms Questionnaire, means and standard errors

Treatment		Combined	Glucose	Caffeine	Placebo
Low[1]	Headache	0.4	-2.5	-3.1	0.2
		(1.7)	(3.0)	(2.5)	(3.5)
	Heart pound	5.7	4.2	4.1	7.0
		(2.9)	(2.5)	(2.7)	(2.4)
	Jittery/Shaky	3.7	4.6	7.8	6.0
		(2.3)	(3.6)	(3.5)	(2.6)
	Light-headed/feeling faint/dizzy	1.9 (2.4)	-2.5 (3.0)	0.7 (3.0)	-2.3 (3.9)
	Hands-trembling	5.4	3.8	6.5	0.4
		(2.1)	(3.2)	(2.6)	(3.1)
	Scared	1.6	2.9	1.6	0.2
		(2.3)	(2.5)	(2.5)	(1.3)
	Feeling hot/sweating	3.3 (2.0)	-1.1 (1.4)	1.3 (1.6)	1.6 (1.5)
	Relaxed	-2.1	-6.7	-2.8	-2.1
		(2.2)	(3.3)	(3.0)	(2.3)
	Clearheaded	-1.2	5.5	5.4	5.9
		(3.2)	(2.6)	(2.7)	(2.4)
	Happy	-0.7	4.3	3.1	2.3
		(2.3)	(2.2)	(2.4)	(2.3)
	Alert	4.7	7.0	10.6	8.3
		(3.0)	(2.9)	(3.4)	(2.8)
Moderate[2]	Headache	2.9	-5.1	5.9	3.5
		(3.4)	(3.0)	(3.7)	(4.0)
	Heart pound	5.4	6.3	7.5	6.6
		(3.8)	(3.6)	(4.0)	(3.5)
	Jittery/Shaky	12.7	9.8	14.1	7.4
		(4.6)	(3.7)	(4.6)	(4.3)

Light-headed/feeling faint/dizzy	-6.5 (3.3)	2.9 (3.4)	1.4 (3.9)	10.9 (3.7)
Hands-trembling	8.1 (4.1)	3.2 (3.0)	8.5 (3.7)	10.2 (3.8)
Scared	2.9 (3.1)	4.4 (1.8)	2.6 (2.4)	0.4 (3.4)
Feeling hot/sweating	2.1 (4.0)	0.8 (4.2)	-0.0 (3.6)	1.2 (4.3)
Relaxed	-11.4 (3.4)	-6.9 (2.6)	-11.9 (3.2)	-9.6 (2.6)
Clearheaded	5.3 (3.2)	-1.0 (3.6)	-0.1 (3.6)	0.5 (2.8)
Happy	-1.0 (2.6)	-1.2 (3.5)	0.7 (2.6)	0.7 (2.2)
Alert	8.1 (3.3)	5.7 (4.2)	6.7 (3.9)	0.1 (3.1)

[1] Change from baseline; covariates appearing in the model for low dose treatments are evaluated at the following average baseline values: Headache = 19.7; Heart pound = 15.8; Jittery/shaky = 18.0; Light-headed/feeling faint/dizzy = 23.2; Hands trembling = 16.3; Scared = 14.5; Feeling hot/sweating (not due to heat) = 13.8; Relaxed = 57.9; Clearheaded = 42.3; Happy = 51.2; Alert = 39.0.

[2]Change from baseline; covariates appearing in the model are evaluated at the following values: Headache = 21.6; Heart pound = 17.7; Jittery/shaky = 18.7; Baseline Light-headed/feeling faint/dizzy = 25.9; Hands trembling = 18.2; Scared = 12.1; Feeling hot/sweating (not due to heat) = 10.7; Relaxed = 60.1; Clearheaded = 44.6; Happy = 52.3; Alert = 41.8.

*Significant compared to placebo at $p < .05$

The Stress Arousal Checklist (SACL)

Stress

There was no significant main effect of the ‘low dose’ treatments on the self-ratings of stress on the SACL, F (3, 59.25) = 0.53, p = .67; or period, F (3, 96.18) = 0.35, p = .79; or their interaction, F (9, 62.65) = 0.290, p = .98.

There was no significant main effect of the ‘moderate dose’ treatments on the self-ratings of stress on the SACL, F (3, 53.30) = 0.15, p = .93; or period, F (3, 95.50) = 0.46, p = .71; or their interaction, F (9, 56.45) = 0.36, p = .95.

There were no significant differences observed following administration of active treatment drinks compared to placebo for either of the dosing regimens.

Arousal

There was no significant main effect of the ‘low dose’ treatments on the self-ratings of arousal on the SACL, F (3, 41.08) = 1.08, p = .37; or period, F (3, 74.20) = 1.94, p = .13; or their interaction, F (9, 56.25) = 1.16, p = .34. There were no significant differences observed following administration of treatment drinks compared to placebo.

There was no significant main effect of the ‘moderate dose’ treatments on the self-ratings of arousal on the SACL, F (3, 38.26) = 0.63, p = .60; or period, F (3, 75.70) = 1.74, p = .17. However, there was a significant interaction effect between treatment and period, F (9, 49.29) = 3.29, p = .003. Participants rated themselves as significantly less aroused on study day 3 (period 3) following the combined drink compared to on study day 4 (period 4), p = .02. Participants also rated themselves as less aroused following the caffeine drink on their first study day (period 1) compared to after the caffeine drink on study day 4, p = .023.There were no significant differences observed following administration of treatment

drinks compared to placebo.. (See Table 2.11 for the Stress Arousal Checklist (SACL), means and standard deviations).

2.11 The Stress Arousal Checklist (SACL), means and standard errors

Treatment		Combined	Glucose	Caffeine	Placebo
Low[1]	Stress	-0.76	-0.95	-0.64	0.06
		(0.82)	(0.85)	(0.80)	(0.50)
	Arousal	1.12	2.68	3.17	1.69
		(2.28)	(2.20)	(2.26)	(2.24)
Moderate[2]	Stress	-1.58	-1.44	-1.05	-1.77
		(0.72)	(0.68)	(0.89)	(0.64)
	Arousal	2.96	2.43	3.39	2.15
		(1.12)	(1.14)	(1.00)	(0.90)

[1] Change from baseline; covariates appearing in the model for low dose treatments are evaluated at the following average baseline values: SACL Stress = 14.23; SACL Arousal = 4.53.

[2]Change from baseline; covariates appearing in the model are evaluated at the following values: SACL Stress = 14.43; SACL Arousal = 4.27.

*Significant compared to placebo at $p < .05$

The Activation-Deactivation Adjective Checklist (AD ACL)

Energy

There was no significant main effect of the 'low dose' treatments on the self-ratings of energy on the AD ACL, F (3, 37.29) = 1.16, p = .34; or period, F (3, 76.92) = 0.64, p =

.59; or their interaction, F (9, 59.09) = 0.90, p = .53. There were no significant differences observed following administration of treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on the self-ratings of energy on the AD ACL, F (3, 54.78) = 0.42, p = .74; or period, F (3, 95.37) = 0.10, p = .96; or their interaction, F (9, 56.92) = 1.09, p = .39. There were no significant differences observed following administration of treatment drinks compared to placebo.

Tired

There was no significant main effect of the 'low dose' treatments on the self-ratings of tiredness on the AD ACL, F (3, 53.23) = 0.13, p = .94; or period, F (3, 103.76) = 0.87, p = .46. There was a significant interaction effect between treatment and period, F (9, 54.64) = 2.19, p = .04. Participants rated themselves as significantly more tired following the glucose treatment on study day 3 (period 3) compared to after the glucose on study day 4 (period 4), p = .031. There were no significant differences observed following administration of active treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on the self-ratings of tired on the AD ACL, F (3, 53.40) = 0.61, p = .61; or period, F (3, 98.16) = 0.30, p = .83; or their interaction, F (9, 57.27) = 0.73, p = .677. There were no significant differences observed following administration of active treatment drinks compared to placebo.

Tension

There was no significant main effect of the ‘low dose’ treatments on the self-ratings of tension on the AD ACL, F (3, 55.20) = 1.27, p = .29; or period, F (3, 100.67) = 0.60, p = .62; or their interaction, F (9, 60.61) = 0.36, p = .95. There were no significant differences observed following administration of active treatment drinks compared to placebo.

There was no significant main effect of the ‘moderate dose’ treatments on the self-ratings of tension on the AD ACL, F (3, 50.78) = 0.43, p = .73; or period, F (3, 96.45) = 1.45, p = .23. There was a significant interaction effect between treatment and period, F (9, 53.27) = 4.74, p < .001. Following the combination treatment participants rated themselves as significantly more tense on study day 4 (period 4), compared to on study day 1 (period 1), 2 (period 2) and 3 (period 3); p = .004, p = .012 and p = .001 respectively. Participants also rated themselves as significantly more tense following the caffeine drink on study day 2 (period 2), compared to on study day 4 (period 4), p = .038. Comparisons of the active drinks compared to placebo showed participants rated themselves as significantly more tense following the combination drink (M = 1.71) compared to after the placebo drink (M = 1.38), p = .002.

Calmness

There was no significant main effect of the ‘low dose’ treatments on the self-ratings of calmness on the AD ACL, F (3, 53.85) = 1.35, p = .27; or period, F (3, 103.83) = 0.15, p = .931; or their interaction, F (9, 57.56) = 0.50, p = .87. There were no significant differences observed following administration of active treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on the self-ratings of calmness on the AD ACL, F (3, 52.93) = 0.93, p = .43; or period, F (3, 93.01) = 0.78, p = .52. There was a significant interaction effect between treatment and visit, F (9, 56.19) = 3.12, p = .004. Following the combination treatment participants rated themselves as significantly calmer at study day 1 (p = .022); study day 2 (p = .035) and study day 3 (p = .006) compared to on study day 4. After consumption of the glucose drink on study day 3 participants rated themselves as calmer compared to after the glucose drink on study day 4, although this did not reach significance, p = .059. On study day 2 participants rated themselves as significantly less calm following consumption of the caffeine drink compared to on study day 4, p = .046. Comparison with placebo showed that participants rated themselves as significantly calmer following the combination drink (M = -1.13) compared to after the placebo treatment drink (M = -1.91), p = .04.

Energetic Arousal

There was no significant main effect of the 'low dose' treatments on the self-ratings of energetic arousal on the AD ACL, F (3, 53.92) = 0.30, p = .83; or period, F (3, 102.62) = 0.91, p = .44. The interaction effect between treatment and period did not reach significance, F (9, 55.45) = 1.85, p = .08.

There was no significant main effect of the 'moderate dose' treatments on the self-ratings of energetic arousal on the AD ACL, F (3, 54.56) = 0.58, p = .63; or period, F (3, 96.52) = 0.19, p = .91; or their interaction, F (9, 58.80) = 0.87, p = .56.

There were no significant differences observed following administration of active treatment drinks compared to placebo for either of the dosing regimens.

Tense Arousal

There was no significant main effect of the 'low dose' treatments on the self-ratings that make up the tense arousal subscale on the AD ACL, F (3, 57.08) = 1.43, p = .24; or period, F (3, 97.89) = 0.31, p = .82; or their interaction, F (9, 59.64) = 0.25, p = .99. There were no significant differences observed following administration of active treatment drinks compared to placebo.

There was no significant main effect of the 'moderate dose' treatments on the self-ratings that make up the tense arousal subscale on the AD ACL, F (3, 47.76) = 0.62, p = .61; or period, F (3, 96.76) = 1.12, p = .35. There was a significant treatment by period interaction, F (9, 50.73) = 4.82, $p < .001$. Participants' tense arousal scores were significantly lower after the combination drink on study days 1, 2, and 3, compared to on study day 4 (p = .006; p = .01; p = .001 respectively). After the glucose drink on study day 3 participants' scores were significantly compared to on study day 4. Participants' tense arousal scores were significantly higher following the caffeine drink on study day 2 compared to on study day 4, p = .02. Comparisons of the means found participants' tense arousal scores were significantly lower following the combination drink (M = 2.83) compared to after the placebo drink (M = 3.278), p = .005. (See Table 2.12 for the Activation-Deactivation Adjective Checklist (AD ACL), means and standard errors).

Table 2.12. The Activation-Deactivation Adjective Checklist (AD ACL), means and standard errors

Treatment		Combined	Glucose	Caffeine	Placebo
Low[1]	Energy	0.89	2.52	1.56	1.61

		(1.15)	(1.13)	(1.08)	(1.12)
	Tired	-1.82	-1.96	-2.15	-1.56
		(0.85)	(0.76)	(0.72)	(0.67)
	Tension	0.79	1.78	0.48	0.35
		(0.50)	(0.59)	(0.66)	(0.50)
	Calmness	-0.42	-2.08	-1.31	-0.71
		(0.61)	(0.67)	(0.64)	(0.52)
	Energetic	2.70	4.49	3.71	3.34
	Arousal	(1.49)	(1.29)	(1.20)	(1.16)
	Tense Arousal	1.21	3.86	1.80	1.06
		(0.84)	(1.16)	(1.23)	(0.91)
Moderate[2]	Energy	2.07	1.61	2.49	1.45
		(0.90)	(0.91)	(0.76)	(0.62)
	Tired	-2.10	-2.39	-2.92	-1.36
		(0.87)	(0.96)	(0.98)	(0.73)
	Tension	1.71 *	1.52	2.20	1.38
		(0.56)	(0.40)	(0.57)	(0.55)
	Calmness	-1.13 *	-2.38	-2.39	-1.91
		(0.65)	(0.74)	(0.46)	(0.57)
	Energetic	4.17	4.00	5.41	2.80
	Arousal	(1.71)	(1.78)	(1.59)	(1.22)
	Tense Arousal	2.83 *	3.89	4.58	3.28
		(1.16)	(0.94)	(0.83)	(1.02)

[1] Change from baseline; covariates appearing in the model for low dose treatments are evaluated at the following average baseline values: AD ACL Energy = 8.10; AD ACL Tired = 14.98; AD ACL Tension = 6.94; AD ACL Calmness = 13.31; AD ACL Energetic Arousal = 18.08; AD ACL Tense Arousal = 18.63.

[2]Change from baseline; covariates appearing in the model are evaluated at the following values: AD ACL Energy = 8.01; AD ACL Tired = 14.88; AD ACL Tension = 6.64; AD ACL Calmness = 13.73; AD ACL Energetic Arousal = 18.13; AD ACL Tense Arousal = 17.92.

*Significant compared to placebo at $p < .05$

2.4 Discussion

Although glucose and caffeine are both widely consumed, few studies have looked at the effects on cognitive performance and mood when they are administered in combination. Moreover, one of the major limitations of previous research is the lack of dose-response investigations and in particular assessment of the lowest efficacy range. Consequently, the aim of this study was to investigate the effects of a 'low' dose (15g glucose and 20mg caffeine), and a 'moderate' dose of glucose and caffeine (25g glucose and 40mg caffeine), in isolation and in combination compared to placebo on cognitive performance, mood and hormonal response in healthy young adults. In terms of the glycaemic response, the findings were as expected and showed that blood glucose levels were significantly higher following the glucose and glucose and caffeine combination drinks compared to the placebo and caffeine drinks, for both the low and moderate dosing regimens.

Evidence for improved performance following any of the active drinks in either dosing regimen ('low' and 'moderate') was limited across all cognitive domains. The primary outcome variable of interest was the effects on attention as measured by the accuracy on the Digit Vigilance task. This was because of the weight of previous evidence that suggested that caffeine and glucose have a beneficial effect on attention. However no effects were found on accuracy on the Digit Vigilance task following any of the treatment drinks. There were no improvements on any measures of attention following any of the active drinks..

There was some evidence for improvement of performance in tasks assessing declarative long-term memory performance. More specifically, participants' accuracy in recognising previously presented words was greater after ingestion of the moderate combination drink compared to placebo. When looking at speed of recognition, both the 'moderate'

combination drink led to participants recognising words faster, whereas for the low dose group, faster performance was seen after administration of 15g of glucose in isolation. A different effect profile was observed for picture recognition, where both 20mg and 40mg of caffeine led to reduced performance decrements compared to placebo. Beneficial effects on working memory were not observed. None of the comparisons between active treatments and placebo were significant.

In terms of mood, the effects were exclusively limited to the moderate dose regimen. The 40mg caffeine drink led to participants rating themselves as more alert. Following the moderate combination drink participants rated themselves as significantly more alert, calmer and had lower overall tense arousal scores. In addition, after consumption of the moderate combination drink, participants rated themselves as feeling less light-headed/dizzy/faint, reported higher levels of tension compared to the placebo drink. There were no effects of the low dose active drinks on any of the mood measures.

There were no effects of the active drinks on any of the measures of attention, and therefore offers no support for previous research findings (Adan & Serra-Grabulosa, 2010; Brice & Smith, 2001a; Chubley, *et al.*, 1979; Gershon, *et al.*, 2009; Howard & Marczinski, 2010; Kennedy & Scholey, 2004; Mets, *et al.*, 2010; Mucignat-Caretta, 1998; Scholey & Kennedy 2004; Smit & Rogers, 2002; Smith, *et al.*, 1992; Smith, *et al.*, 1994; Smith, *et al.*, 1997). Previously for example,Howard and Marczinski (2010) found that reaction time was decreased on a cued go-no-go task after consumption of the 'Red Bull' energy drink. The lowest dose had the greatest effect; this was the equivalent to 45.6mg caffeine and 5g glucose, which in caffeine content is closest to the moderate dose treatments in this study. However as a) both caffeine in isolation and in combination with glucose improved performance and b) the glucose dose was rather small, it is likely that the benefits were driven by caffeine rather than a synergistic effect between glucose and caffeine. The

failure to observe clearer effects on measures of attention in our study, might be due to the nature of the attention tasks employed as they were possibly not 'sustained' enough, most only lasted a few minutes. A longer duration may be necessary in order to see the effects of caffeine in otherwise healthy and well-rested participants. For example Kennedy & Scholey (2004), found that three active combination drinks (68g glucose and 38mg caffeine; 68g glucose and 46mg caffeine; 60g glucose and 33mg caffeine) all improved performance on a rapid visual information processing (RVIP) task. However, this was only evident later in the task after over 30 mins of a demanding cognitive battery. Indeed, the mere act of switching to a different task could be enough to raise attention levels regardless of the treatment consumed (Einother & Giesbrecht, 2013).

The limited beneficial effects found following 15g of glucose could be explained as a result of sub-optimal dosing given that 25g has previously been identified as the most effective in improving verbal declarative memory (Messier, Pierre, Desrochers & Gravel, 1998; Sünram-Lea, Owen, Finnegan and Hu, 2011). However, the failure to observe any beneficial effects of 25g of glucose is unexpected and more difficult to explain. As mentioned earlier, previous studies have shown that in order to demonstrate glucose facilitation of memory performance tasks need to be sufficiently difficult and/or cognitive resources need to be stretched through administration of a dual task design (Foster, *et al.,* 1998; Sünram-Lea, *et al.,* 2002). Although a dual task paradigm was employed in the current study, since task performance on the secondary hand movement task was not monitored, it might have been the case that participants were not equally dividing their attention between the two tasks, which is important for the effectiveness of the dual-tasking paradigm. However, when administered in combination with 40g of caffeine, an improvement in speed of recognition was observed for verbal material. Although, is in line with previous research that has also found combinations of caffeine and glucose to

positively affect performance on this domain (Alford *et al.*, 2001; Horne & Reyner, 2000), support for any synergistic effect is limited as yet again a different effect profile was observed for picture recognition, where none of the combined drinks enhanced performance. Beneficial effects were only observed following 20mg of caffeine and 40mg of caffeine administered in isolation.

The finding that caffeine improved some aspects of long term memory performance supports previous research that caffeine consumption can lead to memory improvements (Kelemen & Creeley, 2001; Smith, Sturgess & Gallagher, 1999). Smith *et al.*, (1999) found that whilst there was no beneficial effect of caffeine (40mg) on a free recall memory task, it did significantly speed up the response times in a delayed recognition task. Kelman & Creeley (2001) found that caffeine did benefit performance on a free recall task. However, it is important to note that they found that when caffeine was administered on both recall and encoding days, recall was significantly better than when it was just administered on one of the days. This is suggestive of state dependent learning (Overton, 1978) rather than caffeine facilitation *per se*. Moreover, they administered a much higher dose of 4mg/kg; which for their average participant weight of approximately 76kg was 304mg of caffeine. With regards to working memory, previous research has found evidence of improvements in working memory following administration of an energy drink containing 75mg caffeine, 37.5mg glucose, 12.5mg ginseng and 2.004mg ginkgo biloba extract (Scholey & Kennedy, 2004). No significant effect of glucose and caffeine administrated in isolation or in combination were observed in the current study on working memory. However, it may be that the tasks used were not sufficiently difficult or demanding enough to tease out the effects of treatment on working memory performance where participants are otherwise performing at an optimum level, particularly given the short (2min) duration of the task.

Whilst evidence for beneficial effect on cognition was limited across all active treatment drinks, the moderate dosing regimen was relatively effective at augmenting participants' mood. Participants rated themselves as significantly more alert following both 40mg of caffeine in isolation or in combination with 25g of glucose. Moreover, this combination drink increased ratings of tension, which is in line with previous research (Gershon, *et al.,* 2009; Glade, 2010; Haskell, Kennedy, Wesnes & Scholey, 2005; Howard & Marczinski, 2010; Kennedy & Scholey, 2004; Mets *et al.,* 2010; Smith, 2002). Interestingly, participants also rated themselves as significantly calmer and had lower tense arousal scores following the combination drink, suggesting that increased feeling of arousal are seen as positive and not accompanied by negative mood states such as increased agitation. The effects of caffeine are likely to be responsible for the combined drinks' alerting effects (Glade, 2010; Smith, 2002), however glucose has been associated with feeling less tense (Benton & Owens, 1993). Other research has found that an energy drink containing 54g glucose and 30mg caffeine reduced anxiety ratings and increased ratings of 'Cheerfulness' (Smit *et al.,* 2006). The amounts of caffeine and glucose in the moderate dosing regimen are similar to the amounts studied in Smit *et al.,* (2006) research.

In conclusion, whilst some of the findings in this study are supportive of previous research they do not demonstrate clear benefits of glucose and caffeine containing drinks on different aspects of cognition and do not allow a clear picture as to the possible synergistic effects of caffeine and glucose when administered in combination. Not all of the previous literature supports the idea for a synergistic effect of caffeine and glucose above those effects of these ingredients in isolation (Jay *et al.,* 2006; Urquiza & Vieyra, 2015). However, for those studies that have found effects one important common denominator is that these are often carried out on a sample of participants who are performing below their optimal potential, for example after sleep restriction, physical exertion or over long periods

of cognitive demand (Alford *et al.*, 2001; Horne & Reyner, 2001; Kennedy & Scholey, 2004). Horne and Reyner (2001) restricted participants to 5 hours sleep the night before testing them on a driving simulator the following afternoon. They found that after consuming an energy drink containing 42g glucose and 30mg caffeine, there were reduced incidents of lane drifting, a proxy measure for attention, and their reaction time to an auditory beep were significantly improved, compared to the placebo drink. This could also be why no mood effects were found in terms of energy and alertness ratings. Smit *et al.*, (2004) found effects of an energy drink (75mg caffeine, 37.5g glucose), maintained self-rated levels of arousal compared to a decline in these following the placebo treatment. However this was following a fatiguing and cognitively demanding task, where the duration and repetition of the tasks were sustained over an extended period (Smit *et al.*, 2004).

As previously mentioned, the vigilance/attention tasks used in this study were all relatively short. It may be that the constant switching between tasks was enough to increase attention and alertness regardless of the treatment consumed. In a similar vein, previous research suggests that the beneficial effects of glucose are enhanced when the tasks require an element of divided attention or they require a high cognitive demand (Foster, *et al.*, 1998; Kennedy & Scholey, 2000; Sünram-Lea, *et al.*, 2002). In particular this effect is found when looking at the performance of healthy young adults where it is generally considered they are performing at an optimum level to begin with (Messier, 2004; Smith *et al.*, 2011). Although a dual-task paradigm was used in this study for the memory task in the hope that this would help to tease out the effects of glucose administration on its' own and in combination with caffeine, performance on the secondary task was not monitored and therefore it might be the case that participants failed to equally divide their attention.

The data suggest that further research should employ more prolonged and difficult tasks which will hopefully tease out the effects of glucose and caffeine. It may be that the state of the participant is also an important mediator for the effectiveness of caffeine and glucose. The psychoactive properties of these substances might be most effective when participants are already fatigued and their performance and mood is below optimal levels. It may be that rather than *improving* performance, these substances are most useful in ameliorating the decline in performance under sub-optimal conditions. For example, in this study both caffeine drinks reduced the decrements seen on a memory task rather than enhancing performance *per se*. Further research should seek to manipulate the state of participants to fully elucidate this effect. It would also be interesting to look at the physiological effects these substances are having, as it may be a case that the participant are performing at a higher level with less effort, i.e. underlying neuro-physiological processes are more cost effective in order to produce the same behavioural response. For example it has been found that although consumption of a glucose and caffeine combination (75g glucose and 75mg caffeine) led to the same performance on a sustained attention task as the components in isolation, there was a decrease in the bilateral parietal and left prefrontal cortex only after the complete drink (Serra-Grabulosa, Adan, Falcon & Bargallo, 2010). As these areas are related to sustained attention and working memory, this suggests that the combination may be increasing the efficiency of the attentional system (Serra-Grabulosa, *et al.*, 2010).

Chapter 3

Dose response investigation into the effects of low doses of glucose and caffeine on hormonal stress response in healthy volunteers.

3.1 Introduction

The popularity of caffeine as a mild stimulant is in part attributable to its effects in the nervous system (Nehlig, Daval & Debry, 1992), including its ability to increase rates of dopamine release in the anterior cingulate gyrus (Daly & Fredholm, 1998). Caffeine activates the two major stress axes, resulting in elevated glucocorticoid and catecholamine output along with increases in blood pressure (al'Absi & Lovallo, 2004). More specifically, activation of the hypothalamic-anterior pituitary-adrenocortical axis (HPA axis) is associated with the release of glucocorticoids from the adrenal cortex (cortisol in humans) and activation of the sympatho-adrenomedullary axis (SAM axis) results an increase in endogenous adrenergic activity, resulting in increased catecholamine activity (adrenaline and noradrenaline). A major physiological role of activation of both endocrine systems is considered to be a temporary increase in energy production and more specifically provision of additional metabolic fuel through increase in glucose availability (Evans *et al.*, 1986). The release of glucocorticoids leads to an increase in blood glucose levels through gluconeogenesis in the liver and decreasing glucose absorption from peripheral tissue. Moreover, the release of adrenaline also produces an increase in circulating blood glucose levels via the liver (Gold, 1992). Consequently, energy mobilization through increases in glucose levels can be seen as a major factor in order to prepare the body for the 'fight or flight' response.

Both caffeine and glucose administration have been shown to affect cortisol and/or catecholamine release (for example Bergendahl *et al.*, 1996; 2000; Gonzalez-Bono, Rohleder, Hellhammer, Salvador, & Kirschbaum, 2002; Graham, Rush, van Soeren, 1994; Kirschbaum, Bono, Rohleder, Gessner, Pirke, Salvador, & Hellhammer, 1997; Vance & Thorner, 1989; James, 2004; Lovallo, Farag, Vincent, Thomas, & Wilson, 2006; Lovallo,

Whitsett, al'Absi, Sung, Vincent, & Wilson 2005; Robinson, Sünram-Lea, Leach, & Owen-Lynch, 2004). For example, Lovello *et al.,* (2006) administered caffeine throughout the day (3x250mg) and found that caffeine increased cortisol levels across the day. Moreover, when cortisol levels were already raised due to a mental stress task, caffeine had an additive effect in raising these further. Some studies suggest that glucose administration may increase the cortisol response to a psychosocial stressor (Gonzales-Bono *et al.,* 2002; Kirschbaum *et al.,* 1997). Kirschbaum *et al.,* (1997) found that administration of 100g glucose one hour before exposure to a psychosocial stressor led to an exacerbated cortisol response that was not seen in participants who consumed water. However, other research observed that administration of a glucose drink (25g) can blunt the cortisol response to a brief naturalistic stressor which has both a psychological and a physical component (fire-fighting training, Robinson *et al.*, 2004).

Whilst cortisol provides a measure of the HPA axis reactivity, more recently salivary alpha-amylase (sAA) has been identified as a measure of SAM axis reactivity (Nater, La Marca, Florin, Moses, Langhans, Koller, & Ehlert, 2006; Nater, Rohleder, Gaab, Berger, Jud, Kirschbaum, & Ehlert, 2005). Salivary alpha amylase (sAA) - a critical protein produced with saliva (Rohleder & Nater, 2009) - has the main function of digesting carbohydrates (Baum, 1993); however, evidence (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996) suggests that it can also be used as an indicator of sympathetic nervous systems (SNS) activity (see Nater & Rohleder, 2009 for a review of the literature). Thus, stressful or demanding situations are argued to activate the SNS with sAA being a useful measure of such activation (Rohleder, Nater, Wolf, Ehlert & Kirschbaum, 2004; Maruyama *et al.,* 2012). There are very few studies that fail to observe a significant increase in the release of sAA in response to stressful situations; higher during both physical and psychological stress (Chatterton *et al.,* 1996). More specifically, sAA has

been suggested as a biomarker of the noradrenergic component of SNS activation (Ditzen *et al.,* 2014; Kuebler *et al.,* 2014; Rohleder and Nater, 2009; Wiemers *et al.,* 2013).

However, the effects of caffeine on sAA activity are not widely known. There are two studies which suggest that caffeine can stimulate sAA activity (Bishop, Walker, Scanlon, Richards, & Rogers, 2006; Morrison, Haas, Shaffner, Garrett, & Fackler, 2003), whereas others found no such effect (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007; Klein, Bennett, Whetzel, Granger, Ritter, 2010; Klein, Whetzel, Bennett, Ritter, Nater, & Schoelles, 2014). Bishop *et al.,* (2006) reported an increase in sAA after caffeine administration in male endurance athletes under prolonged exercise condition. Morrison *et al.,* (2003) found that caffeine intake, but not self-reported stress levels, predicted sAA levels among nurses on a paediatric intensive care unit. However, Nater *et al.,* (2007) did not find an effect of self-reported caffeine intake on diurnal sAA activity. Klein, Bennett, Whetzel, Granger, Ritter, (2010) observed no relationship between basal caffeine levels and basal sAA activity levels in habitual caffeine consumers and recent evidence suggests that sAA activity is not affected by administration of 200mg or 400mg of caffeine in regular caffeine consumers (Klein, Whetzel, Bennett, Ritter, Nater, & Schoelles, 2014).

Thus, the limited literature is inconclusive regarding the effects of caffeine on sAA activity. Moreover, there has been limited investigation into the effects of combined glucose and caffeine administration on hormonal responses and activation. Sünram-Lea *et al.,* (2012) found an increase in cortisol following a stressful fire-fighting training exercise, however there were no effects of glucose and caffeine when administered in combination on cortisol response following either a 40mg caffeine: 50g glucose drink or a 80mg caffeine:12.5g glucose drink. To the best of the author's knowledge there is currently no study reported in the literature that examined the effects of combined administration on sAA activity. However, investigation into the effects of combined administration of

caffeine and glucose on these physiological parameters is important as it may help to further elucidate the underlying mechanisms of the behavioural effects. Consequently, the aim of the study was to investigate the effect of caffeine and glucose administration on neuroendocrine activity in order to further elucidate the mechanisms through which these substances affect behaviour including cognition and mood.

3.2 Method and Materials

3.2.1 Participants

As described in Chapter 2, sixty-four healthy young adults aged 18-35yrs were recruited for the study. There were recruited via the Online Research Participation System (SONA) at Lancaster University. A sample size of 32 participants in each study was deemed to be sufficient as this was comparable to other studies utilising a similar design who had found beneficial effects of caffeine and glucose on attention when co-administered (Kennedy & Scholey, 2004; Scholey & Kennedy, 2004; Smit & Rogers, 2002). All were frequent caffeine consumers, consuming a minimum of 120mg caffeine per day. Participants were excluded if they; had a diagnosis of Diabetes Mellitus; had any intolerance or allergic reaction to substances that contain phenylalanine and/or caffeine; were non-native English speakers; had a history of neurological or psychiatric illness (excluding depression or anxiety); had a current diagnosis of neurological or psychiatric illness (including depression or anxiety); were currently taking medication or nutritional supplements (excluding contraceptive pill); were pregnant, seeking to become pregnant or breastfeeding; had a history of or currently abused drugs or alcohol; smoked. Eligibility was confirmed via a Clinical Records Form (CRF) after the participants had given their signed informed consent to take part.

3.2.2 Design

A double-blind placebo controlled, balanced mixed design was used. With participants randomly allocated to two different dosing regimens (‘moderate’ versus ‘low’), each comprised of three different treatment combinations (glucose, caffeine and a glucose caffeine combination) and a matching inert placebo. There was a 7 day (+/-2) washout

period between treatments. Assessments of cognition, mood, fatigue and hormonal response were completed pre-treatment (baseline) and 20 minutes after (post-dose).

3.2.3 Treatments

Drinks were supplied by GlaxoSmithKline Laboratories in 380ml lightly carbonated taste matched solutions. The 'low' dose regime consisted of a glucose drink (containing 15g glucose, 0mg caffeine); a caffeine drink (containing 0g glucose, 20mg caffeine); and a combined drink (containing 15g glucose and 20mg caffeine). The 'moderate' dose regime consisted of glucose (25g glucose, 0mg caffeine); caffeine (0g glucose, 40mg caffeine); caffeine and glucose (25g glucose, 40mg caffeine). Both regimes also utilised a taste matched placebo (0g glucose, 0mg caffeine).

Participants were instructed to consume one drink per test session within 5 minutes. Post-dose cognitive testing started 20 minutes after the drink administration. A 20-minute delay was chosen as peak plasma caffeine concentration is reached between 15 to 120 min after oral ingestion and brain levels remain stable for at least one hour following peak plasma levels (Nehlig, 1999; Nehlig & Boyet, 2000). Also this time frame was similar to the procedure of previous glucose studies (Foster, Lidder & Sünram, 1998) in order to ensure successful transfer of plasma glucose to the brain.

3.2.4 Procedure

Initial screening was done during sign-up using the Lancaster recruitment on-line system (SONA). At the first visit for screening and training, all participants completed the voluntary written informed consent prior to any study procedures being performed. The participant was screened by the researcher and the outcome of the screening activities was recorded in the CRF. Personal demographic information (such as age, education height and

weight) was also collected at this visit. They also completed a caffeine consumption questionnaire. Training on the cognitive tasks was then completed. No drinks were administered during the practice sessions and performance data from these sessions was not included in the analysis.

Participants then attended the laboratory on a further 4 occasions to complete the testing sessions. Testing was carried out between 8.30am and 12 noon and participants were asked to fast for 12hrs prior to the session (i.e. no food or drink except water) and to abstain from alcohol for 24 hours prior to testing. Due to the cortisol awakening response participants were also asked to wake up no earlier than 6.30am and no later than 8am. There was a 7(+/-2) day washout period between active days of the study. Consequently, participants were required to attend a weekly morning session over a period of approximately five to six weeks. Participants were randomised on arrival at the lab for their first study day. All active study days followed the same procedure.

At the beginning of the study day, a small baseline sample of blood was taken, and further blood glucose measurement was taken 15 and 50 minutes after drink consumption. Immediately after the baseline blood sample two saliva samples were also taken using a Salivette (Sarstedt Ltd.). The first for the measurement of alpha amylase, and the second for cortisol. Further saliva samples were taken 45 minutes post-drink for alpha-amylase and 55 minutes post-drink for cortisol. The first blood and saliva samples were followed by pre-drink baseline evaluation of mood and cognition, using the cognitive test battery. This was followed by administration of the day's treatment (following a double-blind procedure). The post-drink cognitive test session commenced 20 minutes after drink consumption. Each test session comprised of completion of the cognitive test battery (cognitive performance), the Bond-Lader visual analogue scales, the Mood, Alertness and Physical Symptoms (MAPS) Questionnaire, the Stress Arousal Checklist, and the

Activation-Deactivation adjective checklist (mood measures) and all participants received a debriefing sheet at the final day of testing.

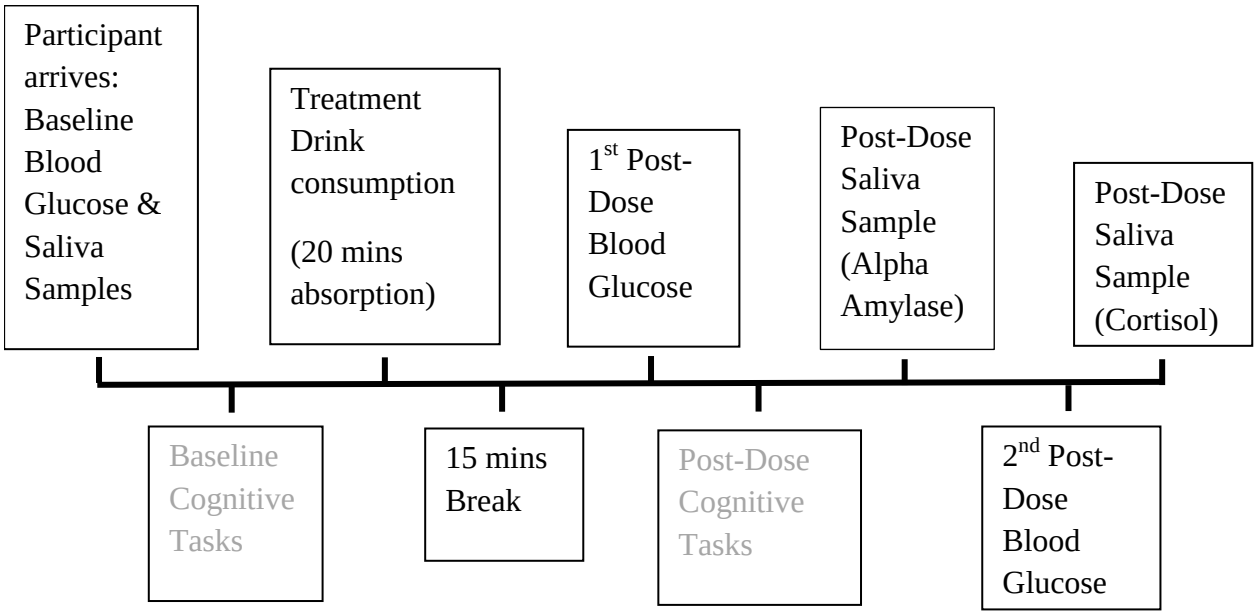

Figure 3.1 Schematic of the study day procedure

3.2.5 Alpha Amylase and Cortisol Measurement

Assessment of salivary cortisol levels is the classic measurement of response to stress, associated with activation of the HPA axis. Salivary alpha-amylase is a measure of adrenergic activity (SAM axis). Collection of saliva, in preference to blood sampling, provided a non-invasive mechanism to collect physiological samples from subjects. Saliva samples were collected using the salivette saliva sampling device (Sarstedt LTD, Leicester, UK). For the measurement of alpha-amylase, participants were instructed to give un-stimulated saliva samples by placing a Salivette in the top right hand corner of their mouth for a timed two-minute period. For the measurement of cortisol, participants were asked to lightly chew on the Salivette for one-minute. Samples were stored at -40oC until analysis. Saliva was recovered by centrifugation and salivary volume determined by weighing. This allows for the calculation of the saliva flow rate. Cortisol concentration

(nmol/l), alpha amylase (μ/mL) concentration in saliva was determined by commercially available kits (Salimetrics, USA).

3.2.6 Statistical Analyses

For hormonal responses (cortisol and alpha amylase), measures were transformed into change from baseline scores and analysed using a linear mixed model. Treatment and period were added as fixed effects and subject as random effect to the model. The model also included baseline measures as a covariate.

3.3 Results

3.3.1 Alpha amylase

There was no significant main effect of the 'low dose' treatments on alpha amylase, F (3, 11.55) = 1.59, p =.24, or period, F (3, 18.16) = 2.69, p = .07; or interaction, F (9,7.05) = 1.33, p = .36. Comparison with placebo showed no significant differences between any of the active treatments.

There was no significant main effect of the 'moderate dose' treatments on alpha amylase, F (3, 46.05) = 0.12, p = .95. There was no main effect of period, F (3, 77) = 2.49, p = .07; or the interaction, F (9, 27.64) = 0.95, p = .50. There were no significant differences observed following administration of treatment drinks compared to placebo.

3.3.2 Cortisol

There was no significant main effect of the 'low dose' treatment, F (3, 14.76) = 0.34, p = .80; period, F (3, 16.85) = 0.35, p = .79, or interaction, F (9, 16.73) = 1.13, p = .40.

For the 'moderate dose' treatment group, there were no significant effects of treatment, F (3, 30.27) = 0.32, p = .81; period, F (3, 24.81) = 0.07, p= .97, or interaction between both factors observed, F (9, 22.05) = 0.61, p = .78.

There were no significant differences observed following administration of treatment drinks from either dosing regimen compared to placebo.

Table 3.1 Alpha Amylase and Cortisol, means and standard errors

Treatment		Combined	Glucose	Caffeine	Placebo
Low[1]	Alpha Amylase (μ/mL)	33.39 (15.48)	37.25 (12.96)	19.90 (12.91)	15.80 (27.61)
	Cortisol (nmol/l)	-0.19 (0.04)	-0.23 (0.04)	-0.24 (0.04)	-0.23 (0.05)
Moderate[2]	Alpha Amylase (μ/mL)	39.61 (27.56)	62.18 (29.45)	64.38 (26.25)	77.19 (26.87)
	Cortisol (nmol/l)	-0.15 (0.07)	-0.16 (0.08)	-0.13 (0.09)	-0.22 (0.06)

[1] Change from baseline; covariates appearing in the model for low dose treatments are evaluated at the following average baseline values: Alpha Amylase = 63.53; Cortisol = 0.53.
[2]Change from baseline; covariates appearing in the model are evaluated at the following values: Alpha Amylase = 80.52; Cortisol = 0.58.
*Significant compared to placebo at $p < .05$

3.4 Discussion

The effects of glucose and caffeine alone and in combination on hormonal response were examined. No significant treatment effects were found for any of the active drinks in either dosing regimen ('low' or 'moderate') on either cortisol or alpha amylase were found. These findings do not support previous research which has found caffeine and glucose can have a modulating effect on hormonal response (Bergendahl *et al.*, 1996; 2000; Gonzalez-Bono *et al.,* 2002; Graham *et al.*, 1994; Kirschbaum *et al.,* 1997; Vance & Thorner, 1989; James, 2004; Lovallo *et al.,* 2006; Lovallo *et al.*, 2005; Robinson *et al.*, 2004). However other research has also found no effects of these substances on either the HPA or SAM axes (Klein *et al.,* 2014; Sünram-Lea *et al.*, 2012).

The lack of effects seen in this study could be due to the relatively low doses of caffeine and glucose administered, i.e. 20/40mg and 15/25g respectively. Previous research found that higher doses of glucose of 100g (Kirschbaum *et al.*, 1997) and three doses of 250mg caffeine across the course of the day (Lovello *et al.*, 2006) increased cortisol reactivity. However, Robinson *et al.*, (2004) found just 25g glucose attenuated the cortisol response to a naturalistic stressor, however in their study glucose was administered after stress exposure. Timing of administration might be an important factor. Another potential moderating factor might be the nature of the task or more specifically whether or not a task is stressful. . Whilst the prolonged cognitive testing battery employed in the current study could be considered a stressor, it probably does not have the stress inducing effects of other stressors that have been employed to elicit a stress response. For example, Kirschbaum *et al.*, (1997) only found the increased cortisol response following glucose consumption when participants completed a psychosocial stressor (Trier Social Stress Test). Similarly, Lovello *et al.*, (2006) found caffeine increased the cortisol response to a

mental stressor which consisted of 15mins demanding attention task and 15mins of a working memory, mental arithmetic task. However, Sünram-Lea *et al.*, (2012) failed to find any effects on cortisol response when two glucose and caffeine combination drinks were administered prior to a naturalistic stressor.

It is also possible that individual participants' characteristics impacted on the results. al' Absi, Lovallo, McKey, Sung, Whitsett, and Wilson (1998) found that caffeine (3.3mg/kg- equivalent to 2/3 cups of coffee) increased cortisol reactivity to a greater extent in individuals with increased central nervous system activation, for example those with a high risk of hypertension. As the participants utilised in this study were young, healthy adults, it is unlikely that they would be presenting with an increased hypertension risk. Similarly, Klein *et al.,* (2014) found that caffeine, at doses of 200mg or 400mg did not affect alpha amylase activity in participants who were regular caffeine consumers. Again, the participants in the current study were regular caffeine consumers. Therefore, the mechanisms of action for any effects via these hormonal pathways may be sensitive to specific individual's characteristics.

Although the effects of these active drinks on cognitive performance and mood (as reported in chapter 2) were limited, the fact that beneficial effects were seen in the absence of hormonal responses suggest that these are not, or at least not solely responsible for the observed behavioural effects. Indeed, it may be that the hormonal mechanisms are only of consequence under conditions of stress.

Chapter 4

The effects of pre-retrieval administration of glucose, caffeine and their combination on memory.

4.1 Introduction

So far, when assessing effects on memory performance the studies reported in this thesis have employed a pre-learning administration approach, in which the treatment drink is administered shortly before the material to be remembered is presented. However, the administration of glucose and/or caffeine just before the learning period does not allow one to distinguish between the effects of glucose on learning (i.e. encoding) or on memory (i.e. storage), as it might be the case that improved memory performance is simply a carry-over effect of improved drink-related encoding of the to-be-remembered target material. If, however, delayed recall performance is also facilitated when glucose and/or caffeine are administered after encoding, depending on the time frame when administration occurs, this would enable one to conclude that these substances improve consolidation or retrieval of the memory material.

To date, research into the facilitative properties of combined caffeine and glucose administration on memory performance has exclusively employed a pre-learning administration approach. However, in terms of the effects of glucose and caffeine administration in isolation, both have been found to improve memory when administered at different stages of the memory process. More specifically glucose has been shown to improve memory when it is administered immediately after encoding and also when administered immediately prior to recall (Kopf & Baratti, 1996; Flint & Riccio, 1998; Manning, *et al.,* 1992; Manning, Stone, Korol & Gold, 1998; Sünram-Lea, *et al.*, 2002a). For example, Kopf and Baratti (1996) obtained evidence for a significant facilitating effect of retrograde peripheral administration of glucose on retention of a habituation response in mice. In this study, male mice were permitted to explore a novel environment (open-field activity chamber) for 10 minutes. This procedure was conducted twice with a 24-h

interval, and the difference in the exploratory activity between the first (training) and the second (testing) exposures to the chamber was taken as an index of retention. The results showed that post-training administration of glucose enhanced retention. The effect of glucose on retention was time-dependent, insofar as glucose administration 180 minutes after training did not result in better memory performance; only glucose injection immediately after the training period resulted in significantly better retention compared to the control group (which received saline injection). Similarly, Flint and Riccio (1998) examined the effects of glucose on infantile amnesia in 17-day old preweanling rats using a retrograde administration approach. Rats were trained to criterion on a passive avoidance conditioning task. They were tested 24hrs later, immediately prior to which they were injected with either a saline or glucose. Following glucose injection the poor performance in retention, suggestive of infantile amnesia which was seen following saline, was attenuated. Stone, Rudd and Gold (1990) also found that performance was enhanced when glucose was administered prior to testing in mice. Manning *et al.*, (1992) examined anterograde and retrograde glucose administration on memory performance in elderly human participants. Participants were asked to listen to a narrative prose passage and their recall was tested immediately afterwards and 24hrs later. The treatment, either 50g glucose or placebo (23.7mg saccharin) was administered either immediately before or directly after hearing the material to be recalled . Both pre- and post-acquisition administration of glucose improved recall 24hrs later compared to placebo. They concluded that, as scores on the immediate recall task following both glucose and placebo were similar, glucose specifically improved memory storage processes, i.e. retention rather than encoding. The results also showed that memory recall at 24hr following pre-acquisition glucose was significantly better compared to placebo. The authors suggest that the enhancing effects of glucose may be due to preventing memory degradation (Manning *et al.,* 1992). Further

evidence that the glucose memory facilitation effect outlasts the rise in blood glucose levels after treatment was provided in a further study by Manning *et al.,* (1998). In this study healthy elderly participants received 50g glucose or 35mg saccharin across four study days, either immediately before acquisition, or immediately before recall 24 hrs later. They found that both anterograde and retrograde administration led to significantly better recall. However participants showed significantly better recall following the pre-acquisition administration compared to after pre-retrieval administration of glucose. The results suggest that whilst glucose can be beneficial for encoding, storage and retrieval, its effects may be less pronounced on retrieval (Manning *et al.*, 1998). Sünram-Lea, Foster, Durlach and Perez (2002a) investigated the effect of post-acquisition glucose administration on memory performance in healthy young adults. Participants consumed 25g glucose or a placebo either immediately before, 15 minutes before, or immediately after the presentation of a word list. The word list was then recalled 30mins and 24hrs later. Both immediate pre-acquisition and immediate post-acquisition administration of glucose improved memory performance compared to placebo at 30min and 24hrs. However, their findings also showed that effect of anterograde glucose administration on memory performance is time-dependent, as the enhancement of retention was decreased when the administration-learning interval was increased.

While there is extensive literature suggesting beneficial effect of caffeine on memory in both humans (Keleman & Creeley, 2001; Smith *et al.,* 1999; Smith, Clark & Gallagher, 1999) and animals (Costa, Botton, Mioranzza, Ardais, Moreira, Souza & Porciúncula, 2008; Prodiger, Batista & Takahasi, 2005) in terms of an anterograde administration mode, some studies have actually reported inhibitory effects on retention after anterograde administration (Childs & de Wit, 2006). Indeed, it has been argued that caffeine is most effective at improving retrieval and storage rather than acquisition. For example,

retrograde administration of caffeine, in particular at lower doses (1 – 30 mg/kg) has been shown to improve memory consolidation (Angelucci, Cesário, Hiroi, Rosalen & Cunha, 2002; Angelucci, Vital, Cesário, Zadusky, Rosalen & Cunha, 1999). In their first study, Angelucci *et al.,* (1999) administered 1, 3, 10, 30 or 100mg/kg of caffeine or saline and measured inhibitory avoidance and habituation to a new environment in rodents. The results demonstrated that for the 10-30mg/kg doses, when caffeine was administered 30mins before the training session, retention scores were impaired. However, caffeine improved the inhibitory avoidance (but not habituation) retention scores when administered immediately after the training or 30 min before the test session at the doses of 1–30 mg/kg or 3–10 mg/kg. The authors conclude that depending on anterograde or retrograde administration caffeine can impair or improve memory, respectively. In a second study, the group investigated the effects of caffeine on spatial memory in rats using the Morris Water Maze (Angelucci, Cesário, Hiroi, Rosalen & Cunha, 2002). Caffeine was administered either at 30mins before training, immediately after training or 30mins before testing, at doses of 0.3, 3, 10 or 30mg/kg. Again, post-training administration of caffeine improved memory consolidation, although this was only observed for the lower doses (0.3-10mg/kg dose). Pre-test administration of caffeine also resulted in a slight memory advantage (shortened escape latencies). Interestingly, whilst pre-training caffeine administration did not significantly improve memory performance, it also did not result in memory impairments as previously reported. The inhibitory effect of caffeine on memory acquisition could be related to a direct impairment effect on learning or to state-dependent learning mechanisms. Angelucci *et al.,* (1999) suggested that the amnestic effects induced by pre-training caffeine administration in rodents submitted to both an inhibitory avoidance task could not be attributed to state- dependent learning as they were not abolished by a subsequent pre-test caffeine administration. A relatively recent study by

Sanday *et al.,* (2013) however suggested that caffeine-induced memory deficits might be due to state dependent learning mechanisms as pre-test caffeine administration abolished the memory impairments effect produced by pre-training injection of caffeine. Despite, the controversy surrounding the potential underlying mechanisms, the data suggest that caffeine is most effective at improving retrieval and storage rather than acquisition (Angelucci *et al.,* 2002).

Since both substances in isolation have been shown to enhance consolidation and retrieval, the aim of the current study was to investigate their combined effects when administration occurs pre-retrieval. Previous research carried out with human participants has found that glucose has beneficial effects on free recall following anterograde and retrograde administration, therefore the primary outcome will be the number correct on the delayed word recall task. The findings of the proposed research will have ecological relevance as glucose and caffeine containing beverages (such as energy drinks) are often consumed when consumers want to boost their performance. In the case of pre-retrieval administration, this could be prior to an exam or prior to an important presentation where the to-be-remembered material has been learned previously. In addition, memory performance will be tested in the afternoon, following a two hour fast. Given that all previously reported studies in the literature examining the effects of combined glucose and caffeine administration were conducted in the morning, it was decided not only to manipulate the administration mode (pre-versus post acquisition) but also to carry out testing in the afternoon. Given that people may consume energy drinks as an afternoon 'pick-me-up' after a period of post- lunch fasting, the aim of this study was to increase the generalisability of the previously observed facilitation effect and to establish its ecological validity. Moreover, there is evidence to suggest that both glucose (Sünram-Lea, Foster, Durlach& Perez, 2001) and caffeine (Smith, Hatfield & Hostetter, 2002; Ryan, Hatfield &

Hoffstetter, 2002; Nehling, 2010) administration in isolation can facilitate performance irrespective of time of day. The memory enhancing effect of glucose was essentially equivalent whether administration and testing occurred in the morning or in the afternoon (2-h after lunch; it was given after an over- night fast or a 2-h fast following breakfast or lunch. For caffeine, there is an indication that effects might be even stronger in the afternoon. This suggestion is in line with the observation that beneficial caffeine effects on mood and performance are more prominent under low arousal conditions. For example, caffeine has been shown to ameliorate the decline in in sustained attention after lunch, and is more effective during night work and prolonged work (Smith, Hatfield & Hostetter, 2002; Nehling, 2010). In older adults caffeine has been shown to ameliorate a decline in cognitive performance in the afternoon, which is commonly observed in most adults over the age of 65 (Ryan, Hatfield & Hoffstetter, 2002). It has been suggested that these effects might be moderated by physiological arousal. In general, caffeine has been shown to mainly improve performance by reducing decrements in performance under suboptimal alertness conditions (Nehling, 2010).

In terms of dosage, 40mg of caffeine was chosen given that previous studies reported in this thesis have shown this dosage to be effective in improving cognitive performance, including memory. For glucose, although 25g has been shown to robustly enhance memory performance when administrated in isolation, this has not been the case in the studies reported here. In the current study 40g of glucose was chosen as the dose of interest to investigate. In summary, the current study aimed to provide further insight into the effects of caffeine and glucose containing drinks using a more realistic testing paradigm with greater ecological validity.

4.2 Method and Materials

4.2.1 Participants

Thirty healthy young adults (10 males, 20 females) took part in the study, recruited via the Online Research Participation System (SONA) at Lancaster University. A sample size of 30 participants was deemed to be sufficient as this was comparable to other studies examining the same domains, both retrograde administration on recall and effects on the attention networks, and where effects of caffeine and glucose had been found (Brunyé, Mahoney, Lieberman, Giles & Taylor, 2010a; Brunyé, Mahoney, Lieberman & Taylor 2010b; Manning *et al.,* 1998). The age range was 18-35 years (mean age 21.53 years). They all consumed at least 120mg caffeine per day, (average consumption was 225.75mg). Exclusion criteria included; a diagnosis of Diabetes Mellitus; any intolerance or allergic reaction to substances that contain phenylalamine and/or caffeine; being non-native English speakers; having a history of neurological or psychiatric illness (excluding depression or anxiety); having a current diagnosis of neurological or psychiatric illness (including depression or anxiety); currently taking medication or nutritional supplements (excluding the contraceptive pill); being pregnant, seeking to become pregnant or breastfeeding; having a history of or currently abusing drugs or alcohol; or smoking. The study was approved by the Department of Psychology Ethics Committee at Lancaster University. Participants gave their signed informed consent prior to taking part and a Clinical Records Form (CRF) was used to confirm their eligibility.

4.2.2 Design

The study followed a placebo controlled, repeated measures design; the within factor being treatment (glucose (40g), caffeine (40mg), glucose/caffeine (40g/40mg), and placebo). There was at least a 48hr washout period between visits. Participants were randomly allocated to a treatment regime using a Latin square which counterbalanced the order of test drinks across the study days.

4.2.3 Treatments

The treatments were supplied by Suntory Food and Beverage Europe in 380ml solutions. There were three active drinks; glucose containing 40g glucose, caffeine containing 40mg caffeine and a glucose and caffeine combination containing 40g glucose and 40mg caffeine. A taste matched placebo was also utilised.

Participants were instructed to consume one drink per test session within 5 min. Cognitive testing started 20 minutes after drink administration. A twenty-minute delay was chosen as peak plasma caffeine concentration is reached between 15 to 120 min after oral ingestion and brain levels remain stable for at least one hour following peak plasma levels (Nehlig, 1999; Nehlig & Boyet, 2000). Also this time frame is similar to the procedure of previous glucose studies (Foster *et al.,* 1998) in order to ensure successful transfer of plasma glucose to brain.

4.2.4 Procedure

Initial screening was done during sign-up using the Lancaster recruitment on-line system (SONA). At the first visit for screening and training, all participants completed the

voluntary written informed consent prior to any study procedures being performed. The participant was screened by the researcher and the outcome of the screening activities was recorded in the CRF. Personal demographic information (such as age, education, height and weight) was also collected at this visit. They also completed a caffeine consumption questionnaire to confirm their daily caffeine consumption. Training on the cognitive tasks was then completed. No drinks were administered during the practice sessions and performance data from these sessions was not included in the analysis.

Participants then attended the laboratory on a further four times to complete their study sessions. All the study days followed the same procedure and were separated by at least a 48hr wash out period. Upon arrival at the morning encoding session, baseline blood glucose measurements were taken. Participants were then presented with the material to be remembered (acquisition phase). Participants were then free leave the laboratory until they returned for their afternoon session, where memory performance was assessed. The afternoon session started 6hrs after encoding. Upon arrival the participant gave a finger prick blood sample. They then consumed their treatment drink for the day (following a double-blind procedure). The post-drink test session commenced 20 minutes after drink consumption. At around 15 mins post treatment a further blood glucose reading was taken. After the full 20 mins absorption period, participants' memory performance on the various tasks was assessed. Following on from this they then completed the Attention Network task, which will be discussed in chapter 5. A final blood glucose reading was taken at the end of the testing session. All participants received a debriefing sheet on the final day of testing.

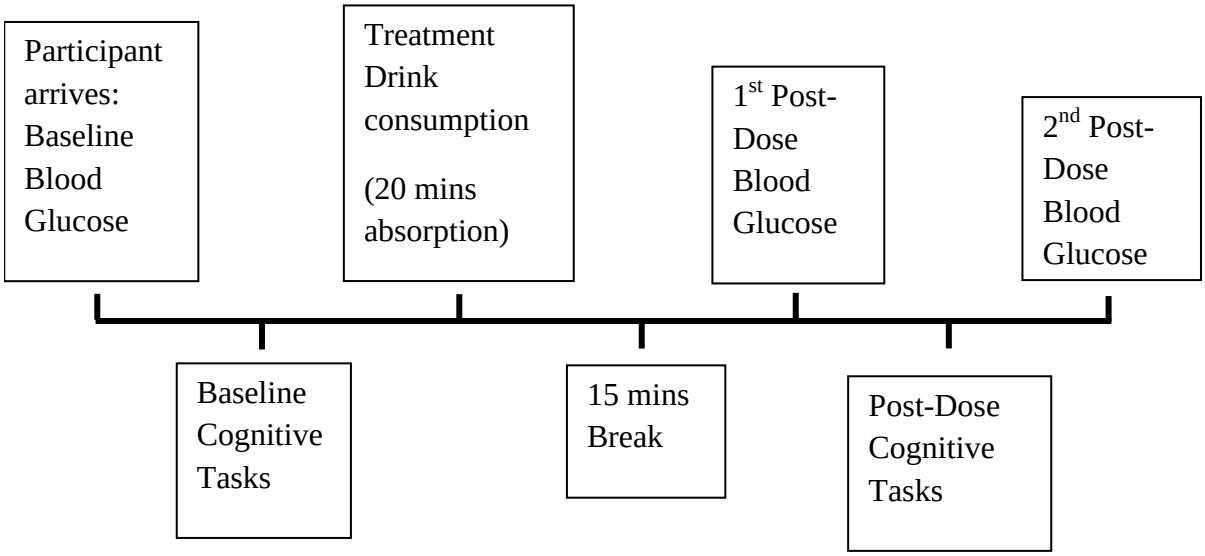

Figure 4.1 Schematic of the study day procedure

4.2.5 Assessments

Computerised assessment was used to evaluate cognitive performance. The memory tasks were delivered within the Computerised Mental Performance Assessment System (COMPASS), which is a purpose designed software application for the flexible delivery of randomly generated parallel versions of standard and novel cognitive assessment tasks. With the exception of the paper and pencil tasks (word recall); all responses were made using the computer keyboard and mouse. In this case the assessment comprised a selection of standard psychometric tasks with stimuli chosen to possess good face validity in an 'everyday' context. The elements of the cognitive assessment are described below.

Encoding Phase:

Word presentation. – A list of 20 words matched for frequency, concreteness and imagery were presented on the monitor at the rate of 1 every 2 seconds for participants to remember. During encoding, participants were required to perform two complex hand-movement sequences (Sünram-Lea, Foster, Durlach & Perez, 2002b). Each sequence was performed using both hands and contained three movements: fist – chop - slap and back-slap – chop – fist. Participants were told to alternate the sequence every fifth word and they were not informed when to change, they had to keep track of this themselves. Hand-movements were performed continually during word presentation. Participants were told that the hand-movements were being video recorded during the task to ensure they fully engaged with this aspect of the task.

Distractor Task. – After viewing the words participants engaged in an arithmetic task, which served to prevent the rehearsal of items in working memory. The Serial 3s task was used, this is where participants are presented with a starting number between 800 and 999 on the screen and they have to serially subtract 3 from this number. The distracter task lasted for 30 seconds. Performance on this task was not used for analysis.

Immediate word recall. - Participants were given 60-seconds to write down as many words as they could from the list they have just seen. Participants' responses were marked according to total number of words recalled correctly and number of errors.

Picture presentation. – 20 pictures were individually, displayed in the centre of the screen at a rate of 1 every 3 seconds. Each picture is displayed for 1 second. Participants were required to remember the pictures.

Face presentation. – 20 faces were individually, displayed in the centre of the screen at a rate of 1 every 3 seconds. Each face is displayed for 1 second. Participants were required to remember the pictures.

Retrieval Phase:

Delayed word recall. - Participants were given 60-seconds to write as many words as they could from the list they have seen at the encoding phase. Participants' responses were marked according to total number of words recalled correctly and number of errors.

Delayed word recognition. – The 20 original words and 20 distractor words were presented individually in a randomised order. Participants were asked to indicate whether each word had been in the original list or not by responding using the 'm' key for 'yes' or the 'z' key for 'no' on their keyboard. Percentage accuracy and reaction times for both distractors and targets were recorded in addition to overall reaction times.

Picture recognition. - The 20 original pictures and 20 distractor pictures were presented, individually in a randomised order. Participants were asked to indicate whether each word had been in the original list or not by responding with the 'm' key for 'yes' or the 'z' key for 'no' on their keyboard. Percentage accuracy and reaction times for both distractors and targets were recorded in addition to overall reaction times.

Face recognition. - The 20 original faces and 20 distractor faces were presented, individually in a randomised order. Participants were asked to indicate whether each word had been in the original list or not by responding with the 'm' key for 'yes' or the 'z' key for 'no' on their keyboard. Percentage accuracy and reaction times for both distractors and targets were recorded in addition to overall reaction times.

Blood glucose measurement

Blood glucose readings were obtained using the ExacTech® blood glucose monitoring equipment (supplied by MediSense Britain Ltd, 16/17 The Courtyard, Gorsey Lane, Coleshill, Birmingham B46 1JA), following the recommended procedure: The researcher swabbed the finger of the volunteer with a Sterets Isopropyl Alcohol BP Pre-injection swab (Seton Healthcare Group, Oldham, UK) and allowed the skin to air dry. The skin was punctured using an automatic lancing devise and a drop of blood was collected onto the analytical test strip. The volunteer applied a tissue to blot any excess blood. Ambidex-HG powder free polymer bonded latex examination gloves were worn by the experimenter at all times during the procedure. Each lancet and cap was only used once and then disposed of into a sharps container. Swabs, test strips, tissues and gloves were placed in a clinical waste sack.

For performance characteristics of ExacTech® blood glucose monitoring equipment see Chapter 2.

4.2.6 Statistical Analysis

The primary efficacy variable was the number of correctly recalled words on the delayed word recall task. The secondary efficacy variables included; Immediate word recall correct and errors, delayed word recall errors, word recognition accuracy and speed, picture recognition accuracy and speed and face recognition accuracy and speed. The absolute values for all the behavioural and mood variables were used in the analysis. Using IBM SPSS Statistics Version 22 a linear mixed model was used to analyse the treatment drinks (Caffeine, Glucose, Caffeine and Glucose, and placebo). Treatment drink, period and treatment by period interaction were added as fixed factors and subject as a random factor. The Estimates of Fixed Effects were used to compare each active treatment with placebo.

For glycaemic measurement comparisons were performed on the blood glucose levels using a linear mixed model. The model included a random effect for subject and fixed effects for period, treatment and time. The Estimates of Fixed Effects were used to compare each active treatment with placebo.

4.3 Results

Table 4.1 Demographics, means and standard deviations

N	Gender	Age (yrs)	BMI (kg/m^2)	Years in Full Time Education	Average Caffeine Consumption (mg)
30	10 Males/ 20 Females	21.53 (2.35)	20.07 (3.08)	15.73 (2.03)	225.75 (57.24)

4.3.1 Blood Glucose

There was a significant main effect of the low dose treatments on blood glucose, F (3, 164.18) = 94.02, $p < .001$. There was a significant effect of time, F (3, 128.25) = 89.87; $p < .001$; and treatment x time interaction F (9, 89.82) = 36.47, $p < .001$ were observed. Comparisons showed that baseline blood glucose levels did not differ, however after administration of glucose containing drinks, higher blood glucose levels were observed at both post dose measures compared to placebo and the caffeine only drink (all $p < .001$) (see Figure 4.1 for glycaemic response as a function of treatment and time).

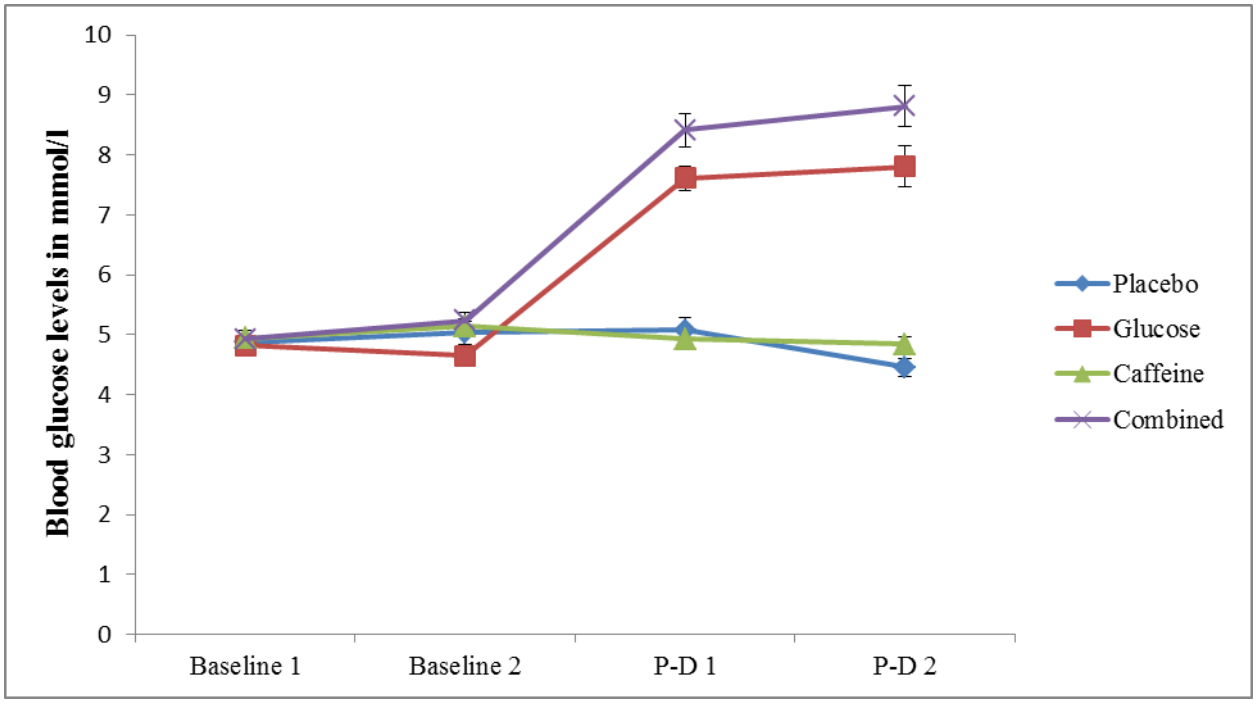

Figure 4.2 Glycaemic response to treatment as a function of drink and time

Table 4.2 Blood glucose, means and standard errors

Time Point	Treatment			
	Placebo	Glucose (40g)	Caffeine (40mg)	Glucose (40g) / Caffeine (40mg)
Baseline	4.87 (0.12)	4.82 (0.10)	4.94 (0.14)	4.93 (0.15)
2nd Baseline	5.03 (0.20)	4.65 (0.13)	5.14 (0.12)	5.24 (0.14)
1st Post Dose	5.08 (0.20)	7.61 (0.21)	4.93 (0.16)	8.41 (0.27)
2nd Post Dose	4.46 (0.15)	7.81 (0.34)	4.84 (0.13)	8.81 (0.34)

4.3.2 Memory Results

Data of all participants were included in the analysis.

4.3.2.1 Primary Outcome

Delayed Word Recall Correct

There was no significant main effect of the treatment on correctly recalled words in the Delayed Word Recall task, F (3, 28.74) = 1.88, p = .16. There was no main effect of period, F (3, 29.30) = 0.43, p = .73; or treatment x period interaction, F (9, 35.26) = 1.20, p = .33. No significant differences in performance were observed following administration of active treatment drinks compared to placebo.

4.3.2.2 Secondary Outcomes - Memory

Immediate Word Recall Correct

There was no significant main effect of treatment on correctly recalled words, F (3, 33.78) = 1.87, p = .15. There was a significant main effect of period, F (3, 34.72) = 2.85, p = .05, with participants remembering fewer words at their first visit, but no significant interaction between treatment and period, F (9, 32.22) = 1.07, p = .41. Comparison with placebo showed no significant differences following administration of any of the active treatment drinks.

Immediate Word Recall Errors

There was no significant main effect of treatment on number of incorrectly recalled words, F (3, 34.77) = 0.58, p = .63. There was no main effect of period, F (3, 25.29) = 1.68, p = .20, and no significant interaction between treatment and period, F (9, 28.19) = 1.03, p = .44. There were no significant differences observed following administration of active treatment drinks compared to placebo.

Delayed Word Recall Errors

There was no significant main effect of the treatment drinks on incorrectly recalled words, F (3, 19.61) = 0.19, p = .90. There was no main effect of period, F (3, 20.20) = 0.98, p = .42, and no interaction between treatment and period, F (9, 21.16) = 1.23, p = .33. However, participants performed significantly better following the placebo drink (M = 1.67) compared to after the caffeine only drink (M = 1.71), p = .04.

Word Recognition Accuracy

There was no significant main effect of treatment on word recognition accuracy, F (3, 33.71) = 1.08, p = .37. There was no main effect of period, F (3, 35.94) = 1.42, p = .25; or interaction between treatment and period, F (9, 35.86) = 1.60, p = .15. There were no significant differences observed following administration of active treatment drinks compared to placebo.

Word Recognition Speed

There was no significant main effect of treatment on reaction time for correctly recognised words, F (3, 29.14) = 0.23, p = .88; no main effect of period, F (3, 28.95) = 0.52, p = .67; and no significant treatment x period interaction, F (9, 32.12) = 0.84, p = .59. There were no significant differences observed following administration of active treatment drinks compared to placebo.

Picture Recognition Accuracy

There was no significant main effect of treatment on the percentage correct responses on the picture recognition task, F (3, 48.67) = 0.29, p = .84. There was no main effect of period, F (3, 43.20) = 0.31, p = .82; or interaction between treatment and period, F (9, 32.99) = 0.66, p = .74.

Picture Recognition Speed

There was no significant main effect of treatment on the reaction time of correctly recognised pictures, F (3, 15.36) = 1.34, p = .30. There was no main effect of period, F (3, 23.55) = 2.61, p = .08; or interaction between treatment and period, F (9, 23.05) = 1.57, p = .18. There were no significant differences observed following administration of active treatment drinks compared to placebo.

Face Recognition Accuracy

There was no significant main effect of the treatment drinks on the correct responses on the face recognition task, F (3, 29.04) = 0.78, p = .51, and no main effect of period, F (3, 31.48) = 1.57, p = .22, or treatment x period interaction, F (9, 20.36) = 1.63, p = .17. Comparison with placebo revealed no significant effects.

Face Recognition Speed

There was no significant main effect of treatment on the correct reaction time responses on the Face Recognition task, F (3, 39.04) = 0.17, p = .92. There was no main effect of period, F (3, 32.65) = 0.35, p = .79; or interaction between treatment and period, F (9,

26.65) = 1.86, p = .10. No significant differences were observed following administration of active treatment drinks compared to placebo.

Table 4.3 Memory Results, means and standard errors

Treatment	Combined	Glucose	Caffeine	Placebo
Immediate Word Recall Correct	5.17 (0.38)	4.42 (0.36)	5.58 (0.40)	5.37 (0.40)
Immediate Word Recall Errors	0.86 (0.16)	1.26 (0.25)	1.02 (0.27)	1.02 (0.20)
Delayed Word Recall Correct	2.48 (0.27)	2.15 (0.24)	2.98 (0.34)	2.58 (0.27)
Delayed Word Recall Errors	1.90 (0.25)	1.82 (0.30)	1.71 (0.33)	1.67 (0.31)
Word Recognition Accuracy	62.79 (10.35)	60.29 (10.35)	63.95 (10.37)	61.96 (10.35)
Word Recognition Speed	888.1 (71.2)	870.2 (69.9)	913.7 (73.7)	913.0 (76.3)
Picture Recognition Accuracy	84.48 (1.78)	84.15 * (2.03)	86.30 (1.75)	85.08 (1.47)
Picture Recognition Speed	833.2 (20.9)	813.3 (20.9)	845.5 (26.2)	840.1 (28.3)
Face Recognition Accuracy	70.42 (2.88)	71.24 (2.91)	72.40 (2.88)	68.40 (2.78)
Face Recognition Speed	927.3 (15.9)	925.5 (30.1)	952.5 (40.8)	943.1 (39.1)

*Significant compared to placebo at $p < .05$

4.4 Discussion

The current study aimed to provide further insight into the effects of caffeine and glucose containing drinks using a more realistic testing paradigm with greater ecological validity. More specifically, we assessed the effects of drinks on memory performance when administered in the afternoon prior to retrieval, primarily on delayed free recall. Whereas previous studies have shown that in isolation, caffeine and glucose improve memory when administered prior to retrieval, in particular when measured by free recall (Angelucci *et al.*, 1999; Angelucci *et al.*, 2002; Kopf & Baratti, 1996; Flint & Riccio, 1998; Manning, *et al.*, 1992; Manning, Stone, Korol & Gold, 1998; Sünram-Lea, Foster, Durlach & Perez, 2002a), the results of the current study provided no evidence for such facilitation effect as neither the delayed free recall task, nor any of the other tasks showed glucose facilitation. For caffeine and for both substances in combination there was no evidence of any enhancement to performance when they were administered prior to retrieval. Indeed there was evidence to show that participants' memory performance was impaired following caffeine, as participants' performance was better following placebo compared to after the caffeine and combination drinks.

Previous research has found that pre-test administration of glucose attenuated retention loss indicative of infantile amnesia in rats (Flint & Riccio, 1998). Manning *et al.*, (1998) found that 50g glucose improved memory performance when administered immediately prior to testing memory of a previously heard passage. It may be that whilst 40g glucose is a similar dose to 50g used in Manning *et al.*,'s study, it is not effective for enhancement of recognition memory. The dose response profile for different tasks may be quite sensitive to changes in dose levels. The dose response profile for glucose has been previously shown to be different for different types of tasks (Sünram-Lea, Owen, Finnegan & Hu, 2011), and so

it may be that the specific stages of the memory process i.e. learning, storage and retrieval are also modulated by different doses.

The caffeine drink was found to have detrimental effects on some of the performance measures, and so it seems that when consumed in isolation, caffeine has a negative effect. This finding does not support previous literature as caffeine has previously been found to impair memory acquisition, but have beneficial effects on memory consolidation and retrieval (Angelucci *et al.*, 1999; Angelucci *et al.*, 2002). One explanation for this finding may be state-dependent memory effects; whereby memory retrieval requires the state is the same as when encoding took place (Bruins Slot & Colpaert, 1999; Ceretta, Camera, Mello & Ruben, 2008). Sanday *et al.*, (2013) found that pre-training caffeine-induced amnesia could be abolished by the administration of pre-test caffeine. This was further supported by the finding that whilst pre-training administration of caffeine impaired memory performance on a subsequent test, it did not alter the way mice learned to avoid the aversive enclosed arm compared to saline (Sanday *et al.*, 2013). Therefore, the pre-training amnesia was not related to impaired learning of the task (Sanday *et al.*, 2013). In the current study caffeine was only administered at recall and not at encoding, whereas in previous studies where caffeine was found to have beneficial effects on memory (see Chapter 2) caffeine was administered prior to encoding; therefore caffeine was present at both encoding and retrieval and this could have resulted in state-dependent memory effects. Another reason why no effects were observed might have been that the time scale between encoding and retrieval was too short to see the beneficial effects of these substances on memory retrieval. In this study the interval was only 6hrs from the start of the encoding session to the start of the retrieval session, whereas in previous research there has been a gap of 24hrs (Flint & Riccio, 1998; Manning *et al.*, 1998) or 48hrs (Angelucci *et al.*, 1999; Angelucci *et al.*, 2002). The longer timeframe would increase the cognitive

demand of the task as participants would have to maintain the to-be-remembered material for longer.

In conclusion, this study aimed to examine the effects of administration of glucose and caffeine and their combination prior to memory retrieval, primarily as measured by the performance of correctly recalled words on the delayed word recall task. No facilitation of memory performance after glucose administration was evident on this task or any of the secondary outcome measures. Consequently, the result of this study do not support previous research which found more robust effects following retrograde glucose administration (Flint & Riccio, 1998; Manning *et al.,* 1998) and beneficial effects when caffeine was administered pre-retrieval (Angelucci *et al.,* 1999; Angelucci *et al.,* 2002). Moreover, there were no effects when caffeine and glucose were administered in combination. However, there are a number of factors that might have led to the failure to observe any effects. These include dose, time of day, level of fasting. For example, it would be useful to establish the dose-response profile of retrograde administration in order to see whether there is an optimal combination dose that is specific to enhancing retrieval. The optimal time for drink administration should also be investigated.

Chapter 5

The effects of glucose, caffeine and their combination on the different attention networks

5.1 Introduction

The effects of caffeine on attention have been extensively reviewed (for reviews see Einöther & Giesbrecht, 2013; Lieberman, 1992; Nehlig, 2010; Ruxton, 2008; Smith, 2002; Smith, 2005; Smith, Osborne, Mann, Jones & White, 2004; Stafford, Rusted & Yeomans, 2006). Although not all studies found effects of caffeine on attention tasks, most studies found improvements in reaction time and there is evidence that caffeine can help sustain attention in demanding tasks (Einöther & Giesbrecht, 2013). For glucose, benefits have preferentially been observed on memory processes over other aspects of cognition including attention (Foster, *et al.,* 1998; Meikle, Riby, & Stollery, 2005; Sünram-Lea *et al.,* 2001). However, this might be due to the fact that effects on attention and vigilance have not been evaluated to the same extent. Studies that have assessed the impact of glucose on non-mnemonic processes including attention have also reported positive effects, especially when tasks are sufficiently difficult (Owens *et al.,* 1994; Reay *et al.,* 2006).

Yet, the studies reported in this thesis which have examined the effects of caffeine, glucose and their combination on measures of simple and complex attention demonstrated no clear effects of these substances on any of these attention measures.

Attention is an essential aspect of cognitive functioning. The concept of attention as central to human performance extends back to the start of experimental psychology (James, 1890). Optimal attention is an important prerequisite for improving other cognitive processes, including memory. Consequently, assessment of effects of attention is crucial when trying to evaluate neurocognitive enhancement, especially when cognitive enhancement is defined as 'the amplification or extension of core capacities of the mind through improvement or augmentation of internal or external information processing

systems' (Bostrom & Sandberg, 2009). It is generally accepted that the concept of attention entails a number of distinct but related processes, but the exact structure of attention remains a matter of scientific debate (Raz and Buhle 2006). Yet, as observed with other psychoactive substances (for example nicotine; Hahn *et al.*, 2009), the attention-enhancing effect of caffeine and/or glucose might depend on the nature of the attentional function. There are currently two main theories of attention; the more traditional, which divides attention into simple and complex attentional processes, and more recently the Attention Network Theory (Posner & Petersen, 1990; Posner & Rothbart, 2007). The Attention Network Theory (ANT) postulates the existence of three distinct attentional networks, which differ both in functionality and anatomically (Posner & Petersen, 1990). These are the alerting, orienting and executive control attention networks (Fan, McCandliss, Sommer, Raz & Posner 2002; Posner & Petersen, 1990; Posner & Rosthbert, 2007). The alerting network helps to achieve and maintain an alert state; the orienting network helps to select information from sensory input; and the executive control network is responsible for resolving conflict between responses (Fan *et al.,* 2002). Fan *et al.,* (2005) used event-related functional magnetic resonance imaging (fMRI) during the ANT to examine the brain areas involved in the three attentional systems. All three systems were shown to be related to separate distinct brain regions. They found the alerting network showed strong thalamic involvement along with the activation of the anterior and posterior cortical sites, whereas parietal sites and frontal eye fields were activated by the orienting network. The anterior cingulate, right and left frontal areas and several other sites e.g. left and right fusiform gyrus were activated by the executive control network (Fan *et al.,* 2005). However, there is evidence for an interdependency of the different networks (Posner, 1994, Funes & Lupiáñez, 2003, Callejas, Lupiáñez & Tudela, 2004).

The ANT allows assessment of several aspects of attention (alerting, orienting, and central-executive function) within a single procedure (Fan *et al.*, 2002). In order to assess the alerting network, participants are measured as to whether cues which alert them to trial onset improve their performance (Fan *et al.*, 2002; Fan, McCandliss, Fossella, Flombaum & Posner, 2005). To assess the orienting network, the extent to which participants' performance is improved when they see cues which orient them to the upper or lower section of the screen in preparation for the trial onset, compared to cues which do not provide spatially relevant information, is measured (Fan *et al.*, 2002; Fan *et al.*, 2005). For the assessment of the executive control network the extent to which participants can respond to the direction of a middle arrow, whilst inhibiting the effects of opposite facing flanker arrows, is compared to their performance when the flanker arrows are congruent and a slowed performance demonstrates the inefficiency of the network (Fan *et al.*, 2002; Fan *et al.*, 2005).

There is evidence to suggest that different attentional networks are sensitive to glucose and caffeine administration. Brunyé, Mahoney, Lieberman & Taylor (2010b) examined the effect of caffeine (0mg, 100mg, 200mg & 400mg) on the ANT using a placebo-controlled, double-blind, repeated-measures design, in low caffeine consumers (M = 42.5mg/day). The results showed that caffeine improved the alerting and executive control networks following both the 200mg and 400mg doses, whereas 400mg of caffeine resulted in performance impairments on the orienting network components. In a further study the group assessed the effects on the attention networks in high caffeine consumers (M = 592.3mg/day; Brunyé, Mahoney, Lieberman, Giles & Taylor, 2010a) using the same experimental design. Whereas no effects on the orienting network were observed, there was a dose dependant increase in improvement on the alerting networks. However, compared to placebo a significant improvement was only seen at the highest dose

(400mg). Similarly, a dose dependant improvement in performance was evident on the executive control network; but only significant compared to placebo at the 400mg dose. There is also evidence that glucose (albeit in combination with taurine) can enhance the orienting network (Giles, Mahoney, Brunyé, Gardony, Taylor and Kanarek, 2012). Giles *et al.,* (2012) evaluated the effects of caffeine, taurine and glucose alone and in combination on the attention networks. Participants received various caffeine and taurine dosage combinations together with either glucose (50g) or placebo. They found that 200mg caffeine improved the performance of the executive control network, and glucose in combination with taurine improved the performance of the orienting network.

Taken together these findings suggest that caffeine and glucose can have beneficial effects on attention. As the ANT allows assessment of several aspects of attention (alerting, orienting, and central-executive function) within a single procedure, using this approach might help elucidate substance specific effects on attention and might help explain why some studies failed to find effects on attention. As previous research has found the most robust effects with caffeine administration on the executive control network, this will be primary efficacy outcome for this study. In addition, attention will be tested in the afternoon, following a two hour fast. Given that people may consume energy drinks as an afternoon 'pick-me-up' after a period of post-lunch fasting; this will increase the generalisability of the previously observed facilitation effect and to establish its ecological validity.

The doses of 40mg caffeine and 40g of glucose where chosen with consideration to the memory tasks reported in Chapter 4 as these two studies utilised the same participants and testing schedule. So far the 25g glucose has not been shown to have beneficial effects on cognitive performance, and therefore 40g of glucose was chosen as the dose of interest to investigate.

5.2 Method and Materials

5.2.1 Participants

Thirty healthy young adults (10 males, 20 females) took part in the study, recruited via the Online Research Participation System (SONA) at Lancaster University. A sample size of 30 participants was deemed to be sufficient as this was comparable to other studies examining the same domains, both retrograde administration on recall and effects on the attention networks, and where effects of caffeine and glucose had been found (Brunyé, Mahoney, Lieberman, Giles & Taylor, 2010a; Brunyé, Mahoney, Lieberman & Taylor 2010b; Manning *et al.,* 1998). The age range was 18-35 years (mean age 21.53 years). They all consumed at least 120mg caffeine per day, (average consumption was 225.75mg). Exclusion criteria included; a diagnosis of Diabetes Mellitus; any intolerance or allergic reaction to substances that contain phenylalamine and/or caffeine; being non-native English speakers; having a history of neurological or psychiatric illness (excluding depression or anxiety); having a current diagnosis of neurological or psychiatric illness (including depression or anxiety); currently taking medication or nutritional supplements (excluding the contraceptive pill); being pregnant, seeking to become pregnant or breastfeeding; having a history of or currently abusing drugs or alcohol; or smoking. The study was approved by the Department of Psychology Ethics Committee at Lancaster University. Participants gave their signed informed consent prior to taking part and a Clinical Records Form (CRF) was used to confirm their eligibility.

5.2.2 Design

The study followed a placebo controlled, repeated measures design; the within factor being treatment (glucose (40g), caffeine (40mg), glucose/caffeine (40g/40mg), and placebo). There was at least a 48hr washout period between visits. Participants were randomly allocated to a treatment regime using a Latin square which counterbalanced the order of test drinks across the study days.

5.2.3 Treatments

The treatments were supplied by Suntory Food and Beverage Europe in 380ml solutions. There were three active drinks; glucose containing 40g glucose, caffeine containing 40mg caffeine and a glucose and caffeine combination containing 40g glucose and 40mg caffeine. A taste matched placebo was also utilised.

Participants were instructed to consume one drink per test session within 5 min. Cognitive testing started 20 minutes after drink administration. A twenty minute delay was chosen as peak plasma caffeine concentration is reached between 15 to 120 min after oral ingestion and brain levels remain stable for at least one hour following peak plasma levels (Nehlig, 1999; Nehlig & Boyet, 2000). Also this time frame is similar to the procedure of previous glucose studies (Foster, Lidder & Sünram, 1998) in order to ensure successful transfer of plasma glucose to brain.

5.2.4 Procedure

Initial screening was done during sign-up using the Lancaster recruitment on-line system (SONA). At the first visit for screening and training, all participants completed the

voluntary written informed consent prior to any study procedures being performed. The participant was screened by the researcher and the outcome of the screening activities was recorded in the CRF. Personal demographic information (such as age, education, height and weight) was also collected at this visit. They also completed a caffeine consumption questionnaire to confirm their daily caffeine consumption. Training on the cognitive task was then completed. No drinks were administered during the practice sessions and performance data from these sessions was not included in the analysis.

Participants then attended the laboratory on a further four times to complete their study sessions. All the study days followed the same procedure and were separated by at least a 48hr wash out period. Upon arrival at the morning encoding session, participants gave a small sample of blood for the measurement of blood glucose. They then completed the presentation phase of the memory tasks. The results of the memory tasks are not reported or discussed here; they form the experimental data for chapter 4. Participants were then free to go until they returned for their afternoon retrieval session. The afternoon session started 6hrs after the start of the encoding session. Upon arrival the participant gave a finger prick blood sample (the results of the blood glucose analysis are discussed in chapter 4). They then consumed their treatment drink for the day (following a double-blind procedure). The post-drink cognitive test session commenced 20 minutes after drink consumption. At around 15 mins post treatment they gave another finger prick blood sample for blood glucose measurement. After the full 20 mins absorption period, they then completed the retrieval phase of the memory tasks. Following on from this they then completed the Attention Network test. A final finger prick blood sample was taken at the end of this testing session. All participants received a debriefing sheet at the final day of testing.

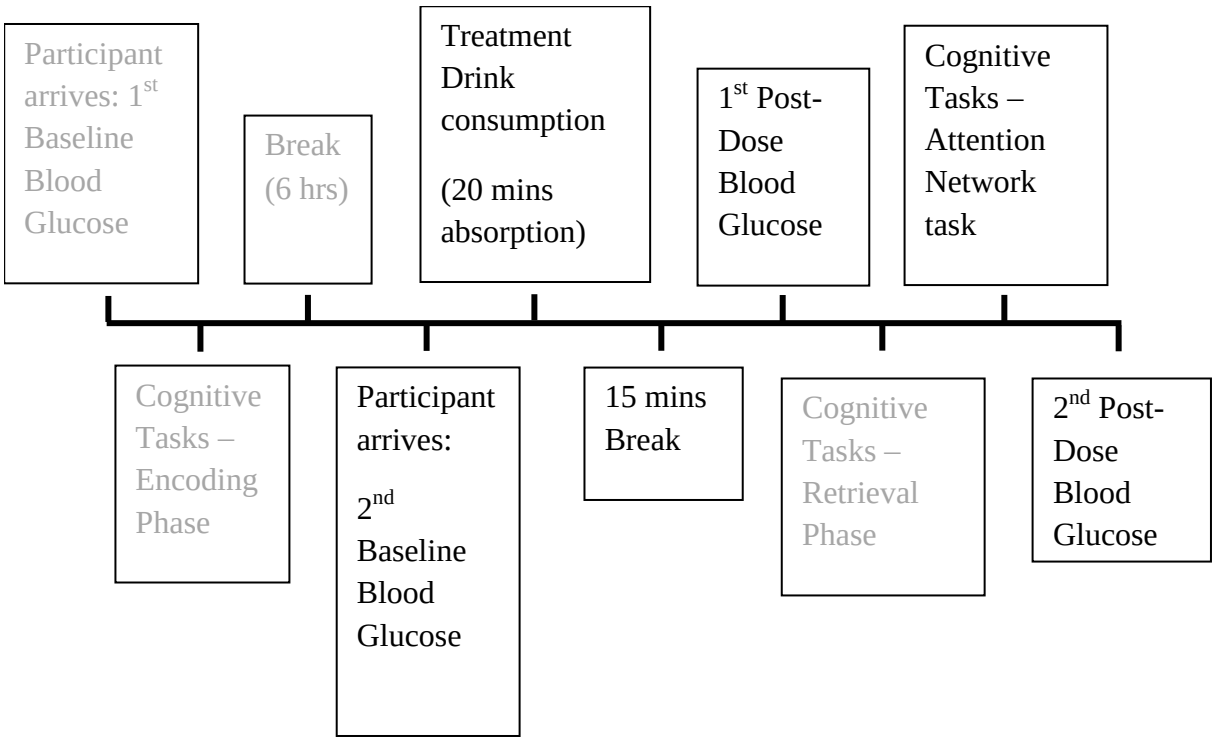

Figure 5.1 Schematic of the study day procedure

5.2.5 Assessments

The *Attention Network Test (ANT)* (Fan *et al.*, 2002) was delivered using E-Prime.

The ANT is used to assess the performance of the three visual attention networks (alerting, orienting, and executive control; Posner & Petersen, 1990), which are all neuroanatomically-defined.

During each trial, the participant fixated on a point (400-1600ms), they then saw a cue (100ms), there was then a continued fixation period (400ms) and then an array of horizontally-aligned arrows appeared, either above or below a central fixation point (maximum 1700ms). The cue could alert the participant that the trial was about to be presented ("centre"), or it could also orient the individual to where the trial would be presented (above or below fixation: "spatial"; both above and below fixation: "double"). A central target arrow was then presented within an array of horizontally-aligned congruent

arrows (same facing direction), incongruent arrows (opposite facing direction), or neutral lines (without arrow heads). There was then a variable intertribal interval (calculated as 3500ms minus first fixation duration minus reaction time).

Three blocks of 96 trials were presented in a random order (total of 288 trials). In each block, there were two trials presented for each of the four cue conditions (none, centre, double and spatial), two target locations (top, bottom), two target directions (left, right), and the three flanker conditions (neutral, congruent, incongruent). Participants responded to the central arrow's direction (left or right) and this reaction time was measured.

In order to assess each of the attention networks, change scores were calculated for alerting, orienting and executive function (Fan *et al.*, 2002).

For the alerting score, the average double-cue RTs was subtracted from the no-cue RTs. For the orienting score, the average spatial RTs were subtracted from the centre cue RTs. Higher scores on both of these would indicate more efficient alerting and orienting functioning. For the executive control, a conflict change score was calculated by taking the incongruent flankers RTs and subtracting the average congruent flankers RTs (across all cue types). Here a lower score would indicate more efficient executive control functioning with conflicting information.

Blood glucose measurement

Blood glucose readings were obtained using the ExacTech® blood glucose monitoring equipment (supplied by MediSense Britain Ltd, 16/17 The Courtyard, Gorsey Lane, Coleshill, Birmingham B46 1JA), following the recommended procedure: The researcher swabbed the finger of the volunteer with a Sterets Isopropyl Alcohol BP Pre-injection swab

(Seton Healthcare Group, Oldham, UK) and allowed the skin to air dry. The skin was punctured using an automatic lancing devise and a drop of blood was collected onto the analytical test strip. The volunteer applying a tissue blotted any excess blood. Ambidex-HG powder free polymer bonded latex examination gloves were worn by the experimenter at all times during the procedure. Each lancet and cap was only used once and then disposed of into a sharps container. Swabs, test strips, tissues and gloves were placed in a clinical waste sack.

For performance characteristics of ExacTech® blood glucose monitoring equipment see Chapter 2.

5.2.6 Statistical Analysis

The primary efficacy variable was the executive control network. The secondary outcome variables were the alerting and alerting and orienting networks. The absolute scores for all the behavioural variables were used in the analyses. Using IBM SPSS Statistics Version 22 a linear mixed model was used to analyse the treatment drinks (Caffeine, Glucose, Caffeine and Glucose, and placebo). Treatment drink, period and treatment by period interaction were added as fixed factors and subject as a random factor. The Estimates of Fixed Effects were used to compare each active treatment with placebo.

For glycaemic treatment comparisons were performed on the blood glucose levels using a linear mixed model. The model included a random effect for subject and fixed effects for period, treatment and time. The Estimates of Fixed Effects were used to compare each active treatment with placebo (the results of glycaemic analysis are reported in Chapter 4).

5.3 Results

Participant demographics are as described in Chapter 3.

5.3.1 Blood Glucose

As reported in Chapter 3.

5.3.2 Attention Network Task Results

5.3.2.1 Primary Outcome

Executive Control

There was no significant main effect of treatment on the executive control component of the ANT, F (3, 36.52) = 0.44, p = .73. There was no significant main effect of period, F (3, 35.43) = 2.40, p = .08, or treatment x period interaction, F (9, 22.55) = 0.61, p = .78. None of the active treatment drinks led to superior performance compared to placebo.

5.3.2.2 Secondary Outcomes - Attention

Alerting

There was no significant main effect of treatment on the alerting component of the ANT, F (3, 39.94) = 1.25, p = .30. The main effect of period, F (3, 38.93) = 2.83, p = .05, failed to

reach significance and no treatment x period interaction was observed, F (9, 25.28) = 0.46, p = .89. Comparison with placebo showed no significant differences following administration of any of the active treatment drinks.

Orienting

There was no significant main effect of treatment on the orienting component of the ANT, F (3, 37.95) = 0.59, p = .63. There was no main effect of period, F (3, 36.77) = 0.77, p = .52; and the interaction between treatment and visit was also not significant, F (9, 33.11) = 1.79, p = .11. As before, no significant differences were observed following administration of treatment drinks compared to placebo.

Table 5.1 Attention Network Results, means and standard errors

Treatment	Combined	Glucose	Caffeine	Placebo
Alerting	31.1	35.7	40.4	41.2
	(4.0)	(5.2)	(4.0)	(4.7)
Orienting	56.9	54.8	50.0	59.8
	(5.6)	(3.6)	(5.7)	(4.7)
Executive Control	75.7	79.7	78.2	83.9
	(4.4)	(5.1)	(6.0)	(5.8)

*Significant compared to placebo at p < .05

5.4 Discussion

The aim of the study was to investigate the effect of caffeine and glucose administered in isolation and combination on attention, using an approach that allows evaluation of different attentional networks and systems. The study found no significant effects of glucose, caffeine and their combination on the primary efficacy variable of the executive attention network, or on either of the secondary efficacy variables (alerting and orienting network) and therefore fails to support previous findings that caffeine has beneficial effects on the alerting and executive control networks (Brunyé *et al.*, 2010a; Brunyé *et al.*, 2010b) and that glucose (in combination with taurine) has beneficial effects on the orienting network (Giles *et al.*, 2012).

Limited research has examined the effects of caffeine and glucose on these attention networks, however there are clear underlying mechanisms of action whereby consumption of glucose and caffeine could assert their effects. For example, the areas of the brain which underlie the alerting and executive control network are rich in dopamine receptors (Ferre *et al.*, 1992; Ferre, 2008; Lumme, Aalto, Ilonen, Nagren & Hietal, 2007). For the alerting network this is the thalamus and bilateral frontal and parietal brain regions (Fan *et al.*, 2005); and for the executive control network this is the anterior cingulate cortex (ACC) and lateral prefrontal cortices (Botvinick, Braver, Barch, Carter & Cohen, 2001; Bush, Luu & Posner, 2000; Fan *et al.*, 2005). Therefore these attention networks are likely to be the most susceptible to caffeine modulation as caffeine has a strong interaction with central dopaminergic systems (Einother & Giesbrecht, 2013). Conversely, the brain areas underlying the orienting network have limited dopaminergic activity and so caffeine is unlikely to modulate this area (Einother & Giesbrecht, 2013). However the parietal lobe, which is the brain area which underlies the orienting network, is involved in the

cholinergic system (Einother & Giesbrecht, 2013) and therefore could be susceptible to modulation by glucose (Messier, 2004). In their review of the literature on the effects of caffeine on attention, Einother and Giesbrecht (2013) concluded that doses ranging from 60-400mg had beneficial effects on the executive control network.

Consequently, dose might be an important factor when trying to explain the lack of effect observed in the current study. Previously quite high levels of caffeine i.e. 200mg and 400mg have been needed to elicit beneficial effects on the attention networks (Brunyé *et al.*, 2010a; Brunyé *et al.*, 2010b). Despite the fact that previous research has found caffeine doses of between 12.5mg to 100mg to have a fairly flat dose response rate on cognitive task performance (Smit & Rogers, 2000), it might be that higher caffeine doses are needed to elicit the effects on the attention networks. With regards to glucose, previous research by Giles *et al.*, (2012) found that 50g glucose in combination with 2000mg taurine improved the orienting network. This dose of glucose is very close to the one used in this research (40g), however it may be that the addition of the taurine has some modulating effect in addition to the glucose.

Also, whilst the task was fairly long (approximately 20mins), participants who were otherwise performing optimally (e.g. fully rested), may not have found it sufficiently difficult to maintain their attention for this period of time. Therefore it may be necessary to deplete participants' cognitive resources prior to the drink consumption and completion of the ANT in order to tease out the potential beneficial effects of these substances. Giles *et al.*, (2012) also suggested that the lack of effects of glucose could be due to low task difficulty, as previously glucose has been shown to preferentially enhance task with a higher cognitive load (Kennedy & Scholey, 2000), so this may be one way to increase the cognitive load of the ANT. Increasing the duration of the task may also have this effect and increase its sensitivity to glucose and caffeine.

Whilst previous research has shown that caffeine and glucose can have beneficial effects on the attention networks (Brunyé *et al.*, 2010a; Brunyé *et al.*, 2010b; Giles *et al.*, 2012), this research failed to find effects on the ANT of either caffeine or glucose or their combination.

Chapter 6

Moderating effects of cognitive resource depletion on cognitive and mood effects of glucose and caffeine in combination

6.1 Introduction

Study 1 showed some improvements following both the low (15g glucose/20mg caffeine) and moderate dose (25g glucose/40mg caffeine) combination treatments on aspects of memory and attention with faster reaction times being observed on several task (e.g. Picture Recognition and Word Recognition). Whilst these findings are in line with previous research that also found combinations of caffeine and glucose to positively modulate performance on these tasks (Alford *et al.*, 2001; Horne & Reyner, 2000), they are not clear cut in terms of which aspects of cognition are improved by combined glucose and caffeine consumption. In general, when administered together they have been found to improve attention and ameliorate fatigue (Adan & Serra-Grabulosa, 2010; Kennedy & Scholey, 2004; Scholey & Kennedy, 2004; Smit, Cotton, Hughes & Rogers, 2004). However, the overall results of the literature remain equivocal and one reason for this could be due to participants' characteristics.

Task demand and more generally the activation state of the consumer may be important effect moderators. The psychoactive properties of caffeine and/or glucose may be most effective when participants are already fatigued and their performance and mood is below optimal levels or when task demand is particularly high. Indeed previous research has demonstrated that tasks, which are more cognitively demanding, are more susceptible to improvement by caffeine and glucose (Brice & Smith, 2001; Donohoe & Benton, 1999; Kennedy & Scholey, 2000; Smith *et al.*, 1999; Brown & Riby, 2013; Brandt, Gibson & Rackie, 2013). For example, Donohoe and Benton (2000) found that higher levels of blood glucose had more beneficial effects on the more cognitively demanding tasks. Indeed, falling blood glucose has also been associated with participants rating themselves as feeling less energetic when performing cognitively demanding tasks (Owens, Parker and

Benton, 1997). Kennedy and Scholey (2000) found that 25g glucose was more effective in enhancing performance on a cognitively demanding Serial 7s task (as rated by the participants). In addition, enhanced performance following glucose administration was observed for the for the most demanding episodic memory and attention task conditions (Brown and Riby, 2013; Meikle, Riby & Stollery, 2005) and the incongruent and therefore more difficult trials in the Stroop task paradigm (Brandt *et al.,* 2013). Moreover, glucose facilitation of memory performance has most robustly been observed under dual task conditions (Sünram-Lea, Foster, Durlach & Perez, 2002). Consequently, tasks that are more cognitively demanding may be particularly sensitive to glucose loading. For caffeine, there is evidence to suggest effects are most pronounced when consumers are fatigued, sleep-deprived (Lorist, Snel, Kok and Mulder 1994; Schweitzer, Muehlbach & Walsh, 1992; Horne & Reyner, 1996), and/or have to sustain performance over longer periods of time (Scholey and Kennedy, 2004). Lorist *et al.,* (1994) examined the effects of 200mg and 50mg caffeine on Event Related Potentials (ERPs) during a selective search task. Participants were either well-rested or fatigued. Although no difference in effectiveness between well-rested or fatigued individuals was observed on behavioural measures (reaction time), the state of the participants determined the neurocognitive effects to caffeine as measured by the P3b component. The effect of caffeine on this component considered to reflect the maintenance in working memory was greater in participants who were fatigued compared to those who were well-rested. Another example is Brice and Smith's (2001) study which demonstrated that when performing two consecutive cognitively demanding tasks (either a simulated driving task or a sustained attention task), performance on the second task was improved following administration of 3mg/kg of caffeine. Specifically it improved steering accuracy in the driving task and increased

accuracy on the sustained attention task. Self-reported alertness was also increased following the caffeine treatment prior to and on completion of both tasks.

With regards to the combined administration literature, where more robust effects of glucose and caffeine have been found, these are often in participants who are performing below their optimal potential, for example after sleep restriction, physical exertion; over long periods of cognitive demand or performing under stress (Alford, Cox & Wescott, 2001; Horne & Reyner, 2001; Kennedy & Scholey, 2004; Sünram-Lea, Owen-Lynch, Robinson, Jones & Hu, 2012). Horne and Reyner (2001) administered an 'energy drink' containing 42g glucose and 30mg caffeine to participants prior to a 2hr drive in driving simulator. To ensure the participants were tired, they were sleep restricted to 5hrs sleep the night before testing. They found that, particularly in the first hour, the 'energy drink' in comparison to placebo, reduced lane drifting incidents and reduced the participants' reaction time to an auditory beep. Kennedy and Scholey (2004) examined the effects of three 'energy drinks' (68g glucose/38mg caffeine; 68g glucose/46mg caffeine; 60g glucose/33mg caffeine) compared to placebo, and they found that the active drinks improved performance and reduced mental fatigue during an extended period (60mins) of cognitively demanding tasks. An 'energy drink' containing 75mg caffeine and 37.5g glucose was found to maintain self-rated levels of arousal compared to a decline in this following placebo (Smit, Cotton, Hughes & Rogers, 2004). This was following tasks which due to their duration and repetition over an extended period of time were fatiguing and cognitively demanding (Smit *et al.*, 2004). The beneficial effects of glucose and caffeine have also been shown when participants have been under conditions of physiological and psychological stress. Sünram-Lea *et al.*, (2012) administered either one of two 'energy drinks' or a placebo to participants before they underwent a fire fighting search and rescue training exercise. Energy drink 1 contained 50g glucose and 40mg

caffeine, and energy drink 2 contained 10.25g fructose/glucose and 80mg caffeine. Memory performance was not impaired following energy drink 1 compared to the other two treatments. Both energy drinks improved information processing performance in comparison to a decrease in performance following the placebo. The participants also reported reduced self-assessed anxiety and stress following energy drink 1. These research findings support the idea that the beneficial effects of caffeine and glucose are most reliably observed when participants are operating at below optimal levels.

However, to date it is unclear whether prior depletion of cognitive and/or energy resources might be important to demonstrate beneficial effects. As demonstrated by time-on-task research, performance on prolonged tasks declines due to depletion of limited cognitive resources (Grier, Warm, Dember, Matthews & Galinsky, 2003; Smit, Eling & Coenen, 2004; Warm, Parasuramen & Matthews, 2008). The importance of the effects of state dependent moderating factor in drug research has been long recognised (Janke, 1983). However, to date no study has specifically addressed this question in the context of combined glucose and caffeine administration by manipulating the level of cognitive resource depletion prior to drink administration.

Consequently, the aim of this study is to further assess the effects of glucose and caffeine administration on cognition and mood and to evaluate whether these substances might have their greatest effects when cognitive resources are under strain due to prior depletion. Given that the most robust findings in the previous combined literature show effects of caffeine and glucose on attentional measures, the primary efficacy analysis will be the accuracy performance on the RVIP task.

The study is also designed to address issues raised around the memory task used previously in this research series (see chapter 2), for example it appears that tasks

involving more complex cognitive processes are more likely to show enhancement following single and combined drink administration (Kennedy and Scholey, 2000; Kennedy and Scholey, 2004; Smit *et al.*, 2004; Smit, Grady, Finnegan, Hughes, Cotton & Rogers, 2006). Therefore a memory task which includes a dual-tasking paradigm to increase the workload, an extended attention/vigilance task and a demanding working memory task were implemented. In addition, two different doses of glucose and caffeine in combination were administered to evaluate potential dose dependant effects.

6.2 Method and Materials

6.2.1 Participants

Fifty-nine healthy young adults aged 18-35yrs took part in the study. The Online Research Participation System (SONA) at Lancaster University was used for recruitment. A sample size of 30 participants in each condition (high and moderate) was deemed to be sufficient as this was comparable to other studies examining the effects of caffeine and glucose on cognitively demanding tasks, (Kennedy & Scholey, 2004; Scholey & Kennedy, 2000). They all consumed at least 120mg caffeine per day. Exclusion criteria included; a diagnosis of Diabetes Mellitus; any intolerance or allergic reaction to substances that contain phenylalamine and/or caffeine; being non-native English speakers; having a history of neurological or psychiatric illness (excluding depression or anxiety); having a current diagnosis of neurological or psychiatric illness (including depression or anxiety); currently taking medication or nutritional supplements (excluding the contraceptive pill); being pregnant, seeking to become pregnant or breastfeeding; having a history of or currently abusing drugs or alcohol; or smoking. Participants gave their signed informed consent to take part and a Clinical Records Form (CRF) was used to confirm their eligibility.

6.2.2 Design

The study followed a placebo controlled, mixed 3x2x2 design; the within factors was treatment (two different doses of glucose and caffeine in combination; 60g glucose/40mg caffeine and 25g glucose/40mg caffeine, and placebo) and time (pre- versus post drink

assessment) and the between factor was the manipulation of resource depletion (depletion versus no depletion). Depending on the resource depletion condition they were assigned to, they either completed a resource depletion task at the beginning of their visits or watched a DVD prior to baseline testing and drink administration. There was a 7(+/-2)-day washout period between visits. Participants were randomly assigned to one of the two resource depletion conditions and treatment order was allocated according to a Latin Square.

6.2.3 Treatments

The treatments were supplied by GlaxoSmithKline Laboratories in 380ml solutions. The "high" dose consisted of 60g glucose and 40mg caffeine; the "moderate" dose consisted of 25g glucose and 40mg caffeine, and a taste matched placebo was also utilised.

Participants consumed one treatment drink per test session. They were instructed to consume these within 5 minutes and post-dose testing started 20 minutes later. A 20 minute delay was chosen as peak plasma caffeine concentration is reached between 15 to 120 min after oral ingestion and brain levels remain stable for at least one hour following peak plasma levels (Nehlig, 1999; Nehlig & Boyet, 2000). Moreover, a recent GSK study has shown that behavioural effects can be observed 14 minutes following caffeine consumption (Rogers; RHS00794). Also this time frame was similar to the procedure of previous glucose studies (Foster, Lidder & Sünram, 1998) in order to ensure successful transfer of plasma glucose to the brain.

6.2.4 Procedure

Initial screening was done during sign-up using the Lancaster recruitment on-line system (SONA). At the first visit for screening and training, all participants completed the voluntary written informed consent prior to any study procedures being performed. The participant was screened by the researcher and the outcome of the screening activities was recorded in the CRF. Personal demographic information (such as age, education, height and weight) was also collected at this visit. They also completed a caffeine consumption questionnaire to confirm their daily caffeine consumption. Training on the cognitive task was then completed. No drinks were administered during the practice sessions and performance data from these sessions was not included in the analysis.

Participants then attended the laboratory on a further 3 occasions to complete the testing sessions. Testing was carried out between 8am and 12 noon and participants were asked to fast for 12hrs prior to the session (i.e. no food or drink except water) and to abstain from alcohol for 24 hours prior to testing. There was a 7(+/-2) day washout period between active days of the study. Consequently, participants were required to attend a weekly morning session over a period of approximately five to six weeks. Participants were randomised to their Depletion/No Depletion group and treatment schedule on arrival at the lab for their first study day. All active study days followed the same procedure.

Upon their arrival participants completed either the resource depletion task or watched a DVD for 30 minutes. All participants then had a blood glucose sample taken via a finger prick to measure their baseline blood glucose levels. Further samples were taken at 15 and 40 minutes after treatment administration. The first blood samples were followed by pre-treatment baseline evaluation of mood and cognition. This was followed by administration of the day's treatment (following a double-blind procedure). The post-treatment cognitive test session commenced 20 minutes after drink consumption. Each test session comprised of the completion of the cognitive test battery (cognitive performance), the Bond-Lader

visual analogue scales, and the Mood, Alertness and Physical Symptoms (MAPS) Questionnaire. All participants received a debriefing sheet at the final day of testing.

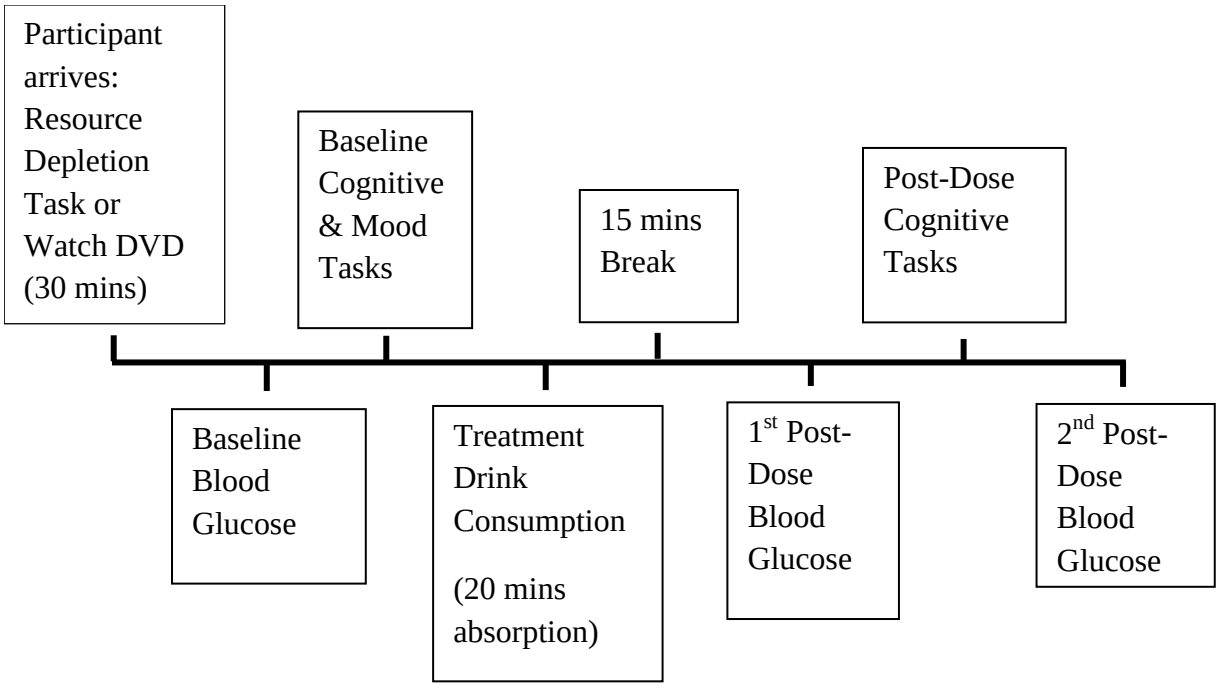

Figure 6.1 Schematic of the study day procedure

6.2.5 Assessments

Cognitive Tasks

Computerized assessment was used to evaluate cognitive performance. All tasks were delivered within the Computerized Mental Performance Assessment System (COMPASS), a purpose designed software application for the flexible delivery of randomly generated parallel versions of standard and novel cognitive assessment tasks. With the exception of the paper and pencil tasks (word recall); all responses were made using the computer keyboard and mouse. In this case the assessment comprised a selection of standard

psychometric tasks with stimuli chosen to possess good face validity in an 'everyday' context. The elements of the cognitive assessment are described below.

Resource Depletion Battery

The manipulation of cognitive-resource depletion was achieved by using two repetitions of two cognitively demanding tasks, each repetition lasting 15 minutes, making the total battery 30 minutes. The tasks were;

Computerised Serial Sevens Task

This task evaluates working-memory performance (Hayman, 1942). Participants were required to compute a running subtraction of 7, starting from a randomly generated number. Participants were given 5 minutes to complete this task. The number of responses, number of correct responses and number of incorrect responses were recorded.

Digit Vigilance

This task measures sustained attention (Lewis, 1995). A single target digit was randomly selected and continuously displayed on the right side of the screen. In the centre a series of rapidly changing digits was displayed. Participants were required to press the space bar button as quickly as possible, whenever the digit in the centre matched the target digit. The task lasted for 10 minutes. Reaction times (milliseconds), percentage accuracy and number of false alarms were recorded.

Pre and Post-dose Cognitive Assessment

Word presentation

A list of 20 words matched for frequency, concreteness and imagery is presented on the monitor at the rate of 1 every 2 seconds for participants to remember. During encoding, participants were required to perform two complex hand-movement sequences (Sünram-Lea *et al.*, 2001). Each sequence was performed using both hands and contains three movements: fist – chop – slap and back-slap – chop – fist. Participants were told to alternate the sequence every fifth word and they were not informed when to change, only that they had to keep track of this themselves. Hand-movements were performed continually during word presentation.

Distractor Task

After viewing the words participants engaged in an arithmetic task, which serves to prevent the rehearsal of items in working memory. The participants completed a short computerised Serial Threes Subtraction task, this was the same as the Serial Sevens subtraction task except it required the serial subtraction of 3s. The distracter task lasted for 30 seconds.

Immediate word recall

Immediately after the words were presented participants were given 60-seconds to write down as many words as they can from the list they have just seen. Participants' responses are marked according to total number of errors and number of words recalled correctly.

Rapid Visual Information Processing (RVIP)

A series of numbers between 1 and 9 will appeared one at a time in quick succession. Participants were asked to press the space bar whenever they saw three odd or three even numbers in a row. The numbers were presented at the rate of 100/min. The task lasts for 10 minutes. Percentage accuracy, reaction time and number of false alarms were recorded.

3-Back Task

Participants were presented with a series of letters one at a time. For each letter they had to decide if it was the same as one presented 3 letters previously in the series and then press the 'm' key for 'yes or the 'z' key for 'no' on their keyboard. The task lasted for 5 minutes. Percentage accuracy and reaction times for both distractors and targets were recorded in addition to overall reaction times.

Delayed word recall

Participants were given 60-seconds to write as many words as they could remember from the list they have seen at the beginning of the battery. Participant's responses were marked according to total number of errors and number of words recalled correctly.

Delayed word recognition

The 20 original words and 20 distractor words were presented individually in a randomised order. Participants were asked to indicate whether each word had been in the original list or not by using the 'm' key for 'yes' or the 'z' key for 'no' on their keyboard. The percentage accuracy and reaction times for both distractors and targets were recorded in addition to overall reaction times.

Subjective Mood

The Bond and Lader visual analogue scales (VAS; Bond & Lader, 1974)

Visual Analogue Scales were presented on the screen immediately after the cognitive tests. Participants used the mouse to position a cross at the point on the scale that represented

their feelings at that moment. The 16 scales were combined as recommended by Bond and Lader (1974) to form three mood factors: ‘alertness’, ‘calmness’ and ‘contentment’.

Mood, alertness and physical symptoms questionnaire (MAPS) (Rogers *et al.*, 2008)

This computerised questionnaire consisted of seven unipolar and four bipolar visual analogue scales adapted from a similar questionnaire used in previous research on caffeine (e.g. Rogers *et al.*, 2005). ‘Headache’, ‘heart pound’, jittery/shaky’, ‘light-headed/feeling faint/dizzy’, ‘hands trembling’, ‘scared’ and ‘feeling hot/sweating (not due to heat)’ were rated on unipolar scales labelled ‘I don’t have this feeling at all’ (left-hand end=0) and ‘I have this feeling strongly (right-hand end=100). The bipolar scales were Relaxed (labelled ‘anxious/tense/nervous/on edge’=0 and ‘relaxed/calm’=100), Clearheaded (labelled ‘muzzy/dazed’=0, and ‘clearheaded’=100), Happy (labelled ‘sad/gloomy/miserable’=0 and ‘happy/cheerful/light-hearted’=100), and Alert (labelled ‘drowsy/sluggish/tired/fatigued’=0 and ‘alert/energetic/lively’=100). Instructions asked participants to rate ‘how you feel RIGHT NOW’. Scores for each item were obtained.

Blood glucose measurement

Blood glucose readings were obtained using the ExacTech® blood glucose monitoring equipment (supplied by MediSense Britain Ltd, 16/17 The Courtyard, Gorsey Lane, Coleshill, Birmingham B46 1JA), following the recommended procedure: The researcher swabbed the finger of the volunteer with a Sterets Isopropyl Alcohol BP Pre-injection swab (Seton Healthcare Group, Oldham, UK) and allowed the skin to air dry. The skin was punctured using an automatic lancing devise and a drop of blood was collected onto the analytical test strip. The volunteer applying a tissue blotted any excess blood. Ambidex-HG powder free polymer bonded latex examination gloves were worn by the experimenter at all times during the procedure. Each lancet and cap was only used once and then disposed of

into a sharps container. Swabs, test strips, tissues and gloves were placed in a clinical waste sack.

For performance characteristics of ExacTech® blood glucose monitoring equipment see Chapter 2.

6.2.6 Statistical Analysis

The absolute pre and post mental fatigue scores from the Resource Depletion Battery analysed using a linear mixed model. Treatment, period and time were added as fixed effects and subject as a random effect to the model. The primary efficacy analysis was attention mentioned by accuracy on the RVIP task. The secondary analyses were; attention (measured by speed and false alarms on the RVIP), memory (measured by immediate word recall correct and errors, delayed word recall correct and errors, word recognition accuracy and speed and 3-Back accuracy and speed) and mood (measured by Bond-Lader and MAPS). All these behavioural and mood variables were transformed into change from baseline scores. Using IBM SPSS Statistics Version 22, the change from baseline scores within each group (No Depletion/Depletion) were analysed using a linear mixed model. Treatment and period were added as fixed effects and subject as a random effect to the model. Baseline average for each individual task were added as a covariate to the model. The Estimates of Fixed Effects were used to compare each active treatment with placebo.

For glycaemic treatment comparisons were performed on the blood glucose levels using a linear mixed model. The model included a random effect for subject and fixed effects for period, treatment and time. The Estimates of Fixed Effects were used to compare each active treatment with placebo.

6.3 Results

6.3.1 Demographics, means and standard deviations

	N	Gender	Age (yrs)	BMI (kg/m^2)	Years in Full Time Education	Average Caffeine Consumption (mg)
No Depletion Group	29	9 Males/ 20 Females	19.97 (1.80)	24.26 (5.19)	14.59 (1.50)	264.65 (86.11)
Depletion Group	30	13 Males/ 17 Females	20.90 (2.94)	23.30 (3.56)	15.30 (2.59)	221.84 (84.29)

Independent samples t tests showed there were no significant differences between the groups in terms of their age, t (57) = -1.47, p = .148; BMI, t (57) = 0.82, p = .41; years in full time education, t (57) = -1.29, p = .20; and average caffeine consumption, t (57) = 1.93, p = .06.

6.3.2 Blood Glucose Results

No Depletion Group

There was a significant main effect of the treatments on blood glucose, F (2, 83.64) = 212.22, p < .001. There was a significant effect of time, F (2, 86.63) = 106.32, p < .001

and treatment x time interaction F (4, 64.08) = 80.27, p < .001 were observed. Comparisons showed blood glucose levels did not differ significantly across any of the time points within the placebo treatment. Following the moderate dose treatment blood glucose levels differed significantly from baseline (M = 4.82) at the second post dose time point (M = 7.43), p < .001, but not at the first post dose time point (M = 6.15) compared to the second post dose time point, p = .17. Following the high dose treatment blood glucose levels differed significantly from baseline (M = 4.86) at the second post dose time point (M = 8.26), p < .001, and at the first post dose time point (M = 6.80) compared to the second post dose time point, p = .004 (see Figure 6.1 for glycaemic response as a function of treatment and time).

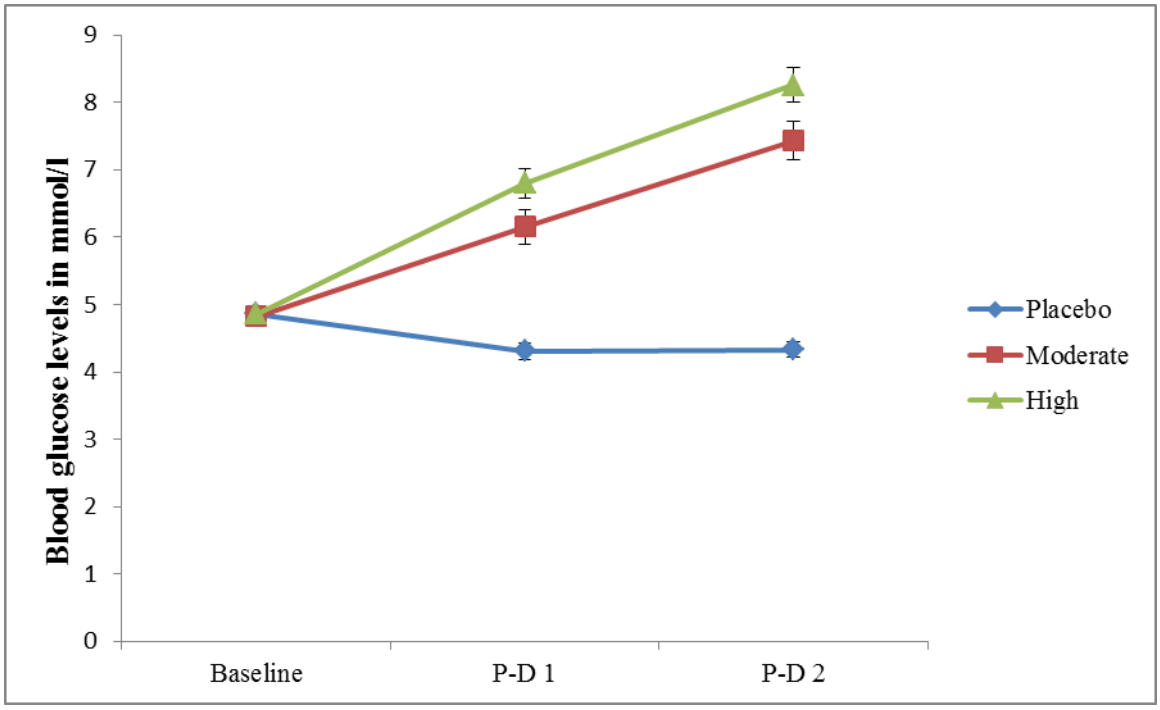

Figure 6.2 Glycaemic response in the No Depletion Group as a function of drink and time

Table 6.1 Glycaemic response to treatments in the No Depletion Group (means and standard deviations)

Time Point	Treatment		
	Placebo	Moderate	High
Baseline	4.86 (0.11)	4.82 (0.12)	4.86 (0.11)
1st Post Dose	4.31 (0.12)	6.15 (0.25)	6.80 (0.22)
2nd Post Dose	4.33 (0.12)	7.43 (0.29)	8.26 (0.25)

Depletion Group

There was a significant main effect of the low dose treatments on blood glucose, F (2, 103.81) = 184.52, $p < .001$. There was a significant effect of time, F (2, 98.57) = 98.05, $p < .001$ and treatment x time interaction F (4, 79.57) = 62.05, $p < .001$ were observed. Comparisons showed blood glucose levels did not differ significantly across any of the time points within the placebo treatment. Following the moderate dose treatment blood glucose levels differed significantly from baseline (M = 5.08) at the second post dose time point (M = 7.55), $p < .001$, but not at the first post dose time point (M = 6.64) compared to the second post dose time point, $p = .28$. Following the high dose treatment blood glucose levels differed significantly from baseline (M = 5.10) at the second post dose time point (M = 8.43), $p < .001$, but not at the first post dose time point (M = 7.09) compared to the second post dose time point, $p = .73$, (see Figure 6.2 for glycaemic response as a function of treatment and time).

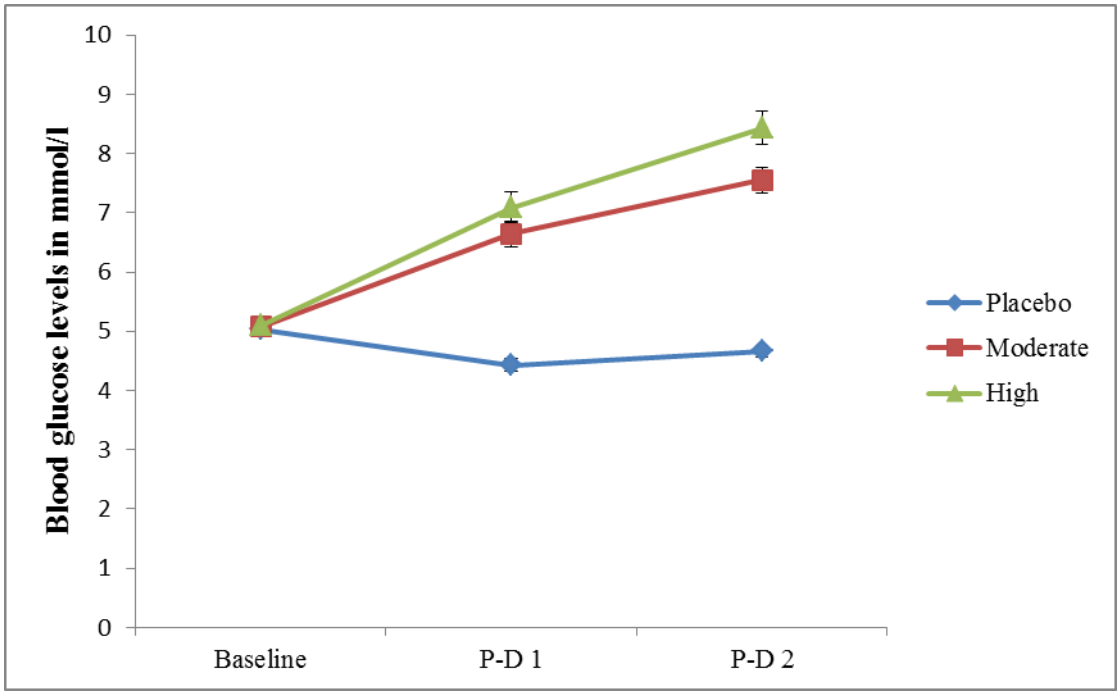

Figure 6.3 Glycaemic response in the Depletion Group as a function of drink and time

Table 6.2 Glycaemic response to treatments in the Depletion Group (means and standard deviations)

Time Point	Treatment		
	Placebo	Moderate	High
Baseline	5.03 (0.08)	5.08 (0.13)	5.10 (0.11)
1st Post Dose	4.43 (0.10)	6.64 (0.21)	7.09 (0.26)
2nd Post Dose	4.66 (0.10)	7.55 (0.22)	8.43 (0.28)

6.3.3 Cognitive Performance Results

Only participants who had not completed the tasks correctly were removed from the analyses for each individual task. In the No Depletion group one participant's scores from the 3 Back task were removed across all study days due to the incorrect completion of the task. In the Depletion group one participant's scores on all outcomes of the RVIP were removed on the day they received placebo due to incorrect completion of the task.

Resource Depletion Battery

The mental fatigue ratings scales completed before and after the Resource Depletion Battery show that the battery was successful in mentally fatiguing the participants, F (1, 133.73) = 30.78, $p < .001$.

Table 6.3 Mental Fatigue ratings pre and post Resource Depletion Battery, means and standard errors

Time Point	Mental Fatigue (VAS)
Pre-RDB	50.0 (2.0)
Post-RDB	61.9 (2.2)

No Depletion Group

6.3.3.1 Primary Outcome

RVIP Accuracy

There was no significant main effect of treatment on the correct responses on the RVIP task, F (2, 49.10) = 0.74, p = .48. There was a significant main effect of period, F (2, 38.63) = 7.02, p = .003, there was a trend for participants to perform better on study day 1 (period 1) compared to on study day 3 (period 3), p = .09. There was no interaction effect,

F (4, 38.12) = 2.36, *p* = .07. There were no significant differences observed following administration of treatment drinks compared to placebo.

6.3.3.2 Secondary Outcomes- Attention

RVIP Speed

There was no significant main effect of treatment on the correct responses on the reaction time of responses on the RVIP task, *F* (2, 41.57) = 0.70, *p* = .50; or period, *F* (2, 40.53) = 2.71, *p* = .08; or their interaction, *F* (4, 29.51) = 0.61, *p* = .66. Significant differences following administration of active treatment drinks compared to placebo were not identified.

RVIP False Alarms

There was no main effect of treatment on the on the false alarm rate on the RVIP task, *F* (2, 44.92) = 0.51, *p* = .61; or period, *F* (2, 48.30) = 0.25, *p* = .78; or their interaction, *F* (4, 41.03) = 0.09, *p* = .90. There were no significant differences observed following administration of treatment drinks compared to placebo.

Table 6.4 RVIP Performance, means and standard errors for the No Depletion Group

Task	Treatment		
	Placebo	Moderate	High
RVIP Correct[1]	-3.12 (2.01)	1.90 (6.44)	5.28 (6.27)
RVIP Speed[2]	13.1 (7.1)	-52.7 (19.7)	-39.9 (15.5)
RVIP FAs[3]	-0.27 (0.44)	-0.59 (0.45)	-0.91 (0.45)

Change from baseline; covariates appearing in the model for treatments are evaluated at the following average baseline values: [1]46.34; [2]524.7;[3]2.84.

*Significant compared to placebo at p < .05

6.3.3.3 Secondary Outcomes - Memory

Immediate Word Recall Correct

There was no significant main effect of treatment on the correctly recalled words in the immediate word recall task, F (2, 53.74) = 0.27, p = .77. There was a significant main effect of period, F (2, 44.42) = 3.96, p = .03, with participants performing significantly better at study day 1 (period 1) compared to study day 3 (period 3), p = .005. There was no significant interaction effect between treatment and period, F (4, 34.97) = 1.02, p = .41. There were no significant differences were observed following administration of any of the active treatment drinks compared to placebo.

Immediate Word Recall Incorrect

There was no significant main effect of treatment on the incorrectly recalled words in the immediate word recall task, F (2, 46.96) = 0.04, p = .96; or period, F (2, 41.02) = 0.04, p = .97; or their interaction, F (4, 38.56) = 0.79, p = .54; There were no significant differences observed following administration of any of the active treatment drinks compared to placebo.

Delayed Word Recall Correct

There was no main effect of treatment on the correct words recall on the Delayed Word Recall task, F (2, 45.46) = 0.09, p = .92; or period, F (2, 48.01) = 2.44, p = .10; or their interaction, F (4, 34.76) = 0.20, p = .94. There were no significant differences observed following administration of treatment drinks compared to placebo.

Delayed Word Recall Incorrect

There was no significant main effect of treatment on the incorrect words recall on the Delayed Word Recall task, F (2, 28.79) = 0.18, p = .84; or period, F (2, 39.79) = 0.11, p = .89; or their interaction, F (4, 26.88) = 0.98, p = .43. Comparison of the active drinks with placebo showed an advantage of the placebo drink compared to the high dose treatment drink (p = .10).

Table 6.5 Word Recall Performance, means and standard errors for the No Depletion group

Task	Treatment		
	Placebo	Moderate	High
Immediate Word Recall Correct[1]	-0.82 (0.32)	-1.02 (0.37)	-1.19 (0.41)
Immediate Word Recall Incorrect[2]	0.21 (0.25)	0.20 (0.16)	0.30 (0.34)
Delayed Word Recall Correct[3]	-1.47 (0.41)	-1.28 (0.42)	-1.24 (0.42)
Delayed Word Recall Incorrect[4]	0.21 (0.43)	.17 (0.16)	.36 (0.27)

Change from baseline; covariates appearing in the model for treatments are evaluated at the following average baseline values: [1]5.74; [2]0.92; [3]4.72; [4]1.35.

*Significant compared to placebo at $p < .05$

Word Recognition Accuracy

The effect of the active treatments on the percentage correct responses on the Delayed Word Recognition failed to reach significance, F (2, 45.07) = 2.10, p = .13. There was no main effect of period, F (2, 36.15) = 0.69, p = .51; or the interaction effect between treatment and period, F (4, 34.21) = 0.52, p = .73. There were no significant differences observed following administration of treatment drinks compared to placebo, but a greater decline in performance was sees following the high treatment drink compared to placebo (p = .10).

Word Recognition Speed

There was no main effect of treatment on the correct reaction time response on the Delayed Word Recognition task, F (2, 44.15) = 0.97, p = .39; or period, F (2, 34.95) = 1.69, p = .20; or their interaction, F (4, 34.43) = 1.19, p = .33. There were no significant differences observed following administration of treatment drinks compared to placebo.

Table 6.6 Word Recognition Performance, means and standard errors for the No Depletion group

Task	Treatment		
	Placebo	Moderate	High
Word Recognition Accuracy[1]	0.76 (1.57)	-0.84 (2.08)	-3.93 (1.69)
Word Recognition Speed[2]	-33.6 (17.7)	10.6 (27.8)	-10.4 (27.8)

Change from baseline; covariates appearing in the model for treatments are evaluated at the following average baseline values: [1]68.68; [2]833.3

*Significant compared to placebo at p < .05

3 Back Accuracy

There was no significant main effect of treatment on the correct scores on the 3 Back task, F (2, 33.87) = 2.04, p = .15; or period, F (2, 33.67) = 2.31, p = .12; or interaction effect, F (4, 36.95) = 1.53, p = .21. There were no significant differences observed following administration of treatment drinks compared to placebo.

3 Back Speed

There was no significant main effect of treatment on the correct reaction time on the 3 Back task, F (2, 39.89) = 1.26, p = .30; or period, F (2, 42.52) = 1.28, p = .29; or their interaction, F (4, 38.81) = 1.35, p = .27. There were no significant differences observed following administration of treatment drinks compared to placebo.

Table 6.7 3 Back Performance, means and standard errors for the No Depletion Group

Task	Treatment		
	Placebo	Moderate	High
3 Back Correct[1]	-1.74 (1.03)	2.31 (1.97)	0.26 (0.93)
3 Back Speed[2]	-79.3 (15.7)	-52.7 (19.7)	-39.9 (15.5)

Change from baseline; covariates appearing in the model for treatments are evaluated at the following average baseline values: [1]86.83; [2]783.0.

*Significant compared to placebo at p < .05

6.3.3.4 Secondary Outcomes - Mood

Bond and Lader Visual Analogue Scale

Alert

There was no significant main effect of treatment on the participants' ratings of alertness on the Bond-Lader VAS, F (2, 44.20) = 2.08, p = .14; or period, F (2, 48.68) = 1.14, p = .33. There was a significant interaction effect, F (4, 38.29) = 3.52, p = .02. Following the

moderate dose drink participants rated themselves as significantly more alert on study day 3 (period 3), compared to on study day 1 (period 1) and study day 2 (period 2), $p = .01$ and $p = .02$ respectively. Comparison of the active drinks to placebo showed that participants rated themselves as more alert following the moderate treatment drink compared to placebo ($p = .002$).

Calm

There was no significant main effect of treatment on the participants' ratings of calmness on the Bond-Lader VAS, F (2, 45.76) = 0.30, $p = .75$; or period, F (2, 36.24) = 0.10, $p = .91$; or their interaction, F (4, 37.88) = 0.90, $p = .47$. There were no significant differences observed following administration of treatment drinks compared to placebo.

Content

There was no significant main effect of treatment on the participants' ratings of feeling content on the Bond-Lader VAS, F (2, 36.37) = 1.02, $p = .37$; or period, F (2, 46.53) = 1.04, $p = .36$; or their interaction, F (4, 34.62) = 0.53, $p = .72$. There were no significant comparisons between the active drinks and placebo.

Table 6.8 Bond and Lader Visual Analogue Scales, means and standard errors for the No Depletion Group

Task	Treatment		
	Placebo	Moderate	High
Alert[1]	7.5	13.7 *	8.9

	(1.9)	(2.4)	(2.1)
Calm[2]	-5.5 (2.1)	-5.3 (2.7)	-7.4 (2.0)
Content[3]	0.1 (1.6)	3.0 (1.4)	2.5 (1.6)

Change from baseline; covariates appearing in the model for treatments are evaluated at the following average baseline values: [1]42.3; [2]63.1; [3]55.6.

*Significant compared to placebo at $p < .05$

Mood, Alertness and Physical Symptoms Questionnaire (MAPS)

Headache

There was no significant main effect of treatment on participants' ratings of headache on the VAS, F (2, 34.23) = 0.13, p = .88; or period, F (2, 37.46) = 0.99, p = .38; or their interaction, F (4, 36.11) = 0.65, p = .63. There were no significant differences observed following administration of treatment drinks compared to placebo.

Heart Pound

There was no significant main effect of treatment on participants' ratings of heart pounding on the VAS, F (2, 43.04) = 0.43, p = .65; or period, F (2, 47.05) = 0.09, p = .92; or the interaction effect between treatment and period, F (4, 34.84) = 0.86, p = .50. No significant differences observed following administration of treatment drinks compared to placebo.

Jittery/Shaky

There was no significant main effect of treatment on the participants' ratings of jitteriness/shakiness, F (2, 40.05) = 1.98, p = .15; or period, F (2, 41.09) = 0.12, p = .89. There was a significant interaction, F (4, 36.80) = 2.91, p = .03, participants rated themselves as significantly less jittery following the high dose drink at study day 3 (period 3) compared to study 1 (period 1), p = .05. No significant comparisons were found between active drinks and placebo.

Light-headed/Feeling faint/Dizzy

There was a significant main effect of treatment on the participants' ratings of light-headedness/feeling faint/dizziness on the VAS, F (2, 37.33) = 4.08, p = .03. There was no main effect of period, F (2, 35.24) = 2.70, p = .08; or interaction effect, F (4, 26.73) = 1.15, p = .36. Comparisons of the means did not reveal any significant differences; however examination of the means shows that participants rated themselves as less light-headed/feeling faint/dizzy after the high dose treatment drink.

Hands-trembling

There was no significant main effect of treatment on the participants' ratings of hands-trembling on the VAS, F (2, 36.94) = 0.12, p = .88; or period, F (2, 34.08) = 0.66, p = .52; or their interaction, F (4, 29.45) = 2.18, p = .10. There were no significant differences observed following administration of treatment drinks compared to placebo.

Scared

There was no significant main effect of treatment on the participants' ratings of sacredness on the VAS, F (2, 43.58) = 0.69, p = .51; or period, F (2, 46.63) = 0.69, p = .51; or their interaction, F (4, 44.99) = 0.59, p = .67. There were no significant comparisons between any of the active drinks compared to placebo.

Feeling hot/sweating (not due to heat)

There was no significant main effect of treatment on the participants' ratings of feeling hot/sweating (not due to heat) on the VAS, F (2, 45.87) = 0.59, p = .56; or period, F (2, 37.06) = 0.35, p = .71; or their interaction, F (4, 34.03) = 0.72, p = .59. There were no significant differences observed following administration of treatment drinks compared to placebo.

Relaxed

There was no significant main effect of treatment on the participants' ratings of feeling relaxed on the VAS, F (2, 40.12) = 0.64, p = .53; or period, F (2, 30.18) = 0.23, p = .80; or their interaction, F (4, 32.46) = 0.76, p = .56. There were no significant differences observed following administration of treatment drinks compared to placebo.

Clearheaded

There was no significant main effect of treatment on the participants' ratings of feeling clearheaded on the VAS, F (2, 44.60) = 1.65, p = .20; or period, F (2, 43.33) = 0.12, p =

.88. There was a significant interaction effect between treatment and period, F (4, 37.51) = 5.08, p = .002. Participants rated themselves as more clearheaded following the moderate drink on study day 3 (period 3) compared to on study day 1 (period 1) and study day 2 (period 2), p = .005 and p = .03 respectively. Participants rated themselves as more clearheaded after the moderate dose drink compared to placebo, p = .005.

Happy

There was no significant main effect of treatment on the participants' ratings of feeling happy on the VAS, F (2, 39.07) = 0.91, p = .41; or period, F (2, 35.38) = 1.03, p = .37; or their interaction, F (4, 32.91) = 1.51, p = .22. Compared to placebo participants rated themselves as happier after the moderate dose drink, p = .04.

Alert

There was no significant main effect of treatment on the participants' ratings of feeling alert on the VAS, F (2, 39.30) = 1.89, p = .16; or period, F (2, 44.76) = 2.45, p = .10. There was a significant interaction effect between treatment and period, F (4, 35.20) = 5.28, p = .002, whereby participants rated themselves as significantly more alert after the moderate dose treatment on study day 3 (period 3) compared to on study day 1 (period 1). Compared to placebo participants rated themselves as more alert following the moderate dose drink, p = .002

Table 6.9 Mood, Alertness and Physical Symptoms Questionnaire (MAPS), means and standard errors for the No Depletion Group

Task	Treatment		
	Placebo	Moderate	High
Headache[1]	-5.0 (2.4)	-4.3 (2.9)	-6.3 (3.0)
Heart pound[2]	6.6 (3.8)	3.8 (3.6)	8.3 (3.2)
Jittery/Shaky[3]	2.7 (3.2)	8.0 (3.4)	11.9 (3.4)
Light-headed/feeling faint/dizzy[4]	-0.1 (3.3)	3.2 (3.1)	-6.8 (2.1)
Hands-trembling[5]	2.5 (3.0)	3.9 (2.6)	1.9 (3.5)
Scared[6]	1.5 (1.7)	-0.7 (0.9)	0.5 (2.1)
Feeling hot/sweating[7]	0.4 (1.6)	2.9 (2.7)	-0.4 (1.3)
Relaxed[8]	-7.2 (2.8)	-10.8 (3.5)	-5.8 (2.9)
Clearheaded[9]	6.3 (3.0)	14.0 * (3.0)	10.1 (2.9)
Happy[10]	1.3 (1.6)	5.2 * (2.6)	1.5 (2.2)
Alert[11]	10.5 (3.7)	19.6 * (3.1)	13.9 (2.9)

Change from baseline; covariates appearing in the model for low dose treatments are evaluated at the following average baseline values: [1]Headache = 26.3; [2]Heart pound = 15.2; Jittery/shaky[3] = 15.4; Light-headed/feeling faint/dizzy[4] = 24.4; Hands trembling[5] = 16.3; Scared[6] = 14.5; Feeling hot/sweating (not due to heat)[7] = 11.7; Relaxed[8] = 63.0; Clearheaded[9] = 41.3; Happy[10] = 51.2; Alert[11] = 36.4.

*Significant compared to placebo at $p < .05$

Depletion Group

6.3.3.5 Primary Outcome

RVIP Accuracy

There was no significant main effect of treatment on the correct responses on the RVIP task, F (2, 52.01) = 1.21, p = .31; or period, F (2, 44.14) = 0.44, p = .64; or their interaction, F (4, 38.58) = 1.22, p = .32. There were no significant differences observed following administration of treatment drinks compared to placebo.

6.3.3.6 Secondary Outcomes – Attention

RVIP Speed

There was no significant main effect of treatment on the Correct Reaction Time scores on the RVIP task, F (2, 21.35) = 0.35, p = .71; or period, F (2, 23.24) = 0.29, p = .75; or their interaction, F (4, 21.66) = 0.77, p = .56. There were no significant differences observed following administration of treatment drinks compared to placebo.

RVIP False Alarms

The main effect of treatment on the false alarm responses on the RVIP task failed to reach significance, F (2, 55.38) = 2.43, p = .10. There was no main effect of visit, F (2, 41.46) =

2.15, p = .13; or their interaction, F (4, 38.16) = 1.70, p = .17. Comparison of the active treatment drinks with placebo found no significant differences.

Table 6.10 RVIP performance, means and standard errors for the Depletion Group

Task	Treatment		
	Placebo	Moderate	High
RVIP Correct[1]	-0.72 (1.08)	1.55 (1.72)	-2.08 (1.61)
RVIP Speed[2]	-20.5 (15.2)	-4.9 (10.7)	-9.7 (7.3)
RVIP FAs[3]	0.38 (0.29)	-0.67 (0.42)	-0.33 (0.48)

Change from baseline; covariates appearing in the model for treatments are evaluated at the following average baseline values: [1]39.92; [2]546.0; [3]2.73.

*Significant compared to placebo at p < .05

6.3.3.7 Secondary Outcomes - Memory

Immediate Word Recall Correct

The main effect of treatment on the correct score of the Immediate Word Recall task just reached significance level, F (2, 36.85) = 3.25, p = .05. There was a main effect of period, F (2, 39.04) = 3.47, p = .04, with participants' performance being worse at their last visit compared to visit 1; however, no treatment x visit interaction was observed, F (4, 45.42) = 1.28, p = .29. Participants performed better following the placebo drink compared to after the moderate drink, p = .01.

Immediate Word Recall Incorrect

There was no significant main effect of treatment on the incorrect score of the Immediate Word Recall task, F (2, 34.14) = 1.86, p = .17; or period, F (2, 37.47) = 0.30, p = .74; or their interaction, F (4, 32.98) = 0.12, p = .97. There were no significant differences observed following administration of treatment drinks compared to placebo.

Delayed Word Recall Correct

There was no main effect of treatment on the correct responses on the Delayed Word Recall task, F (2, 47.83) = 0.34, p = .72. There was no main effect of visit, F (2, 46.70) = 1.16, p = .32; or the interaction effect between treatment and visit, F (4, 34.58) = 1.76, p = .16.

Delayed Word Recall Incorrect

There was no main effect of treatment on the incorrect responses on the Delayed Word Recall task, F (2, 44.55) = .97, p = .39; or period, F (2, 49.45) = 0.92, p = .41; or their interaction, F (4, 41.90) = 1.64, p = .18. There were no significant differences observed following administration of treatment drinks compared to placebo.

Table 6.11 Word Recall performance, means and standard errors for the Depletion Group

Task	Treatment		
	Placebo	Moderate	High
Immediate Word Recall	0.03 (0.43)	-1.35 * (0.40)	-0.80 (0.45)

Correct[1]			
Immediate Word Recall Incorrect[2]	-0.30 (0.21)	0.07 (0.15)	0.17 (.15)
Delayed Word Recall Correct[3]	-0.87 (0.49)	-1.28 (0.42)	-1.40 (0.49)
Delayed Word Recall Incorrect[4]	0.17 (0.22)	-0.30 (0.27)	0.07 (0.15)

Change from baseline; covariates appearing in the model for treatments are evaluated at the following average baseline values: [1]6.06; [2]0.57; [3]4.96; [4]0.86.

*Significant compared to placebo at p < .05

Word Recognition Accuracy

There was no significant main effect of treatment on the correct responses on the Word Recognition task, F (2, 30.96) = 0.21, p = .82; or period, F (2, 30.14) = 1.20, p = .31; or their interaction, F (4, 35.08) = 1.22, p = .32. Comparison of the active treatment drinks with placebo revealed no significant differences.

Word Recognition Speed

There was no significant main effect of treatment on the correct reaction time scores on the Word Recognition task, F (2, 41.27) = 0.92, p = .41; or period, F (2, 44.90) = 1.98, p = .15; or their interaction, F (4, 30.66) = 0.83, p = .52. Comparison of the active treatment drinks with placebo revealed no significant differences.

Table 6.12 Word Recognition performance, means and standard errors for the Depletion Group

Task	Treatment		
	Placebo	Moderate	High
Word Recognition Accuracy[1]	-3.67 (1.41)	-3.00 (1.34)	-4.33 (1.70)
Word Recognition Speed[2]	-37.7 (27.0)	-7.20 (27.8)	-55.7 (23.2)

Change from baseline; covariates appearing in the model for treatments are evaluated at the following average baseline values: [1]72.31; [2]838.6

*Significant compared to placebo at $p < .05$

3 Back Accuracy

There was no significant main effect of treatment on accuracy on the 3 Back task, F (2, 32.68) = 0.26, p = .77. There was a significant main effect of visit, F (2, 34.79) = 5.83, p = .007, with participants performing on study day 1 (period 1) compared to study day 3 (period 3), p = .15. There was no interaction effect between treatment and visit, F (4, 38.58) = 2.06, p = .10. Comparison of the active treatment drinks with placebo revealed no significant differences.

3 Back Speed

There was no significant main effect of treatment on the correct reaction time for the 3 Back task, F (2, 41.11) = 1.34, p = .27; or period, F (2, 39.97) = 1.34, p = .27; or their

interaction, F (4, 32.53) = 2.18, p = .09. Comparison of the active treatment drinks with placebo revealed no significant differences.

Table 6.13 3 Back performance, means and standard errors for the Depletion Group

Task	Treatment		
	Placebo	Moderate	High
3 Back Correct[1]	2.70 (1.38)	4.17 (1.85)	2.77 (1.24)
3 Back Speed[2]	-81.1 (18.9)	-108.8 (14.3)	-73.1 (19.1)

Change from baseline; covariates appearing in the model for treatments are evaluated at the following average baseline values: [1]83.71; [2]872.8.

*Significant compared to placebo at p < .05

6.3.3.8 Secondary Outcomes - Mood

Bond-Lader Visual Analogue Mood Scales

Alert

There was no significant main effect of treatment on the participants' ratings of alertness on the Bond-Lader VAS, F (2, 43.68) = 0.13, p = .88. There was a significant main effect of visit, F (2, 38.33) = 3.30, p = .05, whereby participants rated themselves as more alert at study day 1 (period 1) compared to study day 3 (period 3), p = .13, and less alert on study day 2 (period 2) compared to study day 3, p = .11. There was no significant interaction effect between treatment and visit, F (4, 43.75) = 1.45, p = .23. Participants rated themselves as more alert following the moderate dose treatment drink (M = 6.98), compared to after the placebo drink (M = 5.41), p = .03

Calm

There was no significant main effect of treatment on the participants' ratings of calmness on the Bond-Lader VAS, F (2, 41.63) = 0.75, p = .48; or period, F (2, 36.71) = 0.84, p = .44; or the interaction effect between treatment and visit, F (4, 33.22) = 0.44, p = .78. Comparison of the active treatment drinks with placebo revealed no significant differences.

Content

There was no significant main effect of treatment on the participants' ratings of feeling content on the Bond-Lader VAS, F (2, 35.26) = 0.66, p = .52; or period, F (2, 51.52) = 0.64, p = .53. There was a significant interaction effect between treatment and period, F (4, 38.53) = 3.37, p = .02, participants rated themselves as significantly more content after the moderate drink on study day 3 (period 3) compared to on study day 2, p = .002. Post hoc comparison revealed no significant differences compared to placebo.

Table 6.14 Bond and Lader, means and standard errors for the Depletion Group

Task	Treatment		
	Placebo	Moderate	High
Alert[1]	5.4 (2.0)	7.0 (2.7)	5.4 (2.0)
Calm[2]	-7.5 (1.7)	-8.3 (1.7)	-5.2 (2.0)
Content[3]	1.3	1.7	3.0

	(1.4)	(1.2)	(1.0)

Change from baseline; covariates appearing in the model for treatments are evaluated at the following average baseline values: [1]39.7; [2]63.8; [3]52.1.

*Significant compared to placebo at p < .05

Mood, Alertness and Physical Symptoms Questionnaire (MAPS)

Headache

There was no significant main effect of treatment on the participants' ratings of headache, F (2, 38.14) = 0.09, p = .91; or period, F (2, 34.47) = 0.61, p = .55; or the interaction effect between treatment and visit, F (4, 31.02) = 0.55, p = .70. There were no significant differences observed following administration of treatment drinks compared to placebo.

Heart pound

There was no significant main effect of treatment on the participants' ratings of heart pounding, F (2, 30.17) = 1.32, p = .28; or period, F (2, 31.23) = 0.32, p = .73; or their interaction, F (4, 29.01) = 0.23, p = .92.Compared to placebo, those receiving the moderate treatment drink felt less of an increase in heart pound compared to placebo (p = .03).

Jittery/Shaky

There was no significant main effect of treatment on the participants' ratings of feeling jittery/shaky, F (2, 45.44) = 1.18, p = .32; or period, F (2, 47.87) = 2.26, p = .12. There was a significant interaction effect between treatment and period, F (4, 39.20) = 4.77, p = .003, participants rated themselves as significantly less jittery following the moderate dose

drink on study day 3 (period 3), compared to a study day 1 (period 1), and this just failed to reach significance compared to study day 2 (period 2), $p = .001$ and $p = .05$ respectively. Post hoc comparisons of the active drinks with placebo revealed no significant differences.

Light-headed/Feeling faint/Dizzy

There was no significant main effect of treatment on the participants' ratings of feeling light-headed/feeling faint/dizziness, F (2, 44.10) = 0.51, $p = .61$; or period, F (2, 50.47) = 0.04, $p = .96$; or their interaction, F (4, 40.45) = 1.99, $p = .12$. Participants rated themselves as less light-headed/feeling faint/dizzy following the moderate dose treatment (M = -8.47), compared to placebo (M = -4.00), $p = .006$.

Hands-trembling

There was no significant main effect of treatment on the participants' ratings of hands-trembling, F (2, 38.63) = 0.57, $p = .57$; or period, F (2, 38.57) = 0.53, $p = .59$; or their interaction, F (4, 36.29) = 1.29, $p = .29$; no effect of the baseline average covariate, F (1, 79.23) = 2.26, $p = .18$. No significant differences were observed following administration of active treatment drinks compared to placebo.

Scared

There was no significant main effect of treatment on the participants' ratings of scared, F (2, 29.72) = 0.81, $p = .45$; or period, F (2, 31.49) = 0.39, $p = .68$; or their interaction, F (4,

28.64) = 0.43, p = .79. There were no significant differences observed following administration of treatment drinks compared to placebo.

Feeling hot/sweating (not due to heat)

There was no significant main effect of treatment on the participants' ratings of feeling hot/sweating, F (2, 23.17) = 0.78, p = .47; or period, F (2, 37.05) = 0.12, p = .88; or their interaction, F (4, 35.00) = 2.19, p = .09. There were no significant differences observed following administration of treatment drinks compared to placebo.

Relaxed

There was no significant main effect of treatment on the participants' ratings of feeling relaxed, F (2, 39.37) = 0.03, p = .97; or period, F (2, 36.27) = 0.24, p = .79; or their interaction, F (4, 30.61) = 1.29, p = .29. Comparisons of active drinks with placebo revealed no significant differences.

Clearheaded

There was no significant main effect of treatment on the participants' ratings of feeling clearheaded, F (2, 47.15) = 1.04, p = .36. There was a significant main effect of period, F (2, 40.35) = 4.70, p = .02. There was no significant interaction effect between treatment and period, F (4, 41.12) = 0.84, p = .51. Comparisons of active drinks with placebo revealed no significant differences.

Happy

There was no significant main effect of treatment on the participants' ratings of feeling happy, F (2, 36.58) = 1.27, p = .29. There was no main effect of visit, F (2, 36.41) = 1.27, p = .29; or the interaction effect between treatment and visit, F (4, 32.26) = 1.09, p = .38. Comparisons of active drinks with placebo revealed no significant differences.

Alert

There was no significant main effect of treatment on the participants' ratings of feeling alert, F (2, 45.13) = 0.29, p = .75; or period, F (2, 39.53) = 3.07, p = .06; or their interaction, F (4, 39.72) = 0.70, p = .59. There were no significant differences observed following administration of treatment drinks compared to placebo.

Table 6.15 Mood, Alertness and Physical Symptoms Questionnaire (MAPS), means and standard errors for the Depletion Group

Task	Treatment		
	Placebo	Moderate	High
Headache[1]	-2.3 (2.4)	-3.3 (2.7)	-3.9 (3.1)
Heart pound[2]	7.9 (3.9)	1.2 (2.1)	4.3 (2.6)
Jittery/Shaky[3]	5.1 (2.8)	4.9 (3.3)	-1.3 (3.6)
Light-headed/feeling faint/dizzy[4]	-4.0 (2.7)	-8.5 (3.7)	-4.8 (3.1)
Hands-	6.8	3.2	1.9

trembling[5]	(3.5)	(3.0)	(3.2)
Scared[6]	0.1 (1.0)	-2.0 (2.9)	1.7 (1.4)
Feeling hot/sweating[7]	-0.3 (1.2)	1.1 (2.9)	-2.4 (1.6)
Relaxed[8]	-3.7 (2.2)	-2.9 (2.7)	-3.5 (2.6)
Clearheaded[9]	4.5 (2.5)	10.1 (3.7)	8.4 (2.1)
Happy[10]	0.1 (2.1)	2.1 (2.3)	5.9 (2.9)
Alert	7.1 (2.1)	10.2 (3.1)	8.8 (2.8)

Change from baseline; covariates appearing in the model treatments are evaluated at the following average baseline values: [1]Headache = 19.4; [2]Heart pound = 11.8; Jittery/shaky[3] = 17.5; Light-headed/feeling faint/dizzy[4] = 25.3; Hands trembling[5] = 14.0; Scared[6] = 8.9; Feeling hot/sweating (not due to heat)[7] = 9.9; Relaxed[8] = 57.6; Clearheaded[9] = 35.1; Happy[10] = 47.6; Alert[11] = 31.9.

*Significant compared to placebo at $p < .05$

6.4 Discussion

The aim of this study was to examine whether the effects of glucose and caffeine on cognition and mood would be greatest when cognitive resources were in a sub-optimal state due to prior depletion. In addition to manipulation of participants' state prior to drink administration and behavioural assessment, longer and more demanding working memory and attention tasks were used to help tease out the effects of these substances.

Participants' ratings of their mental fatigue before and after the Resource Depletion Battery showed that the manipulation was successful as participants reported feeling significantly more mentally fatigued. This demonstrates that it is possible to deplete the cognitive resources of participants via the completion of a demanding battery. Therefore it is possible manipulate participants into a sub-optimal state using this method.

With regards to the blood glucose findings, these followed the expected pattern. Following treatment, only the two active treatment drinks increased blood glucose levels. Once raised however, blood glucose did not significantly differ from the first post-dose time-points (pre-post dose completion of the cognitive tasks) compared to the second post-dose time-points (at the end of the study); except for after the 'high dose' combination drink in the No Depletion group where blood glucose was significantly raised again at the second post-dose time-point compared to the first post-dose time-point.

Overall, the active drink effects for both the No Depletion Group and Depletion were limited almost exclusively to effects on mood. This study therefore does not provide support for the notion that the effects of the active treatment drinks would be most beneficial on cognitive performance when participants are in a sub-optimal state, and does not support the literature on combined administration where the most robust effects have

been found on participants who are in a sub-optimal state (Alford, Cox & Wescott, 2001; Horne & Reyner, 2001; Kennedy & Scholey, 2004; Sünram-Lea, Owen-Lynch, Robinson, Jones & Hu, 2012).

There were no beneficial effects of the active treatment drinks on the accuracy performance on the RVIP task for either the Depletion or the No Depletion Group. There were no effects of any of the active treatment drinks on the other measures of attention. Combined administration of glucose and caffeine has previously been shown to improve performance on attention tasks (Adan & Serra-Grabulosa, 2010; Kennedy & Scholey, 2004; Rao, Henglong & Nobre, 2005; Scholey & Kennedy, 2004). Dose may be an important factor here as beneficial effects on attention have been observed after administration of drinks which contained 75mg caffeine (Adan & Serra-Grabulosa, 2010; Scholey & Kennedy, 2004) suggesting that effects on attention might only be observed at higher doses of caffeine. However, Rao *et al.,* (2005) found that a combination of 40mg caffeine and 60g glucose improved performance on a sustained selective attention task both in terms of accuracy and speed. Kennedy and Scholey (2004) also found that 3 'energy drinks' which contained 38mg, 46mg and 33mg of caffeine and 68g, 68g and 60g glucose respectively, improved performance accuracy on an attention task across a cognitively demanding battery. Smit *et al.,* (2006) found that drinks containing 30mg caffeine and 58g glucose were also able to improve performance on attention tasks relative to placebo. However, these beneficial effects were found on tasks that had a much longer duration, and therefore a more demanding period of sustained attention. In Rao *et al.,*'s (2005) study the task lasted for 45mins and in Smit *et al.,*'s (2006) study the attention tasks were repeated over the course of a task battery which lasted for 1.5hrs. Indeed, Kennedy and Scholey (2004) did not find any improvements on attention until the later stages of their cognitively demanding battery, until after at least 35mins. Therefore, whilst the task

in this study was of a longer duration (10mins), compared to the attention tasks in study 1 (Chapter 2) (2mins), it is likely that it is still not sustained enough to elucidate the beneficial effects of glucose and caffeine at these lower doses. Another potential explanation might be that in order for cognitive and/or physiological depletion to occur, consecutive tasks need to pertain to similar domains and structures. Similar to the 'multiple resource' theory which suggests that tasks can be completed simultaneously, unless they are dependent on the same energy resources (Ruiter, Lorist & Snel, 1999). Consequently, it could be argued that for the initial demanding tasks to impact on subsequent performance, tasks need to rely on the same underlying processes in order for these to be fully depleted. The digit vigilance task and the RVIP may be relying on different underlying processes. Whilst the digit vigilance task only requires maintaining attention, the RVIP battery also requires an element of working memory for its successful completion. Apart from the significant drink effects on mood in the No Depletion group, the only other effect was on long-term memory where it was found that performance was better following placebo rather than the high dose drink. A contrasting pattern of results were also seen in the Depletion Group, here only immediate memory was impaired following the moderate dose drink compared to placebo. These findings are in contrast to previous research which has found that an 'energy drink' containing 75mg caffeine and 75g glucose was effective in improving learning/immediate memory as well as long term memory (Adan and Serra-Grabulosa, 2010). These findings are also in contrast to previous literature where 25g has been found to be the most optimal dose for improving verbal declarative memory (Foster *et al.*, 1998; Sünram-Lea, Foster, Durlach & Perez, 2002; Sünram-Lea *et al.*, 2011).

In the Depletion Group, there was no effect of either doses on working memory. The doses may not have been optimal to see these effects. Sünram-Lea *et al.*, (2011) found that dose-

response curves differed across memory domains and were also influenced by participants' individual characteristic e.g. height and weight and suggested that the optimal dose for enhancing performance is task dependent.

In terms of mood effects, both active drinks were able to elicit beneficial effects in both the Depletion and No Depletion groups. These results fit with previous findings in the literature that glucose and caffeine in combination have positive effects on mood (Gershon, *et al.,* 2009; Howard & Marczinski, 2010; Kennedy & Scholey, 2004; Mets *et al.*, 2010; Smit & Rogers, 2002; Sünram-Lea *et al.*, 2012). The moderate drink improved ratings of alertness, across both groups and content/happiness and clearheaded in the No Depletion group. In the Depletion group it also improved ratings of aspects of mood associated with anxiety and light-headedness. The high dose drink had more limited effects only showing some beneficial effects on anxiety and light-headedness in the Depletion group and only improvements in light-headedness in the No Depletion group. The caffeine content in the two drinks was the same but the effects were mainly seen with the moderate dose treatment which contained 25g glucose compared to the high dose treatment which contained 60g glucose. Therefore the amount of glucose must have some moderating or additive effect. This may be related to the inverted U-shaped dose response of glucose (Gold, Vogt, & Hall, 1986), as it may be that 60g is too high to see beneficial effects on some aspects of mood.

Ideally it would have been beneficial to examine each of the constituents of the active treatments in isolation as well as in combination. However, given the primary aim of the study was to compare the effects of depletion versus no depletion on subsequent cognitive performance using accuracy on the RVIP task as the primary measure, this would have resulted in an increase in number of study arms, which would most certainly have impacted on participant recruitment and retention. With regards to the Resource Depletion

Battery itself, it would be useful to ask participants to rate how cognitively demanding they found each task, as well as how mentally fatigued they felt prior to and after the battery. This would be useful if the tasks were also manipulated to see which are most effective at depleting participants' cognitive resources to see if a manipulation of the battery could increase its cognitive demand, thereby elucidating the effects of caffeine and glucose on cognitive function. For example, in this study there were no effects on attention, however it may be that this could be achieved with a more demanding attention task completed in the Resource Depletion Battery. It would also have been useful to have participants rate how mentally fatigued they were prior to and after the DVD, to ensure that they didn't feel significantly more mentally fatigued following this and to ensure it acted as an adequate control.

This study was unable to demonstrate that it is possible to manipulate participants into a sub-optimal state using a cognitively demanding battery. Subsequent to this it has shown that glucose and caffeine administered in combination have greater effects on cognitive performance when the participant is in a sub-optimal state. However, the state of the participant may still be crucial to any enhancing effects of these substances. In this sense their effects could be looked on as restorative, rather than enhancing performance where it is already optimal.

Chapter 7

Effects of caffeine and glucose alone and in combination on neurocognitive processes

7.1 Introduction

Previous studies reported in this thesis have focused on the behavioural effects of glucose, caffeine and their combination. These studies identified beneficial effects on aspects of long-term declarative memory following caffeine administration and combined administration of caffeine and glucose has led to improved aspects of long-term memory performance and attention. In addition, improvements on aspects of working memory have been observed. These results lend support for previous research findings to a certain extent (see for example Smith *et al.*, 1999; Kelman & Creeley, 2001 for effects of caffeine on long-term memory; Alford *et al.*,, 2001; Horne & Reyner, 2000 for effects of combined administration on long-term memory and attention; and Kennedy & Scholey, 2004 for effect on working memory). However, none of the studies reported showed strong evidence for glucose facilitation of memory performance or enhanced level of attention following caffeine administration (Foster *et al.*, 1998; Meikle *et al.*, 2005; Smith, 2002; Sünram-Lea *et al.*, 2001).

One reason for the discrepancies in the findings could be due to the sensitivity of the tasks, especially as the population under study are healthy young adults, who are already performing well on these tasks. However, it is also important to note that behavioural measures only provide indirect information on underlying neuro-cognitive processes (Lorist & Tops, 2003). To help gain a more complete picture of the effects of caffeine and glucose, both alone and in combination the current study aimed to examine the underlying neural mechanisms mediating the behavioural effects. This research used a convergent operations approach that combined cognitive (behavioural) testing with brain imaging using event-related potentials (ERPs). Event-related potentials are voltage fluctuations in the ongoing electroencephalogram (EEG) that are time-locked to an event, such as the

onset of a stimulus or the execution of a manual response. The observed ERP waveform is *a depiction of the* changes in scalp-recorded voltage over time that reflect the sensory, cognitive, affective, and motor processes elicited by a stimulus. The ERP peak can be defined as a reliable local positive or negative maximum in the observed ERP waveform (Kappenman & Luck, 2011). This approach will inform our theoretical understanding of the cognitive and physiological mechanisms involved in the effects of glucose and caffeine administration.

It may be that whilst the effects of these substances cannot be reliably seen behaviourally that they are having effects on the neurocognitive processes in the brain e.g. making performance more efficient so that fewer neural resources are used. Event-related potentials (ERPs) were used as the primary tool to investigate the neural correlates of glucose and caffeine-mediated cognitive processes. In brief, ERPs are a measure of neural activity (derived from EEG, the electroencephalogram, recorded from scalp electrodes) that can be recorded non-invasively from humans whilst performing cognitive tasks. It is a reliable and cost-effective means of tracking mental chronometry in response too various cognitive events. It offers excellent temporal resolution in the order of milliseconds, whereas functional magnetic resonance imaging (fMRI) is much slower (in the order of seconds), and much more costly. ERPs allow the investigation of the organisation and timing carried out, and these measures may be more sensitive to the effects of glucose and caffeine (Lorist & Tops, 2003).

ERP components are defined by their polarity (positive or negative going voltage), timing, scalp distribution, and sensitivity to task manipulations. The P300 component (central to the proposed investigation) has provided a wealth of information on normal and dysfunctional cognition over the last 40 years (see Bashore & van der Molen, 1991 for a review). Early P300 experiments focused on a large positive peak elicited approximately

300-500 ms, maximal over parietal sites. This component follows the presentation of a rare target stimulus, embedded in a train of background stimuli, the so-called oddball task. This deflection, now referred to as the P3b, is widely thought to index memory storage operations. P3b amplitudes are considered to reflect the maintenance in working memory of a stimulus when the mental representation of the stimulus context is updated (Donchin *et al.,* 1986; Polich, 2003), and is closely associated with episodic memory. It is acknowledged that several brain regions contribute to the generation of the P3b, including frontal areas, hippocampal areas of the medial temporal lobe and the parietal cortex (Polich, 2003). Extensive intracranial recordings have also revealed a widespread network of activation for the P3b, with generators in the ventrolateral prefrontal, superior temporal sulcus and posterior superior parietal cortical areas, and hippocampal and perirhinal regions (Halgren *et al.,* 1998). An earlier deflection, with a more fronto-central distribution, is elicited during the oddball task where an additional novel or distracter stimulus is inserted into the background and target sequence. This is referred to as the P3a and is thought to reflect frontal lobe function and orienting of attention (Knight, 1997). Frontal lesion patients exhibit diminished P3a amplitudes (Knight, 1984), and it is therefore considered that frontal lobe engagement is necessary for P3a generation and contributes to attentional control. Intracranial recordings have also implicated the dorsolateral prefrontal cortex as the principle generator, with contributions from the supramarginal gyrus, and the cingulate gyrus (Halgren *et al.,* 1998). The P3a and P3b are characterised as distinct components elicited by the interaction between frontal lobe attentional control over the contents of working memory and the subsequent long-term storage operations (Polich, 2003). These two components are therefore appropriate to investigate the effects of glucose administration on different aspects of attention and memory in older adults.

Indeed, previous research has found that glucose and caffeine alone and in combination affect ERPs (Brown & Riby, 2003; Dixit *et al.,* 2006; Kawamura *et al.,* 1996; Lorist *et al.,* 1994a&b; Lorist *et al.,* 1995; Lorist *et al.,* 1996; Lorist *et al.,*, 2004a; Rao *et al.,* 2005; Riby *et al.,* 2008; Ruijter *et al.,* 2000a&b; Smith *et al.,* 2009). Riby *et al.,* (2008) administered 25g of glucose to participants prior to them completing the Oddball Task. They found that compared to placebo, glucose modulated the P3b component which is the memory updating component of the P300, by reducing the amplitude, latency and duration. They concluded that glucose may enhance memory by reducing the resources needed for memory updating. They also found there was a trend for glucose to enhance the P3a and earlier P2 components, which are associated with novelty detection and orientation of attention. Brown and Riby (2013) again looked at the effects of 25g glucose on episodic memory and attention. They found that compared to placebo, glucose enhances the left parietal old/new effect which is a measure of verbal episodic memory, in particular recollection memory. They also found a trend for glucose to facilitate attentional processes as measured by the frontal-central negativity component.

Caffeine has been found to have effects on the early exogenous N1 component, which is elicited by visual stimuli (Lorist *et al.,* 1994a&b; Lorist *et al.,* 1995). Caffeine affected both the latency and amplitude of the N1 (activity related to perceptual processing) and Lorist *et al.,* (1994a) concluded that it increased the participants' receptivity to external stimuli and increases perceptual processing. However even using ERPs as the measure, the results are still equivocal, with Ruijter *et al.,* (2000a&b), not finding any effects of caffeine on the N1 component. Caffeine also positively modulates the N2b component, which is associated with object recognition and catgorisation, suggesting that caffeine leads to a more effective selection mechanism (Lorist *et al.,* 1994b, 1995, 1996; Ruijter *et al.,* 2000a). Research has also found that 250mg of caffeine can positively modulate P3

amplitude (potentially an indicator for the amount of energy that is used), but not affect latency, which is said to reflect stimulus evaluation time (Lorist *et al.*, 1994; Lorist *et al.*, 1995). Kawamura *et al.*, (1996) found that 500mg caffeine significantly increased the amplitude and area of the P300, but did not reduce latency in response to an auditory stimulus. Dixit *et al.*, (2006) administered 3mg/kg (210mg for a 70kg individual) caffeine and also found it increased the amplitude of the P300, as well as significantly reducing reaction time on the behavioural task and causing a non-significant decrease in latency.

Rao *et al.*, (2005) evaluated the effects of combined caffeine and glucose administration compared to placebo. In their study participants consumed either an energising drink containing 60g glucose and 40mg caffeine or a placebo. Participants then performed a behavioural task where they detected visual targets and the C1, P1, N1, N2 and P3 ERP components were measured throughout the task. The C1 and P1 components reflect early visual cortical processing. The N1 is a visual component, whilst the N2 component is believed to reflect the evaluation of stimuli and the P3 component reflects decision-making and updating. They found that the energising drink lead to a significantly diminished N1 component, although this interacted with scalp hemisphere and was larger over the posterior occipital lobes. The N2 component was also significantly larger over the frontal-central scalp following the energising drink. Finally the P3 component, at the midline sites, was enhanced following the active treatment drink.

Taking that the previous behavioural work reported in this thesis identified more robust effects on memory performance, this study aimed to investigate the effects on the P300 component. The primary outcome measures in this current study was the P3b component for evaluation of memory storage operations was also carried out as well and the P3a components for assessment of fontal lob engagement (secondary outcome measure).

The current study specifically examined the effects of 25g glucose and 40mg caffeine, as these dosage levels have formed the main doses of interest in the previous studies reported in this thesis. Moreover -as outlined above- previous studies have beneficial effects on long-term memory performance following administration of 40mg caffeine, both in isolation and in combination with glucose. In addition, 25g glucose has been shown to modulate ERPs in previous research (Brown & Riby, 2013; Riby *et al.*, 2008). Therefore it seems reasonable to predict that this dosage combination may have effects on these neurocognitive measures.

As prior cognitive depletion and more general the activation state prior to drink administration are still considered to be an important effect moderator (see Chapter 6), all participants completed a series of cognitive tasks prior to drink administration and subsequent assessment of neurocognitive function.

7.2 Method and Materials

7.2.1 Participants

In total, 34 subjects were screened and recruited via the Online Research Participation System (SONA) at Lancaster University. 33 subjects were randomised, received study product and included in the Safety and Intent to Treat (ITT) populations. Thirty (30) subjects completed all four study periods. Based on the data observed in previous studies, a sample size of 30 participants completing all four treatment periods will have >80% power to detect a difference of 3.0 units in mean amplitude for P3b (memory updating component using target stimuli) as assessed at the parietal region (Pz site), between each of the three active treatments (glucose, caffeine, combination) versus placebo. The standard deviation (SD) of the paired differences is assumed to be 4.7 units, derived from the mean squared error 11.2 units, which was reported by Riby (2008).

The age range was 18-35 years (mean age 21.53 years). They all consumed at least 120mg caffeine per day, (average consumption was 225.75mg). Inclusion criteria included; Compliance (understand and is willing, able and likely to comply with all study procedures and restrictions); Good general and mental health, with a) no clinically significant and relevant abnormalities of medical history or physical examination, b) absence of any condition that would impact on the subjects' safety or wellbeing or affect the individual's ability to understand and follow study procedures and requirements; Self-assessed as healthy, confirmed by medical questionnaire during screening; A native English speaker. Exclusion criteria included; individuals who regularly consume less than 120mg/day of caffeine or excessive consumers (>600mg/day) caffeine; a diagnosis of Diabetes Mellitus Types 1 or 2; allergy/intolerance, known or suspected intolerance or hypersensitivity to the

study materials (or closely related compounds) or any of their stated ingredients; any intolerance or allergic reaction to substances that contain phenylalamine and/or caffeine; clinical study/experimentation, a) participation in another clinical study or receipt of an investigational drug within 30 days of the screening visit, b) previous participation in this study; Personnel, an employee of the sponsor or the study site or members of their immediate family; having a history of neurological or psychiatric illness (excluding depression or anxiety); history of heart disease or high blood pressure (≥140/90 BPM) as measured at screening; having a current diagnosis of neurological or psychiatric illness (including depression or anxiety); currently taking medication or nutritional supplements (including vitamins) other than the contraceptive pill and/or asthma inhalers; being pregnant, seeking to become pregnant or breastfeeding; having a history of or currently abusing drugs or alcohol; currently smoke or using nicotine replacement products (i.e. those attempting to quit smoking with the aid of nicotine supplementation. The study was approved by the Department of Psychology Ethics Committee at Lancaster University. Participants gave their signed informed consent prior to taking part and a Clinical Records Form (CRF) was used to confirm their eligibility.

7.2.2 Design

This was a randomised, double blind, placebo-controlled, repeated measures design to examine event-related potentials, cognitive performance and glycaemic response under 4 different drink conditions [glucose (25g), caffeine (40mg), glucose/caffeine (25g/40mg), and placebo]. Participants were randomly allocated to a treatment regime according to a Williams square, for a 4 by 4 crossover study. Within the randomisation list, each treatment was followed by every other treatment an equal number of times.

7.2.3 Treatments

The treatments were supplied by Suntory Food and Beverage Europe in 380ml solutions. There were three active drinks; glucose containing 25g glucose, caffeine containing 40mg caffeine and a glucose and caffeine combination containing 25g glucose and 40mg caffeine. A taste matched placebo was also utilised.

Participants were instructed to consume one drink per test session within 5 min. Cognitive testing started 20 minutes after drink administration. A twenty-minute delay was chosen as peak plasma caffeine concentration is reached between 15 to 120 min after oral ingestion and brain levels remain stable for at least one hour following peak plasma levels (Nehlig, 1999; Nehlig & Boyet, 2000). Also this time frame is similar to the procedure of previous glucose studies (Foster *et al.,* 1998) in order to ensure successful transfer of plasma glucose to brain.

7.2.4 Procedure

Participants attended the laboratory on five different days. Their first visit was a screening session and the other four visits were for study visits. The study visits took place between 9am and 4pm, but each participant took part in the study at the same time of day for all their visits. Participants attended the study visits following a 2 hour fast (i.e. no food or drink except water). The participants were instructed to abstain from drinking caffeine containing drinks and food for 12 hours and abstain from alcohol for 24 hours prior to the start of the study visit. Participants were also instructed not to take part in any strenuous physical activity until after their testing session had been completed each day.

Initial screening was done during sign-up using the Lancaster University recruitment on-line system (SONA). Participants were informed about the inclusion and exclusion criteria and lifestyle restrictions and self-assessed their eligibility to take part in the study.

At their first screening visit, following a discussion between the participant and the researcher about the requirements of the study, voluntary written informed consent was provided by all participants prior to any procedures being performed. All participants then completed the caffeine consumption questionnaire and were screened by the researcher using the completion of the CRF to ensure that they met the inclusion and exclusion criteria. The CRF captured personal demographic information and relevant medical history (including height, weight and confirmation of screening criteria). Participants also had their blood pressure checked to ensure they were not in the hypertensive range (≥140/90). Each participant also completed a training session in order to familiarise them with the cognitive tasks used. They were given instructions for each of the cognitive task assessments. No treatment drinks were administered during the screening session and performance data from those sessions was not included in the analysis.

Once the screening session was successfully completed there were four experimental test sessions (active study days). The first session took place within 14 days of the screening session. Participants were randomised on arrival at the laboratory. All subsequent visits took place a minimum of 48 hours apart.

Upon arrival at the study session the participant gave a blood sample (finger prick) for the measurement of blood glucose, these confirmed their compliance with the 2 hour fasting for food. If baseline glucose was ≤6mmol/L, participants completed the first set of cognitive tasks (Resource Depletion Battery including Serial Sevens task and Digit Vigilance task). The participants then provided a saliva sample to measure their caffeine

levels. Participants then consumed their treatment drink for the day (following a double-blind procedure) in a maximum time of 5 minutes. The post-drink cognitive test session commenced 20 minutes after treatment drink consumption. Whilst the participants consumed their treatment, and during the first 15 minutes of the absorption period, the researcher capped up the participant ready for the EEG recording. After the full 20 minutes absorption period, participants were given a short practice of the next cognitive task (Odd ball task). They then completed the cognitive task whilst the EEG was recording. A further saliva sample was taken at 25 and 55 minutes after the intake of the treatment drink and measuring blood glucose levels was also repeated at 25 and 55 minutes post-intake. All participants received a debriefing sheet at the end of their final study session.

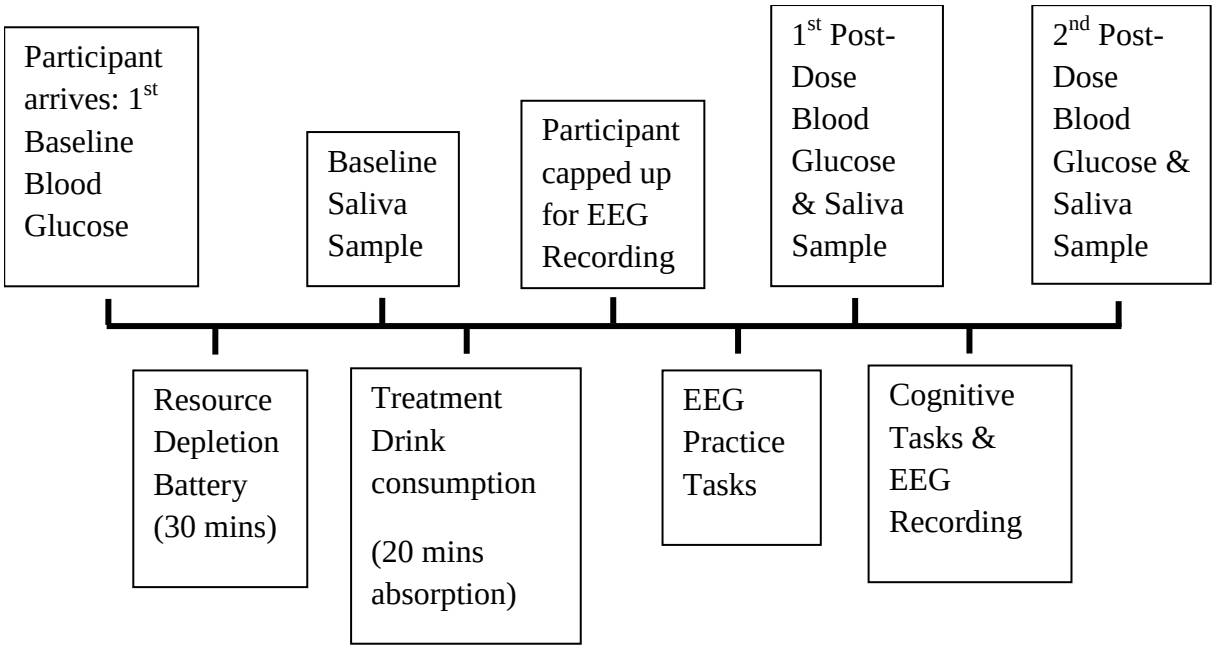

Figure 7.1 Schematic of the study day procedure

7.2.5 Assessments

Depletion Battery

Computerised Serial Sevens Task – Evaluates working-memory performance (Hayman, 1942). Participants were required to compute a running subtraction of 7, starting from a randomly generated number. Participants were given 5 minutes to complete this task. Numbers of responses, number of correct responses and number of incorrect responses were recorded.

Digit Vigilance – Measures sustained attention (Lewis, 1995). A single target digit was randomly selected and continuously displayed on the right side of the screen. In the centre a series of rapidly changing digits were displayed. Participants were required to press the space bar button as quickly as possible, whenever the digit in the centre matches the target digit. The task lasted for 10 minutes. Reaction times (milliseconds), percentage accuracy and number of false alarms were recorded.

These tasks were administered using the Computerised Mental Performance Assessment System (COMPASS), a purpose designed software for the flexible delivery of randomly generated parallel versions of standard and novel cognitive assessment tasks. All responses were made using the computer keyboard and mouse.

Odd Ball Task

The task followed the same procedure as described in Riby *et al.,* (2008). The task was administered via a personal computer on a 14’ monitor. Participants were seated 1.5 m from the computer screen in a semi-darkened room. Three versions of the task were presented in a counterbalanced order in the session. Participants were instructed to press a keyboard every time they identified the designated target stimulus, but that they should ignore all other stimuli. The experimental task comprised of 350 stimuli, with a probability

of 0.8 for the standard stimulus (a large blue square of 16 cm^2 in area), 0.1 for the target stimulus (a smaller blue square 12.82 cm^2 in area), 0.1 for the irrelevant stimuli (neutral photographs selected from the International Affective Picture System, Lang *et al.*, 1988). The stimuli remained on the screen for 100 ms. The inter-stimulus interval was 2000 ms. Prior to the experimental blocks, a practice block of 15 items was administered without the irrelevant stimuli. The Odd Ball task was administered using E-Prime. All the responses were made using the computer keyboard and mouse.

Event-related Potential (ERP) recording

EGI (Electrical Geodesics Incorporated, Eugene, OR) Geodesic EEG System (GES) 250 EEG system with Net Amps 200 amplifier and 128 channel HydroCel Geodesic Sensor Net (HCGSN) were used for EEG recordings. The EEG was recorded and analysed using NetStation software (Electrical Geodesics Incorporated, Eugene, OR). Impedances were kept below 50 kOhm. During the recording, the EEG was referenced to vertex (Cz). The sampling rate was 1000 Hz. Horizontal and vertical eye movements were recorded from around the eyes (channels 8 and 25, 125 and 128 on HCGSN net). Time windows of 320-430ms and 380-700ms was used to capture the P3a and P3b ERP components respectively. Automatic eye-blink correction, artefact rejection (trials where ERPs are outside the range -75uV to +75uV) and ERP averaging were carried out offline using Edit 4.3 (Neuroscan).

Blood Glucose Measurement

Blood glucose readings were obtained using the ExacTech® blood glucose monitoring equipment (supplied by MediSense Britain Ltd, 16/17 The Courtyard, Gorsey Lane,

Coleshill, Birmingham B46 1JA), following the recommended procedure: The researcher swabbed the finger of the volunteer with a Sterets Isopropyl Alcohol BP Pre-injection swab (Seton Healthcare Group, Oldham, UK) and allowed the skin to air dry. The skin was punctured using an automatic lancing devise and a drop of blood was collected onto the analytical test strip. The volunteer applying a tissue blotted any excess blood. Ambidex-HG powder free polymer bonded latex examination gloves were worn by the experimenter at all times during the procedure. Each lancet and cap were only used once and then disposed of into a sharps container. Swabs, test strips, tissues and gloves will be placed in a clinical waste sack.

For performance characteristics of ExacTech® blood glucose monitoring equipment see chapter 2.

Quantification of Caffeine in Saliva

Caffeine saliva concentrations were obtained through saliva samples, which were collected using the salivette saliva sampling device (Sarstedt LTD, Leicester, UK). These consist of a small test tube fitted with an inner receptacle containing a sterile cotton wool bud. Participants were required to remove the cotton wool bud and give unstimulated saliva samples by placing the cotton wool under the tongue for a timed two-minute period and then replacing it in the test tube. Ambidex-HG powder free polymer bonded latex examination gloves were worn by the experimenter at all times during saliva sampling. Excess saliva was removed using Sterets Isopropyl Alcohol BP Pre-injection swabs (Seton Healthcare Group, Oldham, UK). All saliva contaminated waste was placed in a yellow bio hazard bag and disposed of via Lancaster University's Biology Autoclave system. Test tubes were sent to the School of Applied Sciences, University of Huddersfield within 24

hours of collections. There the saliva-cotton wool was analysed and levels of caffeine were determined by high performance liquid chromatography (HPLC) following the procedure described in Child and de Wit (2006).

7.2.6 Statistical Analysis

P3b Memory Component

The primary efficacy variable was the mean amplitude for P3b (memory updating component using target stimuli) as assessed at the parietal region (Pz site). To investigate the effect of glucose and caffeine administration on the P3b component related to memory updating, average amplitude in the 380-700ms region for correct responses to the target were analysed using a linear mixed model. Terms in the model were treatment, period, site (Pz. Cz) and treatment by site interaction as fixed effects and subject as a random effect.

Since each participant had multiple measures (i.e. at the different regions of the brain), the mixed model was set up using a repeated measures framework, in order that to take into account the inherent correlation between the repeat measures on the same participant. An unstructured covariance pattern was used. All active treatment drinks were compared with the placebo drink using two-sided testing and implementing the Dunnett's method to ensure a family wise significance level of 5%. Adjusted 95% confidence intervals are presented for pairwise differences.

The secondary variables were; mean amplitude for P3b (using target stimuli; memory updating component) as assessed at the central region (Cz site); peak latency data for P3b (using target stimuli; memory updating component) as assessed the Pz and Cz sites; mean

amplitude and peak latency data for P3a (using target stimuli; orientation of attention component) as assessed at the frontal and central scalp regions (Fz and Cz sites); Odd Ball task performance, performance are percentage accuracy (%) and reaction time (milliseconds); blood glucose levels (mmol/L) at 0 (baseline), 25 and 55 min, and salivary caffeine levels (µg/ml) at 0 (baseline), 25 and 55 min.

Descriptive summaries and statistical comparisons between study groups were performed for the following secondary parameters:

P3a Attention Component

To investigate the effect of glucose administration on the P3a component related to memory updating, average amplitude in the 320-340ms region for correct responses to the target were analysed using a linear mixed model. Terms in the model were treatment, period, site (Fz,Cz) and treatment by site interaction as fixed effects and participant as a random effect. In addition, an analysis of the repeat latency data using the same mixed model was performed.

Odd Ball Task

Percentage accuracy (data collapsed across experimental blocks) was analysed using a linear mixed model. Terms in the model were treatment and period as fixed effects and participant as random effect. Two measures from the resource depletion task were included as covariates; the percentage accuracy on the Serial Sevens task and percentage accuracy on the Digit Vigilance task.

In addition, an analysis of reaction time using a linear mixed model was performed. Terms in the model were treatment and period as fixed effects and participant as a random effect.

One measure from the Resource Depletion task was included as a covariate; reaction time on the Digit Vigilance task.

For the analyses of the secondary parameters, all active treatment drinks were compared with the placebo drink using two-sided testing and implementing the Dunnett's method to ensure a family wise significance level of 5%. Adjusted confidence intervals are presented for pairwise differences.

7.3 Results

7.3.1 Glycaemic response

Linear mixed model analysis revealed a significant effect of time, F (2, 194.14) = 36.86, p < .001 was observed, but no significant effect of treatment, F (3, 95.82) = 1.37; p =.26, or treatment x time interaction, F (6, 95.93) = 0.61, p = .73. No effect of period was observed, F (3, 68.86) = 0.54, p = .66.

Inspection of the means showed that as expected, blood glucose levels were highest in the test drinks containing glucose. Glucose levels peaked at between 7.25 and 7.50 mmol/l at 25mins for the glucose and glucose & caffeine test drinks (see figure 7.1 for glycaemic response).

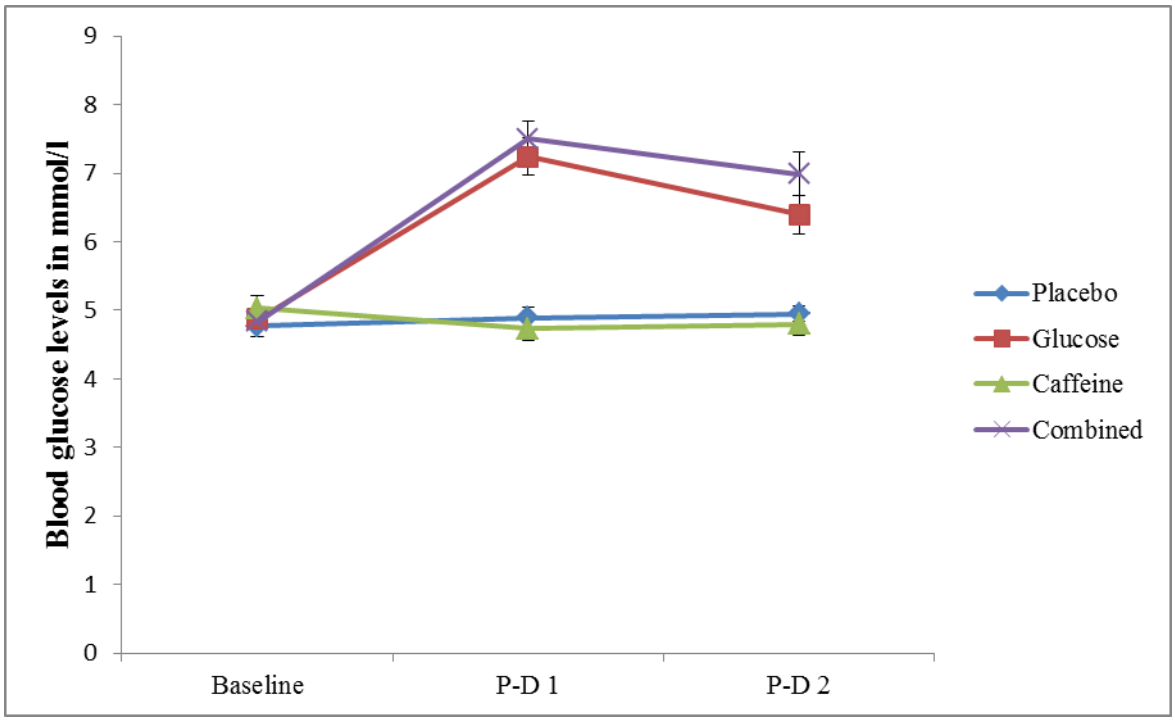

Figure 7.2 Glycaemic response as a function of drink and time

Table 7.1 Glycaemic response to treatments, means and standard deviations

Time Point	Treatment			
	Placebo	Glucose (40g)	Caffeine (40mg)	Caffeine (40mg)/Glucose (40g)
Baseline	4.77 (0.16)	4.87 (0.12)	5.03 (0.18)	4.83 (0.11)
1st Post Dose	4.89 (0.16)	7.24 (0.27)	4.73 (0.17)	7.5 (0.26)
2nd Post Dose	4.95 (0.12)	6.4 (0.28)	4.80 (0.16)	6.99 (0.31)

7.3.2 Salivary caffeine concentration

For caffeine levels, mixed model analysis revealed a significant effect of treatment, F (3, 61.61) = 18.9, $p < .001$, time, F (2, 59.45) = 6.69; $p < .01$, but no significant treatment x time interaction, F (6, 34.49) = 1.98, $p = .10$. No effect of period was observed, F (3, 236.37) = 1.08, $p = .36$.

Salivary caffeine levels were highest following ingestion of caffeine containing drinks. More specifically, caffeine levels peaked at 1240.3 ng/ml at 55 mins for the glucose and caffeine treatment drink. Following the caffeine treatment drink, caffeine levels peaked at 1260.4 ng/ml at 25 mins and then reduced to 746 ng/ml by 55 mins (see figure 7.3 for salivary pharmacokinetics of caffeine).

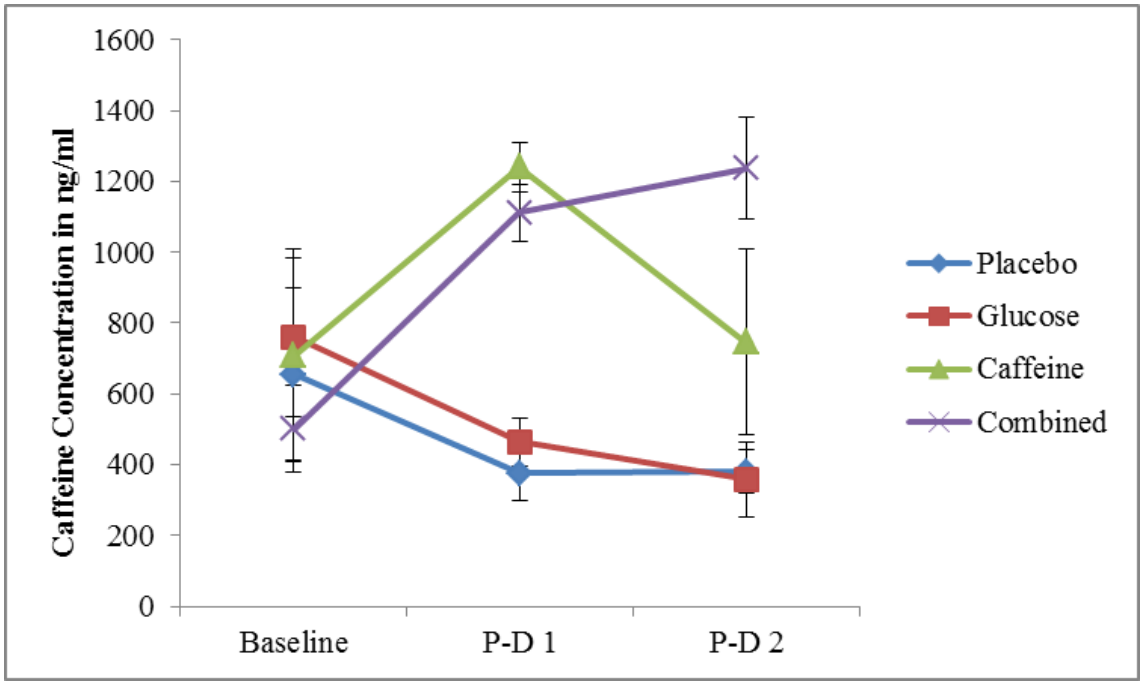

Figure 7.3 Time-caffeine concentration profiles for different drink conditions

Table 7.2 Caffeine concentration response to treatments, means and standard deviations

Time Point	Treatment			
	Placebo	Glucose (40g)	Caffeine (40mg)	Caffeine (40mg)/Glucose (40g)
Baseline	655.6 (243.10)	760.9 (224.85)	708.8 (299.46)	501.3 (122.58)
1st Post Dose	376.5 (78.81)	465 (67.78)	1240.9 (70.65)	1111.4 (80.28)
2nd Post Dose	380.9 (60.74)	359.4 (106.11)	745.9 (262.86)	1237.6 (142.68)

7.3.3 Event-related potentials

7.3.3.1 Mixed model results

P3b: Mixed linear model analysis showed no significant effect of treatment drink on the mean P3b amplitude, F (3, 75.2) = 0.64, p = .59. No effect of period, F (3, 82.5) = 0.56, p = .64, but a treatment x site interaction, F (4, 115) = 9.45, p < .001. For P3b peak latency, no significant effect of treatment drink, F (3, 88.3) = 0.82, p = .45, but a significant effect of period was observed, F (3, 82.2) = 2.81, p = .05, but no significant site x treatment interaction, F (4,115) = 2.37, p = .06.

P3a: Mixed model analysis no significant effect of treatment drink on the mean amplitude for P3a, F (3, 71.5) = 1.08, p = .36. In addition, no significant effects of period, F (3, 81.4) = 0.72, p = .55, or treatment x site interaction, F (4, 115) = 0.69. p = .60 were observed. There was no significant effect of treatment on the peak latency for P3a, F (3, 73.6) = 0.28, p = .84, period, F (3, 83.4) = 2.24, p = .09, or treatment x site interaction, F (4, 115) = 0.83, p = .51.

7.3.3.1 Comparison with placebo based on results from linear model

<u>Mean amplitude for P3b as assessed at the parietal region (Pz site)</u>

No statistically significant differences versus placebo for any of the 3 active treatment drinks were observed for target specific amplitude at parietal region (see table 7.3).

<u>Peak latency for P3b as assessed at the parietal region (Pz site)</u>

No statistically significant differences were observed for any of the three active treatments (see table 7.3).

Mean amplitude and peak latency for P3b as assessed at the central region (Cz site)

Mean amplitude values for each of the three active drinks were not statistically significant (see table 7.3).

Table 7.3 P3b mean amplitude and latency as a function of drink

Region		Glucose	Caffeine	Combined	Placebo
Parietal	Amplitude	-0.02 (0.44)[†]	0.29 (0.44)	-0.14 (0.45)	0.25
	(μV)	$p = .92$[‡]	$p = .10$	$p = .80$	(0.44)
	Latency	432.5 (11.1)	446.7 (11.2)	428.9 (11.3)	449.9
	(ms)	$p = .39$	$p = .99$	$p = .24$	(11.2)
Central	Amplitude	1.76 (0.38)	1.67 (0.38)	2.11 (0.39) p	1.55
	(μV)	$p = .93$	$p = .98$	$= .42$	(0.38)
	Latency	448.6 (16.1)	452.0 (16.1)	475.9 (16.4)	469.9
	(ms)	$p = .61$	$p = .72$	$p = .94$	(16.1)

[†]Adjusted mean (SE) from linear mixed model with terms for treatment, period, site (Pz, Cz) and treatment by site interaction as fixed effects and subject as a random effect.

[‡]Comparison with placebo; results from linear model, p-value adjusted according to Dunnett's for multiple comparisons

Mean amplitude and peak latency for P3a as assessed at the frontal region (Fz site)

None of the differnces in the mean amplitude values following the three active drinks were statistically significant. In addition there was no significant difference between the Peak latency values following any of the three active treatments (see table 7.4).

Mean amplitude and peak latency for P3a as assessed at the central region (Cz site)

The Mean amplitude was no statistically significant following any of the three treatment drinks. There were also no statistically significant differences between the Peak latency values following any of the three active treatment drinks (see table 7.4).

Table 7.4 P3a mean amplitude and latency as a function of drink

Region		Glucose	Caffeine	Combined	Placebo
Frontal	Amplitude	1.57 (0.47)[†]	1.24 (0.47)	1.83 (0.48)	1.17
	(μV)	p = .86[‡]	p = .95	p = .16	(0.47)
	Latency	387.7 (11.9)	399.6 (12.0)	398.3 (12.1)	393.7
	(ms)	p = .96	p = .96	p = .98	(12.0)
Central	Amplitude	2.09 (0.36)	2.21 (0.36)	1.70 (0.37)	1.46
	(μV)	p = .41	p = .27	p = .93	(0.36)
	Latency	373.2 (11.6)	380.4 (11.7)	385.7 (11.8)	382.4
	(ms)	p = .85	p = .99	p = .99	(11.7)

[†]Adjusted mean (SE) from linear mixed model with terms for treatment, period, site (Fz, Cz) and treatment by site interaction as fixed effects and subject as a random effect.

[‡]Comparison with placebo; results from linear model, p-value adjusted according to Dunnett's for multiple comparisons

7.3.4 Behavioural Performance Data

Odd Ball Task Percentage Accuracy

There was no significant effect of treatment drink on the percentage accuracy of the Odd /ball task, F (3, 86.3) = 1.55, p = .21. There was evidence of a notable difference in the percentage accuracy across study days, F (3, 93.6) = 30.45, p <.001. More specifically, performance on study day 1 was significantly lower compared to study days 2 to 4. This is

illustrated in Figure 7.4, which presents the mean percentage accuracy for each group by the study day.

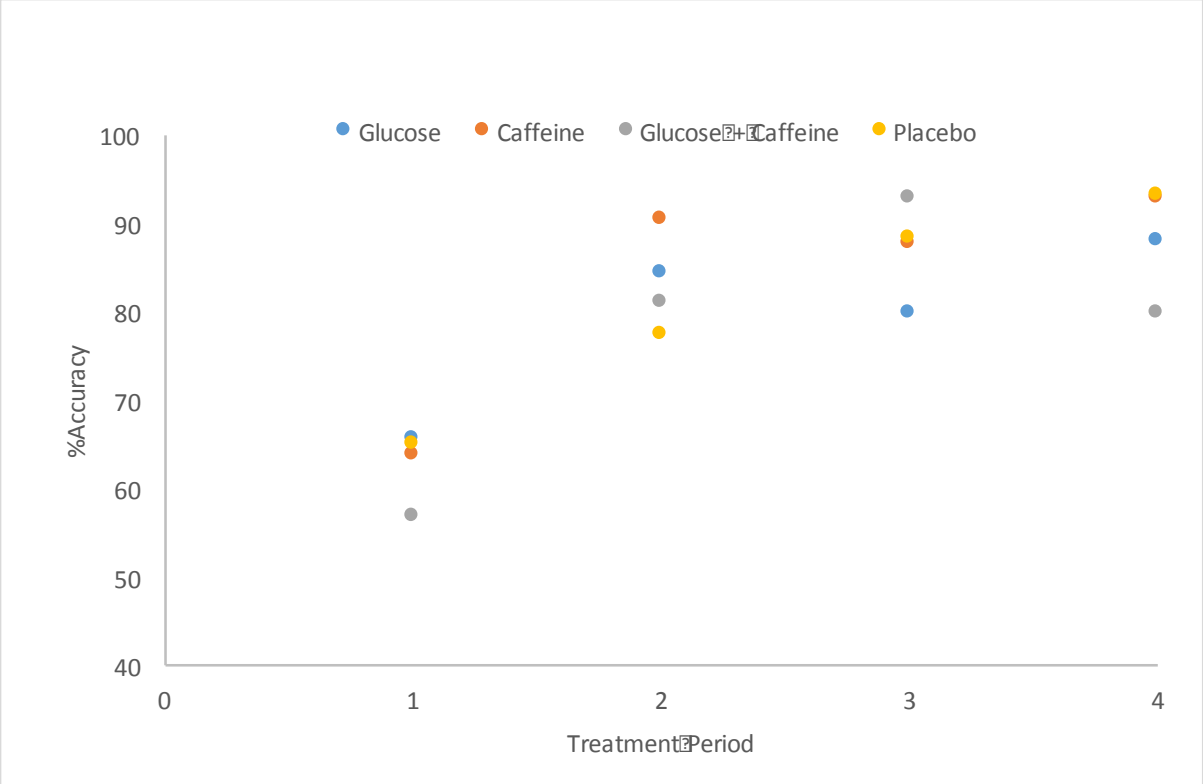

Figure 7.4 Oddball task percentage accuracy as a function of period and drink

In terms of drink effects, percentage accuracy ranged from adjusted means of 77.6% (glucose and caffeine in combination) to 83.59% (caffeine). However, there were no significant differences between any of the active treatment drinks and placebo (see table 7.5).

Odd Ball Task Reaction Time

There was a significant effect of treatment on the reaction time on the Odd /ball task, F (3, 78.4) = 2.91, p = .04. No effect of period was evident, F (3, 79.4) = 0.99, p = .40.

Reaction time was numerically faster in all three active treatment drinks compared to placebo. Following the caffeine treatment drink participants were significantly faster (M = 541.39) compared to after the placebo drink (M = 567.02), p = .03. The glucose (M = 544.86) and glucose and caffeine combination (M = 545.02) treatment drinks failed to reach significance compared to placebo, p = .08 and p = .07 respectively.

Table 7.5 Performance on the Odd Ball Task as a function of drink

Odd Ball Task Outcome	Treatment			
	Glucose	Caffeine	Combined	Placebo
% Accuracy	79.95 (2.72) † p = .99‡	79.95 (2.72) p = .59	79.95 (2.72) p = .66	79.95 (2.72)
Reaction time (ms)	546.9 (13.8) p = .08	541.0 (13.9) p = .03*	546.0 (13.9) p = .07	567.4 (13.9)

†Adjusted mean (SE) from linear mixed model with terms for treatment, period, site (Fz, Cz) and treatment by site interaction as fixed effects and subject as a random effect.

‡Comparison with placebo; results from linear model, p-value adjusted according to Dunnett's for multiple comparisons, * p < .05

7.4 Discussion

The aim of this study was to investigate the effects of glucose and caffeine on the P300 component. The primary outcome measure was the P3b component which relates to memory storage operations, but the P3a component which assesses frontal lobe engagement was also assessed.

For the P3b component, none of the active drinks containing caffeine or glucose had a statistically significant effect on ERPs which reflect memory processes (P3b using the target stimuli) as assessed by mean amplitude at the parietal region (Pz site). There were also no effects of the active drinks on any of the secondary ERP outcomes. The active drinks containing caffeine and/or glucose did alter the behavioural performance on the Odd-ball task. Specifically, reaction time was numerically faster following all 3 active drinks compared to placebo, with participants who received the caffeine drink achieving significantly faster reaction times compared to those on placebo. There were no effects of any of the active drinks on the accuracy outcome on the Odd-ball task.

The lack of active treatment effects does not support previous literature which has found glucose and caffeine to affect the P300 component (Brown & Riby, 2003; Dixit *et al.,* 2006; Kawamura *et al.,* 1996; Lorist *et al.,* 1994a&b; Lorist *et al.,* 1995; Lorist *et al.,* 1996; Lorist *et al.,* 2004a; Rao *et al.,* 2005; Riby *et al.,* 2008; Smith *et al.,* 2009). Administration of 25g glucose has been found to reduce amplitude, latency and duration of the P3b component (Riby *et al.,* 2008) and enhanced the left parietal old/new effect, which is a measure of verbal memory (Brown & Riby, 2013). Caffeine at doses of 250mg (Lorist *et al.,* 1994; Lorist *et al.,* 1995), 500mg (Kawamura *et al.,* 1996) and 3mg/kg (approximately 210mg for a 70kg individual) (Dixit *et al.,* 2006) has been found to increase the amplitude of the P300, but not to reduce latency.

Whilst the dose of glucose previously found to elicit an effect on the P300 component is the same as used in this task, the caffeine dose was much lower at 40mg than the efficacious doses administered in previous studies. It may be therefore that the dose administered in this study was too low to elicit an effect. However the caffeine drink was the only active drink to improve behavioural performance, specifically decreasing reaction time on the Odd-ball task. Potentially, whilst this task elicits a memory updating ERP component, modulation of this particular behaviour outcome, increased speed, could be modulated by a more basic psychomotor process that was not assessed in this study.

In terms of the pharmacokinetics of caffeine, estimated from salivary caffeine samples, the levels of caffeine followed the expected pattern, with the highest levels following the caffeine containing drinks. However, when caffeine was administered in isolation, salivary caffeine levels peaked at 25mins, before declining at 55mins. Conversely, when administered with glucose, salivary caffeine levels peaked at 55 mins. The caffeine results suggest that salivary caffeine peaked later following the combination drink compared to the caffeine drink. These results are similar to findings by Adan and Serra-Grabulosa (2010) whereby they found that the salivary caffeine increase was greater for the caffeine group compared to the caffeine plus glucose group. Rather than the caffeine plus glucose group's salivary caffeine peaking at a lower level however, it may be the same as the finding in the current study, where the peak is just shifted. This is because they only sampled at baseline and 30mins post drink consumption whereas in the current study salivary caffeine was sampled at baseline, 25mins post drink and 55mins post drink. Numerically both the caffeine and caffeine and glucose group reached the same salivary caffeine levels, the caffeine group just did so 25mins post drink and the caffeine and glucose group did so at 55mins post drink. Previous research has found that caffeine can increase glucose uptake and/or release (Graham *et al.,* 2001; Greer *et al.,*, 2001; Keijzers *et*

al.,, 2002; Lee *et al.*, 2005; Petrie *et al.*, 2004; Pizziol *et al.*, 1998; Thong *et al.*, 2002), and affect the intestinal absorption of glucose (Van Nieuwenhoven *et al.*, 2000). However, effects on caffeine absorption by co-administration with glucose has not been explored (Adan & Serra-Grabulosa, 2010).

The potential modulation of glucose on caffeine absorption may impact on the timing of the physiological measurements and cognitive tasks. The task timing is based on the premise that peak plasma concentration is reached around 15 to 120 min after ingestion (Nehlig, 1999; Nehlig & Boyet, 2000). However it may be that when co-administered with glucose this profile is altered and therefore the window of opportunity for the greatest effects of glucose and caffeine in combination may be missed.

This study found that, unlike in previous research glucose, caffeine or their combination did not modulate any parameters of the P300 component despite evidence of beneficial behavioural effects. The concurrent finding that caffeine absorption could be altered when in combination with caffeine leads to the suggestion that perhaps the timing of the tasks misses the window of opportunity for enhancement effects.

Chapter 8

General Discussion

The experiments reported in this thesis were designed to systematically evaluate the effects of combined glucose and caffeine administration in comparison to either glucose or caffeine in isolation in healthy young adults. This was done by examination of dose-response, cognitive domains, evaluation of neural mechanisms and biochemical effects. The following chapter will summarise the findings by discussing cognitive domains affected, summarising effects on biochemical and neurocognitive measures and exploring the effects of different dosages used. Potential moderating and mediating factors will be discussed. Implications and potential methodological issues will be addressed and suggestions for future research directions made.

8.1 Effects on Memory

The first experimental study (as described in chapters 2 and 3), aimed to compare the effects of two dosing regimens, a 'low' dose (15g glucose and 20mg caffeine), and a 'moderate' dose of glucose and caffeine (25g glucose and 40mg caffeine). The active doses of glucose and caffeine were administered in isolation and alone and compared to a placebo drink. Cognitive performance, mood and hormonal responses were assessed. A wide range of cognitive tasks were included to elucidate the cognitive domains most susceptible to modulation by glucose and caffeine in combination. Whilst evidence of improved performance was limited across all cognitive domains for both dosing regimens, there was some evidence of beneficial effects on memory, specifically verbal declarative memory. There were no beneficial effects on working memory.

The second experimental study (as reported in chapter 4) aimed to specifically explore the effects on memory performance when the active drinks (40g glucose and 40mg caffeine)

were administered prior to memory retrieval. There was however no beneficial effect of glucose and caffeine administered in isolation or in combination.

Given the equivocal findings of the research in the series so far, besides temporal relation of drink administration, another aspect of the methodology was of interest: the importance of participants' state before drink administration and testing. Based on previous research, it is possible that a sub-optimal state, i.e. being tired, fatigued might be critical to obtaining an effect. This might suggest that manipulation of participants into a sub-optimal state would provide clearer results. Unfortunately the Resource Depletion battery that was utilized, failed to tease out any effects related to participants potentially altered state.

In general, the findings on memory, in particular verbal declarative memory are equivocal. There is some support for previous research which has found beneficial effects of glucose and caffeine in combination on memory (Adan & Serra-Grabulosa, 2010; Alford *et al.,* 2001; Sünram-Lea *et al.,* 2012). Taken as a whole, the results from this series of studies suggest that caffeine and glucose alone and in combination may preferentially benefit recognition performance rather than recall. The recall and recognition aspects of memory are theorised to be related but separate processes (Flexser & Tulving, 1978; Gillund & Shiffrin, 1984). Whilst by no means exclusive, most of the tasks where no improvements following the active treatments were observed, or where performance was even impaired, were recall tasks. The hippocampus has been most strongly implicated in long-term memory performance (Aggleton & Brown, 1999). More specifically, the hippocampal-diencephalic system has been postulated as important for item recognition during recollection of stimuli; conversely the recognition process suggested to be independent of the 'extended hippocampal system' and related to stimulus familiarity (Aggleton & Brown, 1999). Therefore the modulation of specific memory processes could be facilitated by different mechanisms of action. However, there is evidence to suggest that in

recognition memory itself there are different processes at work. Sünram-Lea *et al.,* (2008) utilised a 'remember-know' paradigm to assess recollection and familiarity components of recognition memory. They found that recognition responses that were based on recollection (remembering responses) were sensitive to beneficial modulation by glucose administration. In contrast responses based on familiarity (know responses) were no sensitive to facilitation by glucose. This suggests that memory processes should be considered in finer detail as indeed should the potential modulation of these processes by glucose and caffeine. These different aspects of memory and their potentially different underlying mechanisms may go some way towards explaining the equivocal findings in this domain of cognition.

With regards to working memory, no supporting evidence was found. This is in support of the previous literature (Aden & Serra-Grabulosa, 2010; Scholey & Kennedy, 2004; Urquiza& Vieyra (2015), which found no effects on working memory.

8.2 Effects on Attention

In the first study (chapter 2) there were no benefits on attention seen following either the low or moderate treatment regimes. Chapter 5 reported the findings of a study which examined the effects of glucose and caffeine administered in isolation and combination on attention. The study utilised the testing paradigm of study 2 (chapter 4), but the task of interest was one which specifically assessed three separate attention networks; alerting, orienting and executive control. These studies also served to examine the effects of caffeine and glucose in a more realistic testing paradigm which had greater ecological validity as testing took place in the afternoon following a 2hr fast. This is more

representative of a typical consumption time as the substances may be consumed in order to provide an afternoon pick-me-up following a slump in performance and/or energy. However, no effects of glucose, caffeine or their combination were found on any of the attention networks. Study 4 (chapter 6) found no beneficial effects on attention following either the 'moderate' or 'high' treatment regime. Behavioural measures in study 5 (chapter 7) found that participants performed significantly faster on the Odd-ball task following 40mg caffeine.

Overall, whilst the effects on attention were very limited they provide some support for previous research that caffeine has a beneficial effect on attention (Brice & Smith, 2001b; Chubley *et al.,* 1979; Hindmarch, *et al*., 2000; Smith *et al.,* 1997; Smith *et al.,* 1994; Smith *et al.,* 1992). In comparison with the effects on memory, beneficial effects were found following the moderate dosing regimens (40mg caffeine). With the moderate 40mg dose beneficial for decreasing reaction time. It appears that caffeine is responsible for the modulation of attention and that there is no evidence from the current research series to suggest that glucose beneficially modulates attention performance. This is because there were no beneficial effects seen following the combination drinks or glucose alone. Previous research for the effect of glucose on attention is limited, although Giles *et al.,* (2012) did find a beneficial effect of 50g glucose on the orienting network, this was in conjunction with taurine administration.

Attention tasks can be divided into simple information processing tasks which merely require a response to a stimulus and have an element of automaticity, and more complex ones which involve executive control (Enother & Giesbrecht, 2013). It has been suggested that simple tasks are more sensitive to effects of pharmacological interventions, in particular on psychomotor components (Enother & Giesbrecht, 2013), and caffeine has previously been found to have its most robust effects on simple attention processes (for

reviews see Glade, 2010; Smith, 2002). As caffeine is the main driver behind the attention result seen here, this could provide an explanation as most of the tasks that have shown beneficial effects are simple attention tasks and no effects were seen on the more complex Attention Network Task. Effects on the more complex attention tasks may be difficult to elucidate due to the myriad of factors e.g. personality and time of day that could influence performance (Smith, 2002).

8.3 Effects on Mood

In terms of mood effects, in the first study (chapter 2) positive modulation of mood was found, although this was almost exclusively limited to the moderate dose regimen, with each of the active treatments improving various self-rated aspects of mood. In the fourth study (chapter 6) both active drinks were able to elicit beneficial effects on mood irrespective whether participants started the session in a state of cognitive depletion or not. In terms of dose, the moderate dose of 25g glucose and 40mg caffeine was more efficacious in eliciting mood effects. Across both studies 40mg caffeine and 25g glucose were found to increase level of alertness, calmness, contentedness/happiness, arousal and reduce anxiety. It also improved ratings on the more physiological based feelings such as reducing light-headedness and increasing clear-headedness. The only negative effect was for the 40mg caffeine and 25g glucose combination treatment to increase one rating of tense arousal. Consequently the studies reported here support previous research findings that consumption of glucose and caffeine engenders mainly beneficial effects on mood (Gershon, *et al.,* 2009; Howard & Marczinski, 2010; Kennedy & Scholey, 2004; Mets *et al.,* 2010; Smit & Rogers, 2002; Sünram-Lea *et al.,* 2012).

Although in one of the two studies in this thesis which assessed mood, glucose and caffeine were only administered in combination, the almost complete absence of any mood effects following administration of either glucose or caffeine in isolation suggests that it is the combination which is responsible for the modulation of mood. Whether this is a truly synergistic effect remains to be elucidated. However, as the findings in this thesis reflect the mood changes found previously following consumption of either caffeine or glucose in isolation, then it is likely that these findings reflect some level of additive effect of each substance at the doses utilised here (Benton & Owens, 1993; Brice & Smith 2001a; Haskell *et al.,* 2005; Hindmarch *et al.,* 2000; Kennedy *et al.,* 2008; Owens, *et al.,* , 1997; Smith *et al.,* 1999).

Previously it has been proposed that mood effects only follow on from changes in cognitive performance (Rusted, 1999). However, given that the cognitive effects found in the current studies were limited, the mood outcomes found would not support this as being a necessity. Yet, emotional arousal state might have mediated some effects. Improvements on memory were seen when administration was prior to encoding, but not when administered prior retrieval. This might be due to specific effects on encoding and storage, or could be explained via state dependent state-dependent memory effects (Bruins Slot & Colpaert, 1999; Ceretta *et al.,* 2008), as in this case the substances (caffeine and glucose) would have been present for both encoding and retrieval. Sanday *et al.,* (2013) suggests that it may be related to the anxiogenic effects of caffeine that modulate the participants' emotional state and thereby elicit a state dependent learning effect.

8.4 Effects on neuroendocrine response

In order to assist with elucidating the underlying mechanisms of action of glucose and caffeine we also investigated the neuroendocrine response to drinks (as reported in chapter 3). Both the hypothalamic-anterior pituitary-adrenocortical axis (HPA axis) and sympatho-adrenomedullary axis (SAM axis) response were examined by measuring both salivary cortisol and alpha amylase. However no effects of the active drinks were found on these mechanisms. Whilst the findings from this study do no support some previous research which has found effects on neuroendocrine responses after glucose and caffeine ingestion (Bergendahl *et al.*, 1996; 2000; Gonzalez-Bono *et al.*, 2002; Graham *et al.*, 1994; Kirschbaum *et al.*, 1997; Vance & Thorner, 1989; James, 2004; Lovallo *et al.*, 2006; Lovallo *et al.*, 2005; Robinson *et al.*, 2004), there are other reports in the literature of a failure to observe effects on these hormonal systems (Klein *et al.*, 2014; Sünram-Lea *et al.*, 2012).

In the study reported in this thesis beneficial effects of the active drinks were found without any concomitant changes in hormonal response. Therefore, our data does not provide evidence that the beneficial effects were elicited via underlying neuroendocrine mechanism.

8.5 Neurocognitive effects

The final study (chapter 7) in the thesis examined the neurocognitive effects of glucose and caffeine. Doses of 25g glucose and 40mg caffeine (chosen as they have been the main doses of interest throughout this thesis), were administered in isolation and combination and their effects on the P3b component of the P300 ERP were examined. This component reflects the memory updating component as improvements in memory have been found

across this programme of studies. The P3a which assesses frontal lobe engagement was also evaluated. No effects of any of the active treatment drinks were found on any of the EEG measures. There was however evidence that the active treatments had a beneficial effect on behavioural performance.

The findings of this study did not support previous research which has found effects of both caffeine and glucose on the P300 component (Brown & Riby, 2003; Dixit *et al.*, 2006; Kawamura *et al.*, 1996; Lorist *et al.*, 1994a&b; Lorist *et al.*, 1995; Lorist *et al.*, 1996; Lorist *et al.*,, 2004a; Rao *et al.*, 2005; Riby *et al.*, 2008; Smith *et al.*, 2009). However, it is important to note that the doses used in this study were much lower than those used previously and this may explain the lack of effects. Alternatively it may be that other factors such as task timings affected the results. Concomitant measurement of salivary caffeine suggested that caffeine absorption was modulated by co-administration with glucose and this may have affected the pharmacokinetic profile of caffeine. Therefore the tasks may have been administered when the ingested caffeine was unable to be utilised fully.

Table 8.1 Summary of the effects on caffeine and glucose administered in combination on outcome measures in this study series

Domain	Evidence Found for Benefits	Evidence Found for Negative Effects	Summary of Evidence
Immediate Memory	No evidence found	No evidence found	No evidence that caffeine and glucose in combination have a beneficial effect on Immediate Memory. Some evidence that Caffeine and glucose in combination can preferentially benefit Recognition based memory.
Delayed Memory	Word Recognition Accuracy* Word Recognition Speed*	No evidence found	
Working Memory	No evidence found	No evidence found	No evidence that caffeine and glucose

			in combination have a beneficial effect on Working memory.
Attention	No evidence found	No evidence found	No evidence that caffeine and glucose in combination have a beneficial effect on Attention.
Improvement in Mood	More alert* Calmer* Lower tense arousal* More clearheaded* Happier* Less light-headed* Reduced heart pounding*	More tense*	Moderate evidence to support that caffeine and glucose in combination have beneficial effects on mood and limited negative effects.
Neuro-cognitive Effects	No evidence found	No evidence found	No evidence that caffeine and glucose in combination have a beneficial effect on Neuro-cognitive effects.
Hormonal Effects	No evidence found	No evidence found	No evidence that caffeine and glucose in combination have a beneficial effect on Hormonal response.

* 40 mg Caffeine and 25g Glucose

8.6 Dose effects

Another important aim of this research was to identify the most effective dose. Overall the 25g glucose and 40mg caffeine doses appeared to be the most effective across memory, attention and mood outcomes. These doses are in line with previous research which has found 25g glucose to be effective at beneficially modulating cognitive performance (Kennedy & Scholey, 2000; Scholey *et al.*, 2001; Sünram-Lea *et al.*, 2002a; Sünram-Lea *et al.*, 2004); and that 50-75mg of caffeine has beneficial effects (Messier, 2004), with evidence of a flat dose response profile at lower doses (Smit & Rogers, 2000). However these doses of caffeine and glucose are lower than those previously found to affect mood

ratings (Benton & Owens, 1993; Haskell *et al.*, 2005; Owens, Parker & Benton, 1997). There is no strong evidence from the current studies of a synergistic effect as often it was found that either caffeine or glucose in isolation also had the same effects as the combination drink.

As found previously for glucose, the results also suggest that the specific dose-response profile is task specific (Sünram-Lea *et al.,* 2011). A lower dose (20mg) of caffeine was more effective at improving performance on attention tasks compared to its ability to modulate memory performance. Equally preferential improvement on recognition performance by 25g glucose and 40mg caffeine compared to their effects on recall performance again is supportive of a different dose-response profile for these aspects of memory.

The salivary caffeine findings from study 5 (chapter 7) provide evidence that the pharmacokinetic profile of caffeine absorption is attenuated by glucose. Specifically it was found that the salivary caffeine peaked later following consumption of a combined glucose (25g) and caffeine (40mg) drink. This finding requires further exploration as little research has examined the effects of glucose on caffeine absorption (Adan & Serra-Grabulosa, 2010). This attenuation of caffeine absorption by glucose could be influential on the most effective doses required when the substances are co-administered.

8.7 Moderating effects

Several factors may be responsible for moderating the effects of glucose and caffeine in the studies conducted in this thesis.

One factor which was the subject of examination in the thesis was the activation state of the participants. Earlier study findings in this thesis of the effects of caffeine and glucose on cognitive performance and mood were equivocal. Previous research had found the most robust effects of these substances in participants who were performing at a sub-optimal level (Alford, Cox & Wescott, 2001; Horne & Reyner, 2001; Kennedy & Scholey, 2004; Sünram-Lea, Owen-Lynch, Robinson, Jones & Hu, 2012). It was postulated that glucose and caffeine may be more effective at ameliorating the decrements in performance of participants in this state, rather than increasing the performance of participants who were already performing at a high level. Study 4 (chapter 6) aimed to examine whether participants activation state would elucidate beneficial effects of caffeine and glucose on performance and also to determine if it was possible to modulate this state using a demanding cognitive battery. The Resource Depletion Battery was found to increase participants' ratings of mental fatigue. However in comparison to a group who did not complete the battery, the active combination treatment drinks were not found to have greater beneficial effects across memory, attention and mood measures.

These findings provide a proof of concept that participants can be cognitively depleted by a demanding task and provides an alternative to fatiguing participants via exercise or sleep restriction that is easier to administer in a laboratory setting. Further work needs to be done to establish the parameters for the task to engender a depletion effect more reliably. The Resource Depletion Battery was also utilised in study 5 (chapter 7) to help elucidate the effects of caffeine and glucose on neurocognitive processes, however only limited behavioural effects of the active treatments were found. The concept is flexible and potentially different tasks could be utilised within the battery depending on the domain under examination.

8.8 Methodological Considerations and Future Research Directions

There are several methodological considerations that should be taken into account when interpreting the findings of this thesis and that warrant further investigation.

Firstly a consideration that should be given is to the length of the testing batteries utilised in this research; in particular the length of the testing battery in the first study (chapter 2). The total length of the battery was quite long at around 30mins and this may be a factor in the limited modulating effects of caffeine and glucose, especially as the tasks were always carried out in the same order. Ullrich *et al.,* (2015) suggest that the lack of effects in their study of 25g glucose and 200mg caffeine on cognitive performance was due to the extensive test battery which lasted for 2hrs as the cognitive resources of the participants may have already been depleted by the time they completed the final task. The effects of this on task outcomes could be moderated by counter-balancing the order of task presentation.

As described above in study 5 (chapter 7), salivary caffeine levels were found to peak later following the caffeine and glucose combined drink in comparison to the caffeine drink. Whilst caffeine has been found to attenuate glucose uptake, release and absorption (Graham *et al.,* 2001; Greer *et al.,* 2001; Keijzers *et al.,* 2002; Lee *et al.,* 2005; Petrie *et al.,* 2004; Pizziol *et al.,* 1998; Thong *et al.,* 2002; Van Nieuwenhoven *et al.,* 2000), to date little research has examined the effect of glucose on caffeine absorption (Adan & Serra-Grabulosa, 2010). The findings from this thesis suggest that further investigation into this effect is warranted. There are implications for the timings of the tasks as the different pharmacokinetic profile in comparison to that expected when caffeine is administered alone (Nehlig, 1999; Nehlig & Boyet, 2000), means that potentially the tasks are completed too early, before the combination of caffeine and glucose is at its most

effective. The salivary caffeine level measured at 55mins post caffeine drink also drop off sharply at the time when the salivary caffeine levels are just peaking following the combination drink. What is unknown, due to the measurement times in this study, is how long it takes before the salivary caffeine levels begin to decline following the combined drink. It is possible that the levels of caffeine remain elevated for a sustained period of time and therefore it may be that if you were to compare cognitive performance after a longer interval that the beneficial effects of combined administration may be greater and therefore elucidated more clearly. Modulation of glucose by caffeine and the effect on the time course of the physiological response should also be investigated further. Young and Benton (2013) found that, compared to administration without caffeine, when 80mg caffeine was administered with either 39g glucose or a yoghurt drink with a low GL (3-6) interstitial glucose levels were increased. The peak of the response was delayed by 10mins and the response was increased and prolonged for 90mins post-drink

Another consideration is an area which has received much attention in the caffeine literature and relatively little in the combination literature and that is whether any beneficial effects seen are due to reversal of caffeine withdrawal rather than any net benefits of caffeine and glucose consumption (James, 1994; James, 1998; James & Rogers, 2005; Rogers & Dernoncourt, 1998; Yeomans, Ripley, Davies, Rusted & Rogers, 2002). In all the studies in this thesis participants were withdrawn from caffeine for at least 12hrs. Previous studies which have investigated this when examining the benefits of combined administration have found mixed results. Warburton *et al.,* (2001) considered the potential effect of withdrawal from caffeine and imposed an abstinence period of only 1hr. They found that administration of a combination drink (80mg caffeine and 26g glucose) had beneficial effects on attention and verbal reasoning in the absence of caffeine withdrawal. Conversely Ullrich *et al.,* (2015) found no effects of 25g glucose and 200mg caffeine in

combination on cognitive performance (logical thinking, processing speed, numeric and verbal memory, attention and ability to concentrate) when participants continued with their habitual caffeine intake prior to the study. They did however find effects of caffeine alone on self-ratings of mental energy and these effects were following a 24hr period of caffeine abstinence. In this study though participants also continued to consume their usual sugar intake and therefore this may also have a separate or possibly combined effect on the findings. Future research should examine the potential for withdrawal reversal effects on co-administration of caffeine and glucose.

Related to the above is the finding that caffeine consumer status of the participant does alter caffeine's effects (Haskell & Kennedy, 2011; Rogers *et al.,* 2013). Differences in cognitive performance, mood and physiological responses have been found between consumers and low/non-consumers of caffeine. The participants who took part in this research were all regular caffeine consumers (at least 120mg per day), and therefore the effects of consumer status on these findings were mitigated. However, future research should explore the combined effects of caffeine and glucose in consumers and non-consumers of caffeine in a more systematic way to elucidate the moderating effects. The differing physiological responses to caffeine in consumers and non-consumers (Haskell & Kennedy, 2011) may provide further information about the underlying mechanisms of action of these substances in combination.

Similarly a moderating factor found in the glucose literature has not been examined here, and that is the effect of participants' glucoregulation. In those with poor glucoregulation (associated with both older adults and high BMI), administration of glucose was associated with impaired cognitive performance (Donohoe & Benton, 1999a; Messier, *et al.,* 2003; Sünram-Lea *et al.,* 2011). Whilst the participants utilised in the current research were all young, healthy adults, the effects of individual glucoregulation cannot be ruled out. As

discussed previously (chapter 1), the effects of glucoregulation have not been examined in the combined literature and therefore further evaluation of its potential mediating effects is warranted. A related point is that individual glucoregulation, and the speed at which individuals metabolise glucose, may make detecting an effect of glucose more difficult when testing takes place shortly after consumption as some participants may not have metabolised it sufficiently (Sünram-Lea *et al.*, 2002)

It is also worth noting that in female participants, both the stage of their menstrual cycle and/or the use of the contraceptive pill, can affect their response to caffeine as estrogen inhibits metabolism and the pharmacokinetics of caffeine (Lane, Steege, Rupp & Kuhn, 1992).

Caveats should also be placed when assessing mood, as this is a subjective measure and therefore interpretations should be made cautiously as it could be affected by a number of external factors. Controlling for baseline mood in the studies reported here goes some way towards protecting pre-existing mood states from carrying over into the study. As discussed earlier in this chapter, the mood effects found in this research were also reasonably consistent and in agreement with previous findings in both the single and combined administration literature, demonstrating reliability and therefore strengthens their meaning.

Whilst the above methodological considerations are of note and worthy of further exploration, it must be remembered that if the findings from research are to be ecologically valid, then it is unlikely that all the potential mediating factors can be controlled. Indeed, if the effects of these substances cannot be found in more naturalistic and realistic settings and without too many parameters controlled for then the value of the effects found could be questioned.

Moving away from participant characteristics, as described above, further exploration into individual aspects of tasks should be further investigated. As discussed, it appears that individual aspects of the memory process may be preferentially enhanced by glucose, caffeine and their combination. Teasing apart these more detailed effects may help to further elucidate the mechanisms that underlie the effects of these substances.

8.9 General summary

In conclusion this research has found some evidence of beneficial effects of caffeine, glucose and their combination on cognitive performance, mood and physiological response. However, no effects were found on neurocognitive measures or neuroendocrine response. The effects found are on the whole supportive of previous findings; in terms of improvements on memory, attention and mood, at doses found previously to be effective, both alone and in combination. The effects of the combined substances appear to be driven by the effects of one or other of the individual substances, depending on the outcome measure and so truly synergistic effects are not seen. Many potential moderating factors and specific cognitive domain effects are yet to be fully explored. Further investigation into these will further elucidate the effects of these substances and their underlying mechanisms of action.

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