EFFECT OF CAFFEINE INGESTION ON ASPECTS OF ENDURANCE PERFORMANCE AND COGNITION IN CYP1A2 HETROZYGOUS A/C MALE RECREATIONAL ATHLETES

By

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ABSTRACT

Background: Globally, caffeine is the most widely accessible psychoactive drug and has been shown to improve endurance performance as well as aspects of cognition, mood and perceptual responses during exercise. However, the ergogenic effects of caffeine between individuals are variable, and the cause of this variability is unknown. The CYP1A2 gene is known to mediate caffeine metabolism and has been suggested as a contributor to the variability of the ergogenic effects of caffeine.

Purpose: To investigate the effects of CYP1A2 genotype on exercise performance (10 km time trial), sleep, mood, cognition and perceptual responses following caffeine ingestion in adult male recreational athletes.

Methods: 16 recreationally trained athletes (age = 26.9 ± 7.93 y; weight = 77.00 ± 9.04 kg) volunteered for this study. Participants completed a familiarisation session at least one week before the first trial and a saliva sample was collected for testing of the participants' CYP1A2 genotype. Participants completed two trials one week apart in a randomised double-blind placebo-controlled cross-over design. Participants were asked to abstain from caffeine ingestion and keep a food diary for 24 h prior to the trial. Participants wore an actigraph, and completed a sleep diary and Leeds Sleep Evaluation Questionnaire (LSEQ) every day for the two week duration of the trials starting 3 days before the first trial and ending 3 days after the second trial. The main trial consisted of a set of pre- and post-ingestion measures which included leg power by vertical jump height (squat jump – SJ; countermovement jump – CMJ), leg strength by maximal voluntary concentric and eccentric contraction of the knee extensors (isokinetic dynamometer), perceptual (feeling scale – FS; felt arousal scale – FAS), mood (profile of mood states – POMS), cognition (digit vigilance – DV; Corsi blocks – CB; rapid visual information processing – RVIP) and heart rate. Pre- and post-ingestion urine, saliva and blood samples were also collected for analysis of caffeine metabolism and genotype. Following completion of pre-ingestion measures, participants consumed a capsule containing either anhydrous caffeine (6 $mg \cdot kg^{-1}$) or placebo (maltodextrin) and were instructed to rest quietly for 50 min. Following post-ingestion measures, participants completed a 10-km time trial run. Perceptual

measures (FS and FAS) including ratings of perceived exertion (RPE) were recorded every 2.5 km and heart rate was recorded every 1 km. A venous blood sample and saliva sample was collected at 5 km and 10 km. At completion of the 10-km time trial all post-ingestion measures were repeated, followed by another 50 min rest period. After the second 50 min rest period the participants completed the perceptual, mood and cognitive measures and further blood, urine and saliva samples were collected. Participants returned 24 and 48 h post-ingestion to repeat all post-ingestion measures and another blood, urine and saliva sample was collected. This protocol was then repeated 1 week later for the alternate treatment (placebo or caffeine). The effect of treatment (caffeine, placebo) and the interaction effect of treatment x time were assessed using a repeated measures ANOVA. A student's t-test was used to measure differences between Leeds sleep evaluation questionnaire (LSEQ) and actigraph data.

Results: Fourteen of sixteen participants were heterozygous A/C CYP1A2 for the CYP1A2 genotype and therefore results based on genotypes could not be compared as originally intended. Plasma caffeine, paraxanthine and theophylline concentrations were all elevated following caffeine ingestion ($P < 0.05$) peaking at 10-km, 1 hour after the 10-km run and 24 hours post caffeine ingestion respectively. Caffeine did not significantly improve 10-km run times. Eccentric leg strength but not concentric leg strength was improved following caffeine ingestion ($P < 0.05$). Squat jump height but not countermovement jump height was improved following caffeine ingestion ($P < 0.05$). Digit vigilance reaction times were decreased significantly following caffeine ingestion ($P < 0.05$) and a trend of decreased rapid visual information processing (RVIP) reaction times were seen ($P < 0.1$), however, no improvements in the accuracy during cognitive tests were seen following caffeine ingestion. A trend of increased heart rate ($P < 0.1$) during exercise was observed following caffeine ingestion, but no significant differences in heart rate before and after exercise were observed.

Conclusions: While no overall, significant improvements in run time occurred following caffeine ingestion, 11 of 14 participants had a faster run time following caffeine ingestion compared to placebo. Caffeine, rather than the metabolites of caffeine, is likely the main cause of any observed ergogenic effects following caffeine ingestion as the improvements in reaction times, mood and

endurance performance occurred when plasma caffeine concentration was elevated but plasma caffeine metabolite concentrations were low. It was found that caffeine ingestion improves endurance performance and reaction times during cognitive tasks. Taken together, the pharmacokinetics of the caffeine and caffeine metabolite peaks suggest that for athletes with the A/C CYP1A2 genotype ingestion of caffeine 1.5 – 2 h prior to an event may be more beneficial for endurance performance compared to the usual recommendations of taking caffeine 1 h prior to exercise.

Keywords: caffeine, endurance exercise, CYP1A2, performance, genetics

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CHAPTER 1 – INTRODUCTION

Caffeine is the most widely consumed psychoactive drug in the world due to its accessibility and the minimal health risks associated with its use (Graham, 2001). It is inexpensive, available in many forms and is both legal and socially acceptable around the world. Caffeine has also been shown to be a powerful ergogenic aid in many populations and across multiple exercise modalities both during competition and training. However, the mechanisms of action of caffeine as an ergogenic aid are still not fully understood despite the large amount of literature on the subject.

Isolation of caffeine from green coffee beans in the 1820's (Runge, 1820) sparked scientific interest in caffeine and its effects on metabolism and has been studied ever since. By 1907 it was reported that caffeine likely had an ergogenic effect and it was classified as a doping agent (Rivers and Webber, 1907). However, there was little research done on the ergogenic effects of caffeine until the 1970's. Caffeine was added to the list of banned substances by the International Olympics Committee (IOC) in 1984 and the World Anti-Doping Agency (WADA) in 2000. Although caffeine was officially removed from the WADA banned substance list in 2004, it is still monitored and athletes are encouraged to maintain a urine caffeine concentration of $< 12\ \mu g \cdot ml^{-1}$ urine which corresponds to $10\ mg \cdot kg^{-1}$ orally ingested (Spriet, 2014).

In a series of experiments by Costill et al. (1978), it was thought that they primary mechanism behind the ergogenic effects of caffeine were due to glycogen sparing and the increase in fat oxidation during submaximal exercise (Costill et al., 1978; Ivy et al., 1978). More recently, others have suggested that caffeine does not have an effect on the rate of lipid oxidation (Graham, 2001; Spriet, 2014). Two studies by Graham et al. (2000, 2008) found that there was an increase in lipid mobilisation after caffeine ingestion, but there was no difference in fat metabolism in muscle, which suggests that the ergogenic effects of caffeine are not related to the sparing of glycogen and fat utilisation as a substrate. While majority of the literature suggests that caffeine improves endurance performance, the mechanisms by which this is achieved are less clear.

Several mechanisms of action, in addition to changes in substrate utilisation, have been identified which may also contribute to the ergogenic effects of caffeine. These potential mechanisms include enhanced ionic balance due to improved function of the sodium-potassium pump (Mohr et al., 2011) and changes in perceived exertion and perception of pain (Meyers et al., 1997; Doherty and Smith, 2005). While several modes of action have been identified, inhibition of adenosine receptors seems to be the most important (Graham, 2001). Caffeine has a similar structure to adenosine and can compete with adenosine at receptor sites. Adenosine receptors are found throughout the body and in all major organs. This allows caffeine to simultaneously affect multiple tissues resulting in a range of interacting responses. However, because it affects many different tissues, determining the exact cause of caffeine's ergogenic effects has proven difficult.

Some studies have also reported significant variance between subjects in response to caffeine ingestion (Doherty et al., 2002; Meyers and Cafarelli, 2005). These studies have reported that approximately 30% of participants derived no ergogenic effect following caffeine ingestion. Therefore, despite the ergogenic effect of caffeine being evident, it is highly variable between subjects. A number of factors have been associated with changes in caffeine metabolism which include pregnancy and use of oral contraceptives (Abernathy and Todd, 1985), smoking (Magkos and Kavouras, 2005), age (Chung et al., 2000) and genetics (Yang et al., 2010). The variance found in these studies (Doherty et al., 2002; Meyers and Cafarelli, 2005) may be due to one or more of these factors.

Research into the effects of genetics on caffeine metabolism and sensitivity is becoming more popular (Loy et al., 2015; Algrain et al., 2016; Pataky et al., 2016). Caffeine is metabolised in the liver by the cytochrome P450 oxidase, and the isozyme CYP1A2 is responsible for the majority of caffeine metabolism which breaks it down into three dimethylxanthines: paraxanthine (81.5%), theobromine (10%) and theophylline (5.4%; Gu et al., 1992). More than 150 single nucleotide polymorphisms have been identified for the CYP1A2 gene (dbSNP database: http://www.ncbi.nlm.nih.gov/SNP/). Three main variants of the CYP1A2 gene have been attributed to caffeine metabolism. A single nucleotide (C→A) polymorphism at position 734 within intron 1 (rs762551) has been correlated with a high

inducibility of the cytochrome P450 in Caucasians, resulting in a higher caffeine metabolism in smokers with the A/A variant (Sachse et al., 1999). It was also found that 46% of the sample population possessed the homozygous A/A (increased caffeine metabolism) variant, 44% had the heterozygous A/C (slow caffeine metabolism) variant while only 10% had the homozygous C/C (very slow caffeine metabolism) variant of the CYP1A2 gene (Sachse et al., 1999). Individuals that possess a C variant have been shown to have an increased risk of myocardial infarction, most likely due to the slower clearance rate of caffeine (Cornelius et al., 2006). These studies (Sachse et al., 1999; Cornelius et al., 2006) indicate that the CYP1A2 gene plays a role in the rate of caffeine metabolism and may explain the variability of ergogenic responses between individuals following caffeine ingestion. There is still much work required to fully understand the role that the CYP1A2 enzyme plays in the metabolism of caffeine.

A study by Womack et al. (2012) investigated the effects of the CYP1A2 gene on the ergogenic effects of caffeine during a 40-km cycle time trial. It was found that caffeine-supplemented homozygous A/A participants had an improved performance of 4.9% compared to 1.8% in individuals with the C allele variants. In contrast, another study (Klein et al., 2012) grouped collegiate tennis players into homozygous A/A and C allele carriers to investigate the effects of CYP1A2 genotype on tennis skill and a 45 min run time trial. Following caffeine ingestion there was an overall improvement in tennis skill performance but no differences were seen between genotypes. These studies suggest that genes such as CYP1A2 influence the ergogenic effect of caffeine, however, the extent to which this occurs is not well understood and more research is required.

The effect of caffeine supplementation on strength and power activities has also been investigated but results are equivocal. Several studies have found significant improvements in strength and power measures following moderate (6-7 $mg \cdot kg^{-1}$ BM) caffeine ingestion (Jacobson et al., 1992; Timmins and Saunders, 2014). However, training status may affect ergogenic responses to caffeine ingestion (Timmins and Saunders, 2014). A review (Astorino and Roberson, 2010) found that 54% of studies reported significant caffeine-mediated improvements in maximal strength and muscular endurance. The mechanism by which caffeine may improve strength and power is not fully

understood. It has been suggested that improvements to strength and power may be due to better signal transduction and the direct effects of caffeine on the muscle rather than changes to metabolism (Graham, 2001). To this author's knowledge no study has investigated the influence of genetics on the effects of caffeine ingestion on strength and power activities. Further research is still needed which assess factors which may influence an individual's ergogenic response to caffeine during strength and power activities.

There has been a large amount of research that has shown that caffeine consumption can have positive effects on perceptual responses, cognition and mood (Lorist and Tops, 2003; Doherty et al., 2004; Ruxton, 2008). It was found that caffeine exhibited stimulant-like behavioural effects on mood and cognitive performance (Lieberman et al., 1987). Earlier studies on caffeine were often carried out with military participants, investigating the effects of caffeine on sleep deprivation, cognitive performance and vigilance (Lieberman et al., 2002). Low to moderate (32 – 256 mg) doses of caffeine, given to sleep-deprived military personnel significantly improved auditory vigilance and visual reaction time while also reducing feelings of fatigue (Lieberman et al., 2002). There were also no observable negative side effects as a result of the doses given in this study. However, several negative side effects such as mental confusion and disturbances to sleep after ingesting caffeine have been documented (Graham and Spriet, 1995; Roehrs and Roth, 2008).

Caffeine has been shown to reduce ratings of perceived exertion (RPE) during exercise. In a meta-analysis of 21 studies (Doherty and Smith, 2005) RPE ratings were found to be significantly reduced (CI 95%) during caffeine trials compared to placebo. Caffeine dosage ranged from 4-10 $mg \cdot kg^{-1}$ with a median of 6 $mg \cdot kg^{-1}$ when all of the studies were included. This meta-analysis included both constant load exercise as well as exercise terminating at exhaustion. In summary, caffeine improved overall exercise performance by 11.2% and RPE decreased by 5.6%. The authors also found that RPE could account for approximately 29% of the variance in the improvement in exercise performance (Doherty and Smith, 2005). To this author's knowledge, only two studies have included RPE as a perceptual response measure when investigating the effects of genetics on caffeine metabolism during exercise (Womack et al., 2012; Klein et al., 2012). No other studies have included

perceptual or cognitive measures whilst investigating the effects of genetics on the ergogenic effects of caffeine. Recent studies (Backhouse et al., 2007; 2011) have investigated alternative measures of perceptual responses during exercise; the use of the feeling scale (Hardy and Rejeski, 1989) and felt arousal scale (Svebak and Murgatroyd, 1985) may provide a more holistic measure of perceptual responses following caffeine ingestion.

Caffeine is known to increase alertness while decreasing tiredness and fatigue (Sokmen et al., 2008); it has also been shown to negatively impact on sleeping patterns when caffeine is ingested shortly before sleep (Sokmen et al., 2008). Large acute doses of caffeine have been shown to lead to sleep disturbances (Smith et al., 1993) and may result in decreases in mental and physical performance due to sleep deprivation. This is particularly pertinent for athletes who may have several consecutive days of competition or training and need to maintain performance throughout. Hindmarch et al. (2000) reported that healthy individuals ingesting repetitive doses of caffeine throughout the day had a significantly impaired onset of sleep, sleep quality and perceived sleep quality. This shows the importance of timing and dosage when ingesting caffeine in order to limit negative effects on sleep which may impair performance on subsequent days. Currently very little literature exists on the potential effects of genetics on responses to caffeine and sleep quality. Only a single study has investigated how genetic variation (ADORA2A) contributes to sensitivity to caffeine effects on sleep (Retey et al., 2007). The ADORA2A gene affects the adenosine receptors in the body making influencing the affinity of the receptors for caffeine and adenosine. This is likely to affect an individual's sensitivity to caffeine (retey et al., 2007).

Despite the large body of work on caffeine research, many of the mechanisms of action remain unclear, particularly those involved during exercise. The role of genetics in caffeine ingestion during exercise has not been thoroughly investigated. In particular the CYP1A2 gene has been shown to affect caffeine metabolism, but the effects on exercise performance are not well understood. Therefore, the overall aim of this thesis was to assess the effects of genotype on exercise performance (10 km time trial), sleep, mood, cognition and perceptual responses following caffeine ingestion in adult male athletes. It is hypothesised that caffeine ingestion will positively influence 10 km time trial

running performance as well improve leg strength and power. It is also hypothesised that CYP1A2 and ADORA2A genotype will affect caffeine metabolism and 10 km time trial performance.

1.1 Hypotheses

1. Caffeine ingestion will improve 10-km time trial performance in adult male athletes.
2. Caffeine ingestion will improve leg strength and power before and after a 10-km time trial run in adult male athletes.
3. Caffeine ingestion will positively affect perceptual responses before, during and after a 10-km time trial run in adult male athletes.
4. Caffeine ingestion will positively affect mood before and after a 10-km time trial run in adult male athletes.
5. Caffeine ingestion will negatively affect sleep quality up to 48 hours after a 10-km time trial run in adult male athletes.
6. Caffeine metabolism will be increased in homozygous A/A CYP1A2 gene carriers and caffeine metabolism will be decreased in C allele carriers.
7. A/C CYP1A2 gene carriers will perform better during the 10-km time trial in comparison to A/A CYP1A2 gene carriers following caffeine ingestion.
8. Caffeine ingestion will improve cognitive function before and after a 10 km time trial run in adult male athletes.

CHAPTER 2 – LITERATURE REVIEW

This review serves to provide background to caffeine metabolism and its central and peripheral mechanisms of actions. In addition, it examines the current literature regarding the ergogenic effects of caffeine on endurance performance as well as the effects of caffeine on mood, sleep quality, perception and cognition. The role of genetics on the ergogenic effects of caffeine will also be examined.

2.1 Caffeine use as an ergogenic aid

Caffeine, unlike other psychoactive drugs such as cocaine, LSD and cannabis, stands in a unique position as a drug, as it is highly accessible and an accepted part of the diet of many people around the world (Graham, 2001; Spriet, 2014). Traditionally, caffeine has been used to a large extent by military personnel and shift workers to ameliorate fatigue and tiredness (Liebermann et al., 2002; Burke, 2008), however, since the early 1900's it has been used as a stimulant to improve performance and prevent fatigue during sports and exercise. Due to its widespread accessibility (found in coffee, tea, chocolate, soft drinks and energy drinks) caffeine was removed from the World Anti-Doping Agency (WADA) banned list in 2004, although it is still monitored and a urinary level of above 12 $\mu g \cdot ml^{-1}$ (approximately equal to 10 $mg \cdot kg^{-1}$).

Research on the effects of caffeine on human performance began in the early 1900's (Rivers and Webber, 1907), but it was not until the 1970's when the focus moved primarily to the effects of caffeine as a stimulant (Costill et al., 1978). Up until the early 1990's some studies were still reporting inconclusive results as to the ergogenic effects of caffeine (Conlee, 1991). However, since then a significant amount of research has been conducted and caffeine is widely considered a potent ergogenic aid (Graham, 2001). Better control of confounding variables such as nutritional and training status of participants, caffeine doses, and exercise modality provided more consistent results from studies and provided stronger evidence for the ergogenic effects of caffeine.

2.2 Mechanisms of action

2.2.1 Fat oxidation

Early studies had found that caffeine ingestion increased free fatty acid (FFA) mobilisation and utilisation as a result of elevated catecholamines, which would create a glycogen sparing effect during exercise and decrease the rate of fatigue (Costill et al., 1978; Ivy et al., 1978). The first of these studies (Costill et al., 1978) had nine competitive cyclists perform two modes of cycling exercise. The first mode had participants cycling to fatigue at 80% $\dot{V}O_{2max}$ after ingesting either coffee or decaffeinated coffee one hour before the trial. Measures of FFA, glycerol and respiratory exchange ratio (RER) showed that there was a significantly increased rate of lipid metabolism in participants who had consumed caffeine prior to exercise. There was also an increase in the total work done by the participants when they consumed caffeinated compared to decaffeinated coffee. However, it was found that carbohydrate utilisation (CHO) was similar for both trials.

In the second study (Ivy et al., 1978), cyclists performed a 120 min isokinetic cycling exercise. Participants were given caffeine (250 mg) one hour before the trial and smaller doses (41.67 mg), as well as a glucose polymer (12.8 g) every 15 min during the first 90 min of the trial. The tastes of all drinks were disguised with an artificial sweetener and the control contained only the artificial sweetener. The results showed that caffeine consumption led to a 7.4% increase in the total work done compared to control, and during the last 30 min of the trial lipid metabolism increased by 31%. These results (Ivy et al., 1978) support the glycogen sparing hypothesis which suggests that improvements in performance following caffeine ingestion are due to the increased mobilisation and utilisation of lipids as a substrate for energy production during exercise, thus sparing glycogen in muscle cells. It is well known that fatigue can occur as a result of depleted muscle glycogen stores (Coyle et al., 1986), thus glycogen sparing has been attributed to delayed onset of fatigue in endurance events.

More recently the glycogen sparing hypothesis has come under scrutiny. There have been many studies which have reported no difference in fat metabolism after caffeine ingestion or have found large differences in responses to caffeine ingestion between subjects, results which have yet to

be fully understood. In one study (Graham et al., 2000), 10 healthy male participants performed one hour of steady state cycling exercise at 70% $\dot{V}O_{2max}$ after ingesting 6 $mg \cdot kg^{-1}$ caffeine or placebo (dextrose) in a double blind placebo controlled study. Leg muscle metabolism was measured using blood samples and muscle biopsies, while expired air was analysed for CO_2 and O_2 content. Increased plasma fatty acid and glycerol concentrations were found following caffeine ingestion, which suggests increased adipose tissue lipolysis. However, there was no change in respiratory exchange ratio (RER) between trials, thus it is unlikely that any glycogen sparing effect occurred due to increased utilisation of fats during exercise. It was unclear whether the increase in FFA mobilisation was due to the effects of increased circulating catecholamines or the direct adenosine antagonism by caffeine on the A_1 receptors of adipose tissues.

Caffeine at low doses (3 $mg \cdot kg^{-1}$) has been found to be just as effective at increasing endurance performance as moderate to high doses (6-9 $mg \cdot kg^{-1}$; Spriet, 2014). This is despite low doses not exhibiting the same physiological responses found with moderate and high doses, such as increased noradrenaline, adrenaline, FFA and glycerol levels (Graham and Spriet, 1995). In one study (Graham and Spriet, 1995), 8 trained male runners performed a running trial to volitional fatigue at 80% $\dot{V}O_{2max}$ after consuming one of three different doses of caffeine (3, 6 or 9 $mg \cdot kg^{-1}$) or a placebo 1 h before exercise. Trials were performed one week apart. It was found that ingesting higher levels of caffeine lead to progressive increases in circulating caffeine levels. The highest dose of caffeine produced significantly higher levels of plasma FFA, but the moderate and low dose did not produce any significant changes in plasma FFA. Despite this, a significant increase in endurance performance was found in well trained athletes following caffeine ingestion at low and moderate doses. Therefore the ergogenic effects of caffeine are unlikely to be as a result of substrate metabolism or glycogen sparing, but instead more likely to involve the central nervous system (CNS) as discussed later (Section 2.2.6). Results from studies which used low doses of caffeine (~3 $mg \cdot kg^{-1}$) have shown similar improvements in exercise performance without increased plasma FFA, which suggests that caffeine may act directly on the muscle through changes in ionic balance in the muscle (Ca^{2+} and K;

Section 2.2.4) which result in improved performance (Graham and Spriet, 1995; Pasman et al., 1995; Jenkins et al., 2008).

2.2.2 Blood glucose and lactate

It is known that during endurance running, glucose uptake by the active muscles increases, while uptake in inactive muscles decreases (Fujimoto et al., 2000) and that most constant-pace running studies have shown an increase in blood glucose levels during exercise (Wilbur and Moffat, 1992; Tsintzas et al., 1993). Caffeine has been shown to have two possible effects that may lead to increased blood glucose levels after caffeine ingestion; decreased insulin sensitivity as a result of increased catecholamines, particularly cortisol (Keijzers et al., 2002), or it may stimulate an increased rate of glycogenolysis and gluconeogenesis due to increased levels of adrenaline and cortisol.

There have been conflicting results about whether caffeine increases blood glucose levels during exercise. Several studies have reported significant increases in circulating blood glucose levels following caffeine ingestion (Spriet et al., 1992; Graham and Spriet, 1995; Graham et al., 2000; Marriott et al., 2015), while others show either decreased or no change in circulating blood glucose levels following caffeine ingestion (Raguso et al., 1996; Greer et al., 2001; Thong and Graham, 2002; Battram et al., 2006). In the study by Graham et al. (2000), 10 male participants performed one hour of exercise at 70% $\dot{V}O_{2max}$ on a cycle ergometer one hour after ingesting either caffeine (6 $mg \cdot kg^{-1}$) or placebo. This study found that caffeine caused a significant increase in arterial blood glucose and lactate levels. Despite the increase in arterial blood glucose there was no increase in RER, which suggests that the carbohydrate metabolism of the active leg was unchanged and that other tissues were responsible for the changes in arterial glucose and lactate.

The study by Raguso et al. (1996) showed that the rate of glucose appearance was not affected by theophylline (a caffeine metabolite) at rest or during exercise at 70% $\dot{V}O_{2max}$ after glucose infusion. However, the rate of glucose uptake had decreased after theophylline infusion compared to

placebo (31.6 ± 4.1 vs. 40.4 ± 5.0 μmol·min^{-1}·kg^{-1}), which the authors suggested was due to decreased glucose uptake by the active muscle.

Greer et al. (2001) examined the effects of caffeine on glucose uptake using a hyperinsulinemic-euglycemic clamp (insulin is raised and maintained at a constant concentration via continuous infusion of insulin) in 9 healthy resting male participants who ingested either caffeine (5 mg·kg^{-1}) or placebo. Glucose uptake rate was measured every 30 min from the time caffeine was ingested for 180 min in total. After caffeine administration, glucose uptake was 6.38 ± 0.76 mg·kg^{-1} compared with 8.42 ± 0.63 mg·kg^{-1} after the placebo trial. Significantly higher levels of plasma adrenaline were recorded during the caffeine trials compared to the placebo. The authors attributed these finding to the adenosine-receptor antagonism of caffeine. The findings of this study support the theory that adenosine is an important regulator of insulin-mediated glucose uptake and potentially glycogen synthesis.

In contrast to majority of the literature a study by Hulston and Jeukendrup (2008) had 10 endurance trained cyclists perform three trials of steady state cycling at 62% $\dot{V}O_{2max}$ followed by a 45-min time trial; during exercise, participants consumed either a glucose solution (6.4% CHO), glucose plus caffeine solution (6.4% CHO plus 5 mg·kg^{-1}) or a placebo (water). Solutions were consumed throughout the steady state exercise at a rate of 2 ml·kg^{-1} every 15 min with a 5.5 ml·kg^{-1} bolus at the start of exercise. Results of this study showed that the rate of appearance and disappearance of glucose was not significantly different between glucose solutions (glucose and glucose plus caffeine) and placebo. This indicates that caffeine did not have an effect on glucose kinetics and as a result is unlikely to produce any ergogenic effects due to changes in circulating glucose levels.

A recent review (Shearer and Graham, 2014) examining the effects of caffeine on performance and glucose tolerance reports that on average caffeine creates a 30% decrease in glucose uptake. The adenosine A_1 receptor has been shown to be directly involved in insulin signalling (Han et al., 1998) and may be largely responsible for the decrease in glucose uptake by the muscles. Thong et al. (2007) examined glucose uptake by rat soleus muscles exposed to combinations of insulin, adenosine and adenosine antagonist. It was found that under insulin-simulated conditions, adenosine

removal was responsible for a 50% decline in glucose uptake by the muscle. This supports the idea that caffeine consumption may lead to decreased glucose uptake by the muscle and may be responsible for elevated levels of plasma glucose following caffeine ingestion.

Thus, caffeine acts at multiple sites to decrease skeletal muscle insulin sensitivity and glucose uptake by the muscle and stimulates release of glucose from various tissues. However, it is unlikely that the increase in blood glucose will result in the enhanced exercise performance that is usually associated with caffeine supplementation as there is normally a decrease in glucose uptake by the active muscle (Shearer and Graham, 2014).

2.2.3 Catecholamines

Caffeine consumption has been shown to increase sympathetic nervous system activation, which leads to an increase in circulating levels of adrenaline compared to placebo (van Soeren et al., 1993; Graham and Spriet, 1995; Jackman et al., 1996; van Soeren and Graham, 1998; Graham et al., 2000). However, it has been noted that only moderate and high doses (6-13 $mg \cdot kg^{-1}$) of caffeine lead to increased noradrenaline and adrenaline levels, while low doses (≈3 $mg \cdot kg^{-1}$) do not result in the same increase (Graham and Spriet, 1995).

A study (Jackman et al., 1996) carried out on 14 recreationally active athletes (3 females, 11 males) showed an increase in plasma adrenaline levels and time to fatigue following caffeine ingestion. Participants performed 2 min of cycling at a power output requiring $\dot{V}O_{2max}$ followed by 6 min of rest, repeated twice more. During the third cycle bout the participant cycled until volitional exhaustion. Ten of the 14 subjects increased time to exhaustion during the caffeine trial (4.93 ± 0.60 min) compared to the placebo trial (4.12 ± 0.36 min). There was a significant increase in plasma adrenaline concentration but there was no change in muscle glycogen levels between trials. It was also found that at least 50% of the original glycogen concentration remained in the muscle. This indicates that fatigue did not occur as a result of depleted glycogen stores and the increase in exercise performance was not associated with glycogen sparing as glycogen stores would not be depleted in

the time taken to complete the trial. The authors suggested that the increase in performance was a result of a direct effect of caffeine on the muscle rather than the increase in catecholamine levels after caffeine ingestion.

The above results and mechanisms of action are supported by several studies in quadriplegic athletes, whose levels of plasma catecholamines are normally very low (van Soeren et al., 1996; Mohr et al., 1998) due to the negative impact on the sympathoadrenal response caused by severe damage to the cervical vertebrae (Mathias et al., 1976). The first study (van Soeren et al., 1996) tested the adrenaline responses of 6 male quadriplegics (lower spinal cord lesions C4-C6) at rest for three hours following caffeine ingestion (6 $mg \cdot kg^{-1}$). There was no increase in plasma adrenaline levels after ingesting caffeine, but there was an increase in plasma FFA and glycerol, which both remained elevated throughout the trial. The authors concluded that caffeine has a direct effect on tissues and the effects are independent to an increase in adrenaline.

Mohr et al. (1998) found similar results when testing 7 male quadriplegic (C5-C7 and two paraplegic T4) participants during electrically stimulated cycling to fatigue. The participants' paralysed legs were electrically stimulated via skin electrodes attached to the quadriceps, hamstrings and gluteal muscles to perform involuntary cycling on a modified cycle ergometer. Fatigue was defined as the inability to maintain 35 rpm of the flywheel. Participants consumed caffeine (6 $mg \cdot kg^{-1}$) or a placebo in a double blind fashion one hour before exercise. Catecholamines levels did not change after 15 min of exercise, but there were significant increases in plasma FFA concentration (caffeine 81.42 ± 8.16 μM vs placebo 76.99 ± 5.40μM) but no increase in RER. Exercise time to exhaustion was increased by 6% during the caffeine trial compared to the placebo trial. Similar to van Soeren et al. (1996), Mohr et al. (1998) suggested that caffeine has a direct effect on both the active muscle and adipose tissues which lead to increased FFA concentration, and that the response is not mediated through the effects of increased catecholamine concentrations.

It has been noted that caffeine ingestion may cause a modest increase in adrenaline concentrations but it is debatable as to whether it would have any metabolic significance. Increases in sympathetic activity would support the glycogen sparing hypothesis as this would lead to increased

lipid mobilisation. However, evidence over the last three decades has refuted the glycogen sparing hypothesis of caffeine, as most studies have not found a decrease in RER following caffeine ingestion. Thus it is likely that the ergogenic effects of caffeine are not a result of increased circulating catecholamine concentrations and glycogen sparing, but rather that caffeine has a direct effect on the active muscle thus leading to increased exercise performance.

2.2.4 Ionic balance

Fatigue has been defined as a failure to maintain the required or expected force (Edwards, 1981) or a decreased force generating capacity (Vollestad and Sejersted, 1988). Muscle fatigue may occur as a result of disruptions to the homeostasis of a muscle cell, shortage of substrates or an accumulation of metabolites within the muscle fibre (Allen et al., 2008). As the muscle contracts potassium [K^+] exits the muscle cell, while sodium [Na^+] and water flow into the cell as a result of depolarisation during contraction. Continued muscle contraction leads to an increase in extracellular [K^+] concentrations and a reduced resting membrane potential which is known to decrease muscle force production and contribute to muscular fatigue (Lindinger and Heigenhauser, 1991).

Several studies have investigated the role caffeine plays in muscle homeostasis during exercise and fatigue. Lindinger et al. (1993) had 8 trained cyclists (cycled on cycle ergometer) and 8 trained runners (ran on treadmill) exercise to exhaustion one hour after ingesting caffeine. Caffeine was shown to attenuate the increase in plasma [K^+] with exercise, as well as increase adrenaline concentrations (1.4 - 2 fold) compared to placebo. The authors showed that caffeine consumption led to increased regulation of intracellular and plasma [K^+] by stimulating Na^+/K^+ pump activity in skeletal muscle which leads to increased intracellular and decreased extracellular [K^+]. Physical training has shown to enhance K^+ regulation in the muscle cell (McKenna, 1995), thus it is likely that the ergogenic effects of caffeine may be due to the improved regulation of K^+ during exercise. However, it was not clear if the results were a direct effect of caffeine consumption or from an increase in adrenaline due to the caffeine supplementation.

The effects of caffeine on ionic balance in skeletal muscle were further investigated in a study by van Soeren et al. (1996). Six male paraplegic participants were tested at rest for three hours to investigate the effects of caffeine consumption (6 $mg \cdot kg^{-1}$) on participants with impaired adrenaline responses. The authors found that there was a decrease in plasma $[K^+]$ after caffeine intake compared to placebo ingestion, but no increase in circulating adrenaline concentrations. This suggests that caffeine had a direct effect on the tissues to increase reuptake of K^+ into the cell and supports the findings by Lindinger et al. (1993).

More recently Mohr et al. (2011) investigated the attenuation of interstitial [K+] as a result of caffeine supplementation. Six recreationally active male participants performed one low intensity (20 W) and three high intensity (50 W) one-legged knee-extensions 70 min after caffeine supplementation (6 $mg \cdot kg^{-1}$) or placebo. It was found that interstitial $[K^+]$ was consistently lower during the caffeine trial (5.5 ± 0.3, 5.7 ± 0.3, 5.8 ± 0.5, and 5.5 ± 0.3 $mmol \cdot L^{-1}$ at the end of the 20-W and three 50-W periods respectively) compared to the placebo (7.0 ± 0.6, 7.5 ± 0.7, 7.5 ± 0.4, and 7.0 ± 0.6 $mmol \cdot L^{-1}$ respectively). The authors suggested that the decrease in interstitial $[K^+]$ may have been a response to the direct effect of caffeine on the Na^+/K^+ pump, since it has been shown that high doses of caffeine restore force in potassium-inhibited muscle *in vitro* (Cairns et al., 1997).

Another potential factor which may lead to enhanced exercise capacity is the improved mobilisation of calcium (Ca^{++}) from the sarcoplasmic reticulum (Tarnopolsky, 1994). It is well known that Ca^{++} is a vital part of the excitation contraction coupling process which enables muscle contraction (Sandow, 1965). During this process membrane potential is depolarised by an action potential, from the motor neuron, which activates non-gated voltage sensors and in turn activates ryanodine receptors. As ryanodine receptors open, Ca^{++} is released from the sarcoplasmic reticulum into the local junctional space and diffuses into the sarcoplasm. The released Ca^{++} binds with troponin C on actin which enables cross bridge cycling to occur, producing force. Ca^{++} ATPase actively pumps Ca^{++} back into the sarcoplasmic reticulum and the Ca^{++} levels decline allowing muscle relaxation. Therefore, an increase in Ca^{++} mobilisation or the increased sensitivity of myofibrils to Ca^{++} could potentially cause an ergogenic effect.

Caffeine has been reported to influence intramuscular Ca^{++} in three ways: increased release of Ca^{++} from the sarcoplasmic reticulum (Lee, 1993), increased sensitivity of the myofibrils to Ca^{++} (Lee, 1993), and inhibition of phosphodiesterase (PDE) leading to an increase in cyclic adenosine monophosphate (cAMP) in the muscle (Nehlig and Debry, 1994). *In vitro* and *in situ* studies have shown that caffeine leads to increased Ca^{++} release from the sarcoplasmic reticulum and that caffeine lowers the threshold potential for excitation and extends the active period of muscle contraction (Bianchi, 1961). However, this has still yet to be definitively proven *in vivo*.

More recently, the ergogenic effects thought to be due to caffeine-induced Ca^{++} release from the sarcoplasmic reticulum and PDE inhibition have been ruled out due to the high, supra-physiological caffeine concentrations needed (>100μM), and this effect is now thought to primarily result from adenosine-receptor antagonism (Magkos and Kavouras, 2005).

Overall, it has been shown that caffeine does improve interstitial K^+ clearance, which can lead to increased force production in muscles. An increased intracellular and decreased extracellular $[K^+]$ maintains resting membrane potential and muscle cell contractility. While there is potential for Ca^{++} to have an ergogenic effect on exercise performance, it is unlikely to result from any caffeine-mediated effects and more likely to be due to adenosine-receptor antagonism.

2.2.5 Adenosine receptor antagonism

Adenosine metabolism is mostly controlled through the breakdown of the adenosine compounds adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) (Costa et al., 2001). As energy is expended during exercise, the levels of adenosine in the skeletal muscle (Latini and Pedata, 2001), and circulating in the brain will increase due to the breakdown of ATP, ADP and AMP during muscle contraction (Daly, 1982).

In humans there are four types of adenosine receptors; A_1, A_{2A}, A_{2B} and A_3. Receptors A_1 and A_{2A} play an important role in the brain and regulate neurotransmitters (Fuxe et al., 2007). Receptor type A_1 is found ubiquitously throughout the body; and when adenosine binds to it an inhibitory effect

is normally achieved in most tissues. Receptor A_{2A} also has a high affinity for adenosine, but differs from the A_1 receptor in that it has an excitatory effect on tissues. The A_{2B} and A_3 receptors both have a low affinity for adenosine (Dunwiddie and Masino, 2001).

Adenosine primarily causes lower motor activity, decreased wakefulness and vigilance as well as inhibiting most major neurotransmitters such as dopamine, serotonin, glutamate, acetylcholine and noradrenaline through binding to receptors found on many tissues throughout the body (Fredholm, 1995). Caffeine has a similar structure to adenosine and thus competes with adenosine binding at the site of the receptor. Due to the high affinity of the A_1 and A_{2A} receptors for adenosine, these receptors also have a high affinity for caffeine and the caffeine metabolite theophylline, which both act as antagonists. Caffeine has a lipophilic structure and can thus pass through the blood-brain barrier easily and bind with the adenosine receptors in the brain (Spriet, 1995). Caffeine has an excitatory effect on the tissues as it inhibits adenosine binding and leads to increased vigilance and motor activity while decreasing fatigue and tiredness (Fredholm, 1995). Caffeine also increases the concentration, synthesis and turnover of all the major neurotransmitters (serotonin, dopamine, glutamate, acetylcholine and noradrenaline) which, as mentioned earlier, are normally inhibited by adenosine (Fredholm, 1995).

Much of the ergogenic effects of caffeine have been attributed to adenosine receptor antagonism and more recent studies have provided strong evidence for the ergogenic effect of caffeine in due to adenosine receptor antagonism (Davis et al., 2003). A study (Davis et al., 2003) had rats run to exhaustion following an injection of caffeine, placebo or adenosine. It was found that after running to exhaustion muscle glycogen, blood FFA, glucose and corticosterone levels were similar across all treatments. Caffeine increased the run time by approximately 60% while adenosine decreased the run time by 68%. This indicates that caffeine can act on the central nervous system to delay fatigue in part by blocking adenosine receptors.

2.2.6 Pain perception

Pain has been shown to negatively affect motor unit recruitment and skeletal muscle force generation proportional to the intensity of the pain (Farina et al., 2004). Experimentally-induced pain has been shown to lead to decreased surface electromyography (EMG) in plantar and dorsiflexion synergists (Ciubotariu et al., 2007). In this study (Ciubotariu et al., 2007), 10 participants performed sustained contractions of plantarflexion and dorsiflexion at two different levels of maximal voluntary contraction (40% and 80% MVC). Pain was induced via injection of a 6% hypertonic saline solution into one synergist muscle, while the control had no induced pain. It was found that the EMG signal decreased across all active muscles and not only in the muscle in which pain had been induced. Time to fatigue was significantly decreased during induced pain compared to the control. This study showed that pain had an influence not only on the muscles directly affected by the localised pain but also the synergist muscles.

The detrimental effect of pain on muscle force generation is likely a centrally-mediated mechanism (Lund et al., 1991; Graven-Nielsen et al., 2002; Farina et al., 2004). Activation of the A_1 adenosine receptor increases pain suppression, while binding to A_{2A} receptors increases pain sensations (Sylven et al., 1986, 1988; Pappagallo et al., 1993; Sawynok, 1998). Adenosine concentrations in skeletal muscle increase during moderate to high intensity exercise, and A_1 and A_{2A} receptors are found on the sensory nerve endings in skeletal muscle, which may influence pain signalling (Sawynok, 1998). As caffeine is a potent adenosine receptor antagonist that supresses pain perception (Meyers et al., 1997), this suggests that caffeine will attenuate pain perception during intense exercise and thus muscle output should be maintained or improved.

A number of studies have investigated the effects of caffeine ingestion on pain perception during exercise, but the results are equivocal (Table 2.1). There is evidence that higher doses (> 5 mg·kg^{-1}) caffeine may attenuate pain perception during moderate to high intensity exercise in a dose-dependent fashion (O'Connor et al., 2004). It has been shown that both the central nervous system and peripheral actions play a role in the reduction of pain perception but it is still unclear as to the extent each part plays.

Table 2.1. Summary of literature on to the effects of caffeine ingestion on pain during exercise.

Study	Participants	Caffeine dosage ($mg \cdot kg^{-1}$)	Exercise protocol	Findings
Motl et al., 2003	16 low caffeine consuming college aged males	10	30 min cycling at $\dot{V}O_{2peak}$	11 of 16 participants reported lower pain intensity ratings following caffeine ingestion
O'Connor et al., 2004	12 college aged males	5 or 10	30 min cycling at $\dot{V}O_{2peak}$	Caffeine had dose dependent response to reducing leg muscle pain during moderate intensity exercise
Maridakis et al., 2007	9 college-aged females	5	Maximal voluntary isometric contraction (MVIC) and submaximal voluntary contraction of the quadriceps 24-48h after muscle injury	Caffeine attenuated perception of pain after eccentric-induced delayed onset muscle soreness
Hudson et al., 2008	15 college aged males	6	Leg extension and arm curls performed to volitional fatigue for 4 sets	Caffeine did not significantly affect pain perception or RPE
Jenkins et al., 2008	13 male cyclists	1, 2 or 3	Cycling for 15 min at 80% at $\dot{V}O_{2max}$ followed by 15 min performance ride	Caffeine had no significant effect on any pain related measures or RPE
Astorino et al., 2011	15 active males	5 or 2	2 bouts of 40 knee extension and flexion at $180° \cdot s^{-1}$	No effect of caffeine on pain perception and RPE
Duncan and Oxford, 2012	18 moderately trained males	5	Bench press to failure at 60% of 1 rep max	Caffeine reduced upper body muscle pain perception during resistance exercise to failure
Astorino et al., 2012	8 endurance trained and 8 active males	5	10 km cycle time trial	Caffeine had no effect on pain perception or RPE

2.2.7 Ratings of perceived exertion (RPE)

Logically, attenuation of pain during exercise as a result of caffeine supplementation would result in a decrease in the rating of perceived exertion (RPE) during exercise. Reductions in pain have been shown to correlate with increases in work done during exercise time trials, due mostly to a reduction in RPE (Gonglach et al., 2015). A meta-analysis (Doherty and Smith, 2005) identified 21 studies using mostly healthy male subjects (74%) between the ages of 20 and 35 years, and examined the effects of caffeine consumption on constant-rate exercise RPE which met the inclusion criteria. The main finding was that compared to placebo, there was a 5.6% reduction in RPE during constant rate exercise following the ingestion of caffeine. An average improvement in performance of approximately 11% was reported across all exercise modalities. However, there was no difference in RPE between placebo and caffeine trials at the end of exhaustive exercise, which suggests that the effort sense and fatigue at the end of "all out" exercise is the same. This reduction in RPE may, at least in part, explain some of the performance increases found after caffeine supplementation.

Several studies investigated the effects of caffeine during high intensity intermittent activity and reported no reductions of RPE during exercise (Schneiker et al., 2006; Glaister et al., 2008). However, these studies used multiple sprints as a measure of performance and there is debate as to whether caffeine consumption reduces RPE during high intensity intermittent exercise. One study investigated the effects of caffeine on perceptual responses during high-intensity cycling (Doherty et al., 2004). This study had 11 male cyclists perform 2 min of cycling at 100% power output followed immediately by an "all-out" effort. One hour before testing, participants ingested either caffeine (5 $mg \cdot kg^{-1}$) in an artificially sweetened drink, or the placebo (artificial sweetener). It was reported that RPE (recorded every 30 s) was lowered by approximately 1 point during the constant rate phase of the exercise but was not different during the "all-out" effort between caffeine and placebo trials. Mean power output and lactate was increased in the caffeine trial compared to placebo.

Caffeine is likely to reduce RPE during constant-rate aerobic exercise. However, during high intensity intermittent or resistance exercise there appears to be a lesser effect of caffeine consumption

on RPE. The mechanism by which RPE is reduced after caffeine ingestion is still poorly understood and warrants further investigation.

2.2.8 β-endorphins

Beta-endorphins have been shown to increase during exercise in a curvilinear fashion (Dinas et al., 2011), particularly during high intensity or long duration exercise (Goldfarb et al., 1990). Beta-endorphins are endogenous opioid neuropeptides that bind to μ-opioid receptors to produce a euphoric or pain-relief effect. A meta-analysis (Dinas et al., 2011) showed that moderate intensity exercise produces the greatest increase in β-endorphin concentration compared to low and high intensity exercise. Additionally, the improvement in mood as a result of exercise and increased β-endorphin concentration was greater in recreational and marathon runners compared to sedentary individuals (Dinas et al., 2011).

Laurent et al. (2000) reported an almost doubling of β-endorphin levels following caffeine ingestion (caffeine 53 $pg \cdot ml^{-1}$ vs placebo 30 $pg \cdot ml^{-1}$) during a two hour cycle at 65% $\dot{V}O_{2max}$ followed by high intensity sprints. Participants ingested caffeine (6 $mg \cdot kg^{-1}$) or a non-nutritive placebo 90 min before exercise. The authors suggested that caffeine lowers the threshold for exercise–induced β-endorphin and cortisol release, which may lead to improved mood, decreased pain perception and an ergogenic effect. More recently, Ivy et al. (2009) reported a trend for an increase in β-endorphin levels ($P = 0.10$) in 12 trained cyclists (6 male, 6 female) after ingesting an energy drink and performing a cycle time trial simulating 1 h of work at 70% $\dot{V}O_{2max}$. However, the caffeine amounted to 160 mg, which is considered a low dose (2.28 $mg \cdot kg^{-1}$ for 70 kg individual) and may not have been sufficient to show significant effects on the β-endorphin threshold as was shown in the previous study (Laurent et al., 2000) which used a larger caffeine dose. The effect of caffeine on β-endorphin may contribute to the reported benefits of caffeine on endurance performance but warrants further study.

2.2.8 Caffeine metabolites

Caffeine is metabolised in the liver by the cytochrome P450 oxidase system, and the isozyme CYP1A2 is responsible for the majority of caffeine metabolism, which breaks it down into three dimethylxanthines: paraxanthine (81.5%), theobromine (10%) and theophylline (5.4%; Gu et al., 1992). Paraxanthine has previously been shown to be at least partly responsible for the lipolytic effects of caffeine which results in increased plasma FFA concentrations (Hetzler et al., 1990), as well as acting as an enzymatic effector for the Na^+/K^+ pump which increases $[K^+]$ uptake into the skeletal muscle tissue (Hawke et al., 1999) and increasing $[Ca^{++}]$ concentrations in muscle (Hawke et al., 2000). The increase in plasma FFA concentration does not mean that there is an increase in lipid metabolism and does not lead to improved exercise performance. However, paraxanthine, like caffeine, is a non-selective adenosine receptor antagonist (Daly et al., 1986) and affects a number of tissues by binding to the adenosine receptors and is thus most likely the reason for improved performance during paraxanthine and caffeine supplementation. Theophylline, like paraxanthine, is also a potent adenosine receptor antagonist, and thus has similar effects to caffeine and paraxanthine (Raguso et al., 1996).

Several studies have shown that theophylline can be ergogenic. Hawke et al. (2000) reported that theophylline, along with theobromine and paraxanthine, lead to increased intracellular $[Ca^{++}]$ in skeletal muscle while Greer et al. (2000) found that theophylline (4.5 $mg \cdot kg^{-1}$) was ergogenic, but not to the same extent as caffeine. In this study, designed to measure the ergogenic effects of theophylline (Greer et al., 2000), participants were given either caffeine (6 $mg \cdot kg^{-1}$), theophylline (4.5 $mg \cdot kg^{-1}$) or a placebo in two separate experiments. Trial A had 8 men cycling at 80% $\dot{V}O_{2max}$ 90 min after ingesting caffeine, placebo (dextrose) or theophylline. Plasma concentrations of theophylline were similar between caffeine and theophylline treatments, however, while performance increased during both the caffeine and theophylline trials, performance following caffeine consumption was significantly better compared to theophylline ingestion alone (Greer et al., 2000). Trial B used the same supplementation protocol but had participants cycle for 45 min at 70% $\dot{V}O_{2max}$ to measure the effects of theophylline on muscle metabolism. It was reported that neither caffeine nor theophylline

affected muscle glycogen utilisation, but both led to increased blood glycerol levels and caffeine increased plasma adrenaline levels (Greer et al., 2000). The authors suggest that theophylline is likely to be ergogenic but is not responsible for all of the performance-enhancing effects of caffeine. However, this level of theophylline is unlikely to occur naturally or through caffeine supplementation, as a caffeine dose of 6 $mg \cdot kg^{-1}$ will only result in 0.324 $mg \cdot kg^{-1}$ of theophylline, far below the 4.5 $mg \cdot kg^{-1}$ used in the study described above.

Theobromine is also an adenosine receptor antagonist but is significantly weaker than caffeine, paraxanthine or theophylline (Hardman and Limbird, 2001) and thought to have approximately one fifth of the stimulant effect of caffeine but with a significantly longer half-life (~7 hours). This suggests that theobromine has a lesser effect on the central nervous system and has a greater effect on tissues such as the lungs and heart (Howell et al., 1997) and causes vasodilation (Caleb, 2005). There has been very little research on the effects of theobromine on exercise performance.

Very little research exists on the effects of paraxanthine, theophylline or theobromine on exercise performance individually or in addition to caffeine. Paraxanthine and theophylline both have a similar, but weaker, ergogenic effect to caffeine whereas theobromine has a greater effect on peripheral tissues and blood vessels but has not been shown to be ergogenic. It has been suggested that quantities of theobromine and theophylline in commercially available products are too low to have any form of significant ergogenic effect (Pearce et al., 2012). Future studies could investigate the ergogenic effects of caffeine metabolites individually and in combination with caffeine.

2.3 Effects of caffeine on mood, perceptual responses and cognition

2.3.1 Mood

There is strong consensus that exercise has a positive effect on mood, and exercise has been rated as among the top behavioural techniques used to self-regulate mood (Thayer et al., 1994). It is no surprise that the profile of mood states (POMS) has been used extensively in exercise settings over

the past three decades (Berger and Motl, 2000). The POMS consists of seven subscales (fatigue, anger, esteem, depression, vigour, tension and confusion), and while the original version (McNair, 1971) consisted of 60 items, abbreviated 30 (McNair, 1971; McNair et al., 1981, 1992) and 40-item versions have since been developed and proven to be reliable and valid measures of mood before, during and after exercise (Grove and Prapavessis, 1992).

While earlier studies (Lieberman et al., 1987) observed mixed results as to whether caffeine ingestion alone improved mood, more recent studies tend to report a positive effect. In a double-blind placebo-controlled study the effects of low-to-moderate doses of caffeine ingestion (64-256 mg) on mood in young, elderly, male and female participants was examined (Amendola et al., 1998). It was found that the fatigue and vigour subscales of the POMS decreased and increased, respectively, following caffeine ingestion. The mood scales were significantly increased following caffeine ingestion and all sub groups tested (male, female, young and elderly) responded similarly. Caffeine also seems to have a greater impact on a wider variety of behavioural parameters when individuals are fatigued (Lieberman, 2001).

Lieberman et al. (2002) found that caffeine ingestion improved self-reported feelings of fatigue and sleepiness in U.S. Navy SEAL trainees. Participants were randomly assigned to orally ingest a 100, 200 or 300 mg caffeine or placebo capsule after 72 hours of sleep deprivation and continuous exposure to other stressors such as withstanding the adverse effects of cold water, sustained high levels of physical activity and maintaining high levels of physical and mental functioning. Mood state was assessed using a 65-item POMS questionnaire one and eight hours post caffeine ingestion. While caffeine was expected to affect four subscales (tension, depression, vigour and fatigue) only fatigue was significantly reduced after caffeine ingestion, there were no significant changes in all other subscales. It was noted that 200 mg provided a significant increase in mental and physical performance measures when compared with the 100 mg dose, however, there was no difference between 200 mg and 300 mg doses. Lieberman et al. (2002) concluded that moderate doses of caffeine can improve mood state, and also found improvements in cognitive function, vigilance, learning and memory.

Similarly, Penetar et al. (1993) reported that ingestion of caffeine had significant alerting and long-lasting beneficial mood effects in individuals who were sleep deprived for 48 hours. In this trial, (Penetar et al., 1993) caffeine (0, 150, 300, and 600 per 70 kg) was administered in a double-blind manner to 50 healthy adult males and was shown to reverse the effects of sleep deprivation on three POMS subscales (vigour, fatigue and confusion). Vigour was found to have a positive relationship with caffeine dose while fatigue had a negative relationship with caffeine dose. Anxiety scores were higher after the two highest doses of caffeine.

Penetar et al. (1993) also reported that low users of caffeine were more likely to experience anxiety when compared to moderate users after consuming moderate-to-high doses of caffeine. An inverted-U relationship has been noted with higher doses of caffeine ingestion eliciting feelings of anxiety and jitteriness (Griffiths and Mumford, 1995; Rogers et al., 2007). Alsene et al. (2003) reported that a caffeine dose of 150 mg significantly increased anxiety in healthy low-caffeine consumers. This study also found that polymorphisms of the A_1 and A_{2A} adenosine receptor gene (ADORA2A, 1976 C>T) may account for inter-individual variations in anxiety after caffeine administration. This suggests that low and moderate doses of caffeine may negatively impact mood in habitually low consumers of caffeine, and not only high doses as was previously thought (Roache and Griffiths, 1987; Loke, 1988).

Thus, it appears caffeine supplementation will likely cause a change in mood in participants. However, a net positive or negative change will depend on each individual's response to caffeine intake relative to their normal consumption patterns.

2.3.2 Perceptual responses

Several methods have been employed to measure perceptual responses during exercise. The exact mechanisms responsible for the ergogenic effects of caffeine remain elusive, reduced RPE and changes in affect and arousal have been widely acknowledged (Davis et al., 2003). The notion that caffeine alters RPE is supported by the findings of a meta-analysis (Doherty and Smith, 2005).

However, caffeine's influence on the dimensions of affect (experience of feeling or emotion) has not been thoroughly investigated, despite previous research which reported that a favourable affect profile is beneficial to exercise performance (Acevedo et al., 1996). To the author's knowledge, only two studies have investigated the alteration to affect with caffeine intake during exercise (Backhouse et al., 2011; Astorino et al., 2012)

The feeling scale (FS; Hardy and Rejeski, 1989) has been used as a measure of affect (pleasure or displeasure) using an 11 point single-item bipolar rating scale. Moreover, the felt arousal scale (FAS; Svebak and Murgatroyd, 1985) has been used as a 6-point single item measure of perceived arousal/activation. Both of these measures are non-intrusive, simple measures of the participant's subjective perceptive responses to exercise, but are based on single responses, which are vulnerable to participant errors compared to multi-response measures (Backhouse et al., 2007). Repeated measures of the FS and FAS scales allow the use of a 'circumplex model' (Russell, 1980) for further analysis. This model suggests that emotions are distributed in a two-dimensional circular space containing arousal and valence dimensions covering high and low arousal, along with pleasure and displeasure. The circumplex model enables the participant's perceptual responses to be categorized into four quadrants (Fig. 2.1). The two primary dimensions of the circumplex model are affective valence (pleasure and displeasure) and activation. This can be broken up further into four categories; (a) 'high activation and pleasure', which reflects excitement and enthusiasm, (b) 'low activation and pleasure', which reflects relaxation and calmness, (c) 'low activation and displeasure', which reflects boredom and fatigue (d) 'high activation and displeasure, which reflects anxiety, tension and distress.

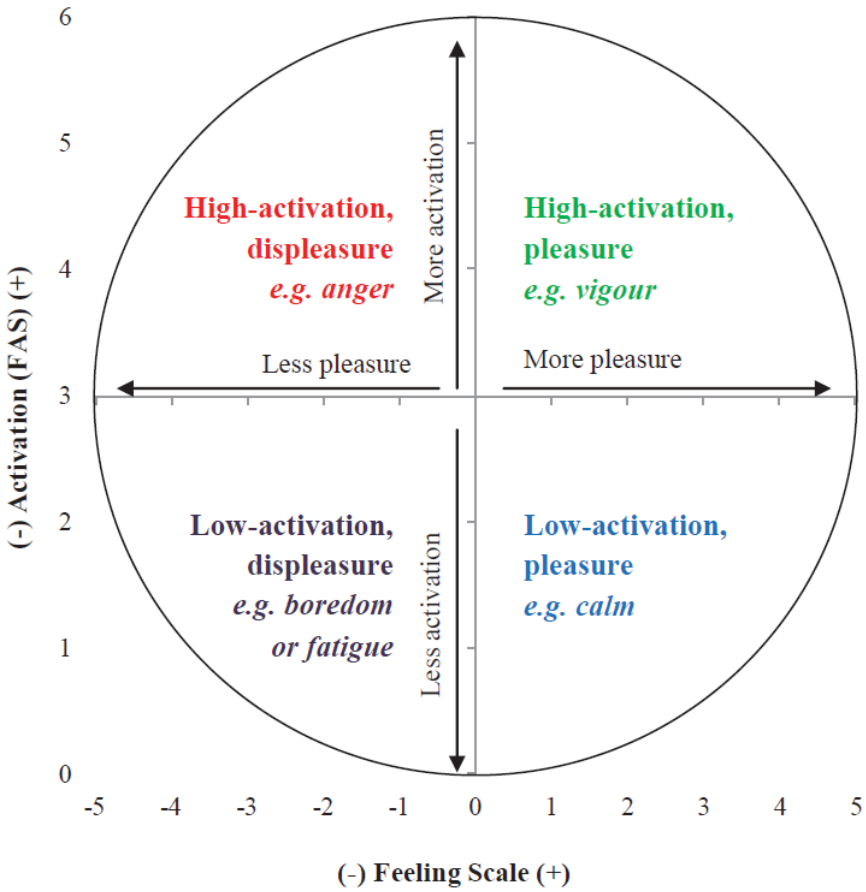

Figure 2.1 – A visual depiction of the affect circumplex model used for plotting the perceptual response to exercise (O'Donnell, 2012).

Backhouse et al. (2011) required 12 endurance trained cyclists to perform 90 min of cycling at 70% $\dot{V}O_{2max}$ one hour after ingesting caffeine (6 mg·kg^{-1}) or placebo with participants providing FS and FAS ratings after every 15 min of exercise. Whereas FS ratings were higher in the caffeine trial (indicating increased ratings of pleasure), perceived arousal did not change between trials but remained elevated during exercise. It was concluded that ingestion of caffeine helped the participants to maintain a more positive subjective experience during prolonged cycling which may contribute to the ergogenic effect of caffeine (Backhouse et al., 2011).

Similarly, Astorino et al. (2012) reported that feelings of pleasure were significantly improved after ingesting caffeine (relative to placebo) in active but not endurance-trained men during a 10 km cycle time trial one hour after ingesting caffeine. As with the previous study (Backhouse et al., 2011) FAS increased as exercise duration continued but there was no difference between

treatments. There is evidence to suggest that ingestion of caffeine prior to exercise may create a more pleasurable 'feel-good' experience when exercising, which may contribute to the ergogenic effects of caffeine. However, more research is needed to better understand the interaction of affect, effort sense and exercise performance.

2.3.3 Cognition

The effects of caffeine consumption on cognition, particularly in the areas of attention, reaction time and task-related arousal have been well documented (Scholey and Kennedy, 2004). The digit vigilance test (DV; Lewis and Rennick, 1979) has previously been used to measure both speed and accuracy of attention during caffeine supplementation (75-150 mg) in young adults (Haskell et al., 2005). While no significant improvement in rapid visual information processing (RVIP) reaction time or accuracy after caffeine supplementation was recorded, there was improvement in DV reaction time. Caffeine habituation also had no effect on reaction times. These results support the findings that caffeine improves reaction time and vigilance on some cognitive tests (Lieberman et al., 1987; Kruk et al., 2001).

Smit and Rogers (2000) observed an improvement in both the reaction time test and RVIP test after caffeine ingestion compared to placebo. This study had 23 participants (11 males, 12 females) complete a cognitive test battery before and 30 min after treatment. Treatments consisted of four different doses of caffeine (12.5 mg, 25 mg, 50 mg and 100 mg) or placebo, with tests performed one week apart and each trial lasting 1.5 h. Smit and Rogers (2000) reported a 'flat' dose-response relationship as the low doses of caffeine improved performance on the cognitive tests to a similar extent as higher doses, a result supported by other studies (Maridakis et al., 2009). Smit and Rogers (2000) suggested that this may also be due to ceiling effects (i.e. increases in dosage would lead to progressively smaller incremental effects) and lack of sensitivity of the tests.

Hogervorst et al. (2008) reported participants were significantly faster at performing both the computerized Stroop test and RVIP after cycling to exhaustion during the caffeine supplementation

trial compared to glucose and placebo trials. In this study twenty-four participants consumed either a caffeinated energy bar (100 mg caffeine, 45 g CHO), or a non-caffeine isocaloric energy bar or placebo (isocaloric artificially sweetened water, 300 ml). Participants performed a 2.5 h cycle at 60% $\dot{V}O_{2max}$ followed by an exhaustion time trial at 75% $\dot{V}O_{2max}$. Cognitive tests were performed before exercise, at 70 and 140 min of exercise and 5 min after completion of the time trial. Time to exhaustion was significantly longer following caffeine ingestion compared to both CHO and placebo trials (Hogervorst et al., 2008). This shows the potent effect caffeine can have on cognitive tasks as participants exercised for longer and performed better on the cognitive tests following caffeine supplementation compared to placebo. This study (Hogervorst et al., 2008) lends evidence to support the idea that caffeine improves cognitive performance and reaction time.

Research indicates that small-to-moderate doses of caffeine will likely lead to improvements in cognition, particularly in areas of attention and reaction time. Moderate intensity exercise is also known to improve cognitive functions such as reaction time and vigilance tasks. In addition, cognitive performance appears to be improved with caffeine consumption following exhaustive or strenuous exercise. The combination of exercise and caffeine supplementation has not yet been thoroughly researched and future studies could investigate dose responses to caffeine and exercise combination.

2.4 Effects of caffeine ingestion on strength and power

It has been suggested that caffeine may improve myoneural function and contractility (Eke-Okoro, 1982; Jacobson et al., 1992) and thus lead to improvements in strength and power during exercise. However, many studies (Beck et al., 2006; Williams et al., 2008; Foskett et al., 2009; Astorino et al., 2010) have reported mixed results and the evidence remains equivocal.

Summaries of the pertinent literature which highlight the mixed results of the effects of caffeine on power and strength are shown in Tables 2.2 and 2.3 respectively. Caffeine doses ranged from 2– 10 $mg \cdot kg^{-1}$ with the most commonly ingested amount of 6 $mg \cdot kg^{-1}$ used to assess the effects of caffeine on a range of high intensity exercise such as sprinting, cycling and resistance training.

While a number of studies (Collomp et al., 1991; Paton et al., 2001; Jacobs et al., 2003; Greer et al., 2006; Astorino et al., 2008; Beck et al., 2008; Trevino et al., 2015) have found no effect of caffeine on high intensity exercise performance, many of the studies that found little difference used untrained to moderately trained individuals and training status has been suggested to be a factor which could influence the effects of caffeine on exercise performance (Astorino and Roberson, 2010). However, more research is needed to determine the strength of the relationship between training status and the ergogenic effects of caffeine.

Another possible explanation of the varied results (Tables 2.2 and 2.3) may be due to the participant's individual responses to caffeine during short-term high-intensity exercise. The CYP1A2 genotype has been linked to an individual's speed of caffeine metabolism (Yang et al., 2010) and may affect the ergogenic response to caffeine. Similarly the ADORA2A gene, which is related to caffeine metabolism and responses to caffeine, has been linked to caffeine habituation and thus may affect responses to caffeine (Yang et al., 2010). Genotype may be partially responsible for the mixed results observed between some of these studies (Tables 2.2 and 2.3). Future studies should examine the effects of various genes related to caffeine metabolism and the influence they have on the ergogenic effect of caffeine.

A review of the effects of caffeine consumption on short-term high-intensity exercise (Astorino and Roberson, 2010) reported that 54% of the studies revealed 9.4 ± 5.7% caffeine-mediated improvements in muscular strength and muscular endurance. It was also found that caffeine doses of 2.5 - 7 $mg \cdot kg^{-1}$ were the most likely to improve performance during short-term high-intensity exercise. Further research is still needed to determine the exact mechanism which explains the ergogenic effect of caffeine on short-term high-intensity exercise.

Table 2.2: Summary of literature on the effects of caffeine ingestion on power exercise.

Study	Participants	Caffeine dosage	Exercise protocols	Findings
Collomp et al., 1991	6 healthy males	5 mg·kg^{-1}	Wingate test	No effect on performance
Paton et al., 2001	16 male team sport players	6 mg·kg^{-1}	Ten 20-m sprints	No effect on performance
Stuart et al., 2005	9 male rugby players	6 mg·kg^{-1}	Tests of sprint speed, drive power and passing accuracy	Increase in performance in all tests
Greer et al., 2006	18 healthy males	5 mg·kg^{-1}	Wingate tests	No effect on performance
Schneiker et al., 2006	10 male team sport players	6 mg·kg^{-1}	Eighteen 4-s cycle ergometer sprints	Increase in total work and mean power
Forbes et al., 2007	15 young males and females	2 mg·kg^{-1}	3 sets of bench presses @70% 1RM + 3 Wingate tests	Increase in bench press reps No effect on power output
Foskett et al., 2009	12 male football players	6 mg·kg^{-1}	Counter movement jump height, 15m sprints	Increase in jump height, no effect on 15m sprints
Glaister et al., 2015	14 well trained males	5 mg·kg^{-1}	Series of 6 s cycle sprints followed by 5 min passive rest	Increase in peak anaerobic power output

Table 2.3: Summary of literature on the effects of caffeine ingestion on strength exercise.

Study	Participants	Dosage	Exercise protocol	Findings
Jacobson et al., 1992	20 male football players	7 mg·kg^{-1}	3-15 reps of knee extensions and flexion	Increase in peak torque
Jacobs et al., 2003	13 strength trained men	4 mg·kg^{-1}	Superset leg and bench press	No effect on performance
Beck et al., 2006	37 strength trained men	2.5 mg·kg^{-1}	Wingate, strength and endurance test	Increase in 1RM bench press, no effects on other parameters
Beck et al., 2008	31 untrained men	2.5 mg·kg^{-1}	1RM bench press + running @85% $V\dot{O}_{max}$	No effect on performance
Woolfe et al., 2008	18 males	5 mg·kg^{-1}	Leg/bench press to fatigue + Wingate test	Increase in weight lifted and power output
Hudson et al., 2008	15 strength trained males	6 mg·kg^{-1}	4 sets of arm curl and leg extension to fatigue	Increase in number of leg extensions, no effect on arm performance
Astorino et al, 2008	22 strength trained males	6 mg·kg^{-1}	1 RM bench/leg press and reps to fatigue	No effect on performance
Timmins et al., 2014	16 resistance trained males	6 mg·kg^{-1}	Maximal voluntary contraction (MVC) of lower and upper body muscle groups	Increased MVC in upper and lower body muscle groups
Trevino et al., 2015	13 active males	5 or 10 mg·kg^{-1}	Maximal voluntary contraction of elbow flexors	No effect on performance

2.5 Effects of caffeine ingestion on endurance performance

The effects of caffeine on endurance performance have been well researched and there is little doubt that ingestion of caffeine is likely to have an ergogenic effect. Only rarely has it been found that caffeine supplementation did not lead to an ergogenic effect (Butts and Cromwell, 1985; Falk et al., 1990). Doherty and Smith (2005) undertook a meta-analysis of 40 double-blind studies (published between 1985 and 2004) to examine the effects of caffeine on exercise performance. Ten of these

studies used long-distance running to determine the effects of caffeine on endurance performance and found an improvement of 19.0 ± 13.6%, whereas 28 cycling studies showed an improvement of 22.3 ± 13.3%. Only one of these studies used a time trial (10 km) as a mode of exercise and found a 1.7% change in performance after caffeine supplementation (Bell and McLellan, 2002). Future studies may wish to investigate the effects of caffeine supplementation using a time trial protocol instead of time to exhaustion method, as it has been shown to have less variability (Laursen et al., 2007) and more closely resembles real world competition.

A more recent systematic review (Ganio et al., 2009) evaluated studies that examined the effect of caffeine intake on endurance exercise. Studies which used a graded form of exercise lasting longer than 5 minutes were also included as well as studies which had any component of a time trial, with the total exercise times ranging from 5 min to 250 min. Of the 21 studies that met the inclusion criteria, there were a total of 33 identifiable caffeine treatments (i.e. if the effect of caffeine administered in any form could be isolated; caffeine dosage ranged from 1 - 9.3 $mg \cdot kg^{-1}$). The average performance improvement with caffeine, independent of timing of ingestion, was 3.2 ± 4.3% in caffeine trials compared to placebo (Ganio et al., 2009). It was found that mean improvement for time trial performance was 1.1 ± 0.5% across all modes of exercise. A mean improvement of 0.9 ± 0.7% was found for studies which used running (n=6) as a mode of exercise compared to 4.4 ± 5.0% for cycling studies (n = 21). However, of the 33 treatments that reported improvements in performance, only 15 were found to be statistically significant. It was noted that the degree of improvement following caffeine ingestion does not seem to be consistent with the mode of caffeine delivery, exercise mode, timing of caffeine or total exercise time which suggests that additional factors may affect responses to caffeine supplementation during endurance exercise (Ganio et al., 2009).

The majority of evidence suggests that caffeine consumption, even at low doses (~2 $mg \cdot kg^{-1}$), will likely improve endurance performance, with a mean improvement in endurance performance of 3.2 ± 4.3 % (Ganio et al., 2009). However, there is a significant amount of variation in response to caffeine supplementation, which may be dependent on a number of factors such as pregnancy and use of oral contraceptives (Abernathy and Todd, 1985), smoking (Magkos and Kavouras, 2005), age

(Chung et al., 2000) and genetics (Yang et al., 2010). Further research should seek to quantify the degree of improvement on endurance performance following caffeine ingestion and isolate the factors that mediate the large range of responses commonly observed after caffeine ingestion.

2.6 Effects of caffeine ingestion on sleep quality

The negative effects of caffeine consumption on sleep have been well documented (Roehrs and Roth, 2008). In an early study, (Karacan et al., 1976) 18 young adult males participated in a 13-night laboratory-based sleep study where they consumed one of four different doses of caffeine in beverage form (0, 1.1, 2.3, 4.6 $mg \cdot kg^{-1}$) 30 min before bedtime. Caffeine increased latency to sleep, reduced total sleep time and reduced stage 3-4 sleep in a dose-related manner. Similarly, caffeine ingestion (150 mg) was found to increase sleep latency and reduce total sleep time in young adult males (Okuma et al., 1982).

Hindmarch et al. (2000) used a protocol which mimicked the dose and time that caffeine is most often consumed to assess cognitive and psychomotor performance and sleep quality at night. Thirty healthy individuals were administered either tea (containing 37.5 mg or 75 mg caffeine), coffee (75 mg or 150 mg caffeine) or water in a randomised five-way crossover design. The drinks were consumed at four times during the day (9am, 1pm, 5pm and 11pm). The Leeds sleep evaluation questionnaire (LSEQ; Parrott and Hindmarch, 1980) was completed every morning and a sleep actigraph was worn on the wrist for the duration of the study. Caffeine was reported to have a dose-dependent negative effect on sleep quality, sleep time and sleep onset with the largest changes in these variables seen with the highest (150 mg) caffeine dose. However, it was noted that this study (Parrott and Hindmarch, 1980) was not blinded and so responses to caffeine may have been subjective and thus may not reflect the actual effects of caffeine, but instead what the participants expected to experience as a result of caffeine consumption.

Youngstedt et al. (2000) examined whether exercise could attenuate nocturnal sleeping disturbances brought about by ingestion of high doses of caffeine. Eight moderately fit young males

(24.5 ± 4.3 y) consumed caffeine (1200 mg) and performed four different exercise protocols on separate days: 60 min of cycling at 60% $\dot{V}O_{2max}$ after placebo (lactose) or quiet rest after placebo; and cycling at 60% $\dot{V}O_{2max}$ after caffeine or quiet rest after caffeine. Caffeine doses (400 mg) or placebo were taken three times during the day: upon waking, at 4pm and 2 h before bedtime. All exercise protocols were completed between 4:15-5:15pm. Caffeine related sleep disturbances were less than previously reported (Bonnet and Arand, 1992), and exercise had little to no effect on sleep whilst on caffeine compared to placebo.

Therefore, while caffeine has been shown to have stimulant properties that enhance vigilance and arousal, the use of caffeine may lead to sleep disturbances and are likely to cause a decrease in performance as a result due to fatigue and sleepiness.

2.7 Effects of genetics on caffeine pharmacokinetics

Caffeine is metabolised in the liver by the cytochrome P450 oxidase enzyme system which is coded for by the CYP1A2 gene (Lelo et al., 1986; Miners and Birkett, 1996). The variation of CYP1A2 activity accounts for a major source of the variability of caffeine metabolism between individuals (Yang et al., 2010). Previous studies have found that caffeine clearance can vary up to 40-fold within and between individuals (Kalow and Tang, 1991; Kashuba et al., 1998). However, caffeine clearance rates may be affected by exogenous factors such as smoking, recreational drugs and medication (Grosso and Bracken, 2005) as well as endogenous factors which include pregnancy, ethnicity (Gunes and Dahl, 2008) and genetics (Yang et al., 2010). Studies have found that monozygotic twins share a closer kinetic profile than dizygotic twins for caffeine metabolism, with a heritability of 0.725 (Rasmussen et al., 2002). This suggests that genetic variation can account for a significant part of the difference in caffeine metabolism between individuals.

A single nucleotide (C/A) at position 734 within intron 1 (rs762551) influences the inducibility of the CYP1A2 gene. Sachse et al. (1999) investigated how much of the variability of CYP1A2 activity is explained by the polymorphisms at intron 1 (A/A, A/C and C/C). Caffeine (100

mg) was administered orally and monitored for 5 h in urine and plasma to determine caffeine metabolism rates between individuals with different genotypes. Among the healthy non-smokers, there was no significant difference in the ratio of paraxanthine to caffeine between CYP1A2 genotypes after ingesting caffeine, thus it could be concluded that caffeine was metabolised at a similar rate between all genotypes in non-smokers. In contrast, smokers with the A/A genotype metabolised caffeine 1.6 times faster compared to other genotypes.

Genetic testing of 185 German healthy non-smokers and 51 smokers showed that 46% of the total sample population was homozygous for variant A (A/A), 44% were heterozygous (A/C), and 10% were homozygous for the C variant (C/C) (Sachse et al., 1999). Renda et al. (2012) found that out of 110 Italian participants 37% were homozygous for variant A (A/A), 64% were heterozygous (A/C) and 2% were homozygous for variant C (C/C), indicating that more research needs to be done in different ethnic groups to determine genetic variation of the CYP1A2 gene in diverse populations.

Recent genetic studies in animals and humans have implicated polymorphisms in adenosine A_1 (ADORA1A) and A_{2A} (ADORA2A) receptors in caffeine response (Yang et al., 2010). Mice lacking A_{2A} receptors did not show increased wakefulness after caffeine ingestion compared to mice that did possess the A_{2A} receptors (Huang et al., 2005). This suggests that the A_{2A} receptors mediate caffeine response to some extent. In addition, human homozygous ADORA2A C/C carriers rate themselves as caffeine sensitive more frequently, compared with those with the homozygous ADORA2A T/T variation who rated themselves more often as caffeine insensitive (Retey et al., 2007), suggesting that ADORA2A may be related to caffeine habituation or caffeine response (Cornelius et al., 2006).

Adenosine A_{2A} receptors have also been associated with the rewarding properties of caffeine. El Yacoubi et al. (2005) reported that knockout A_{2A} mice self-administer less caffeine compared to wild-type mice. This could have implications for performance as a rewarding or positive feeling during exercise could be conducive to enhanced performance by lowering perceived exertion and improving mood allowing the individual to exercise at a higher intensity.

2.8 Effects of genetics on the ergogenic effect of caffeine during endurance exercise

To the author's knowledge only one study has investigated if the polymorphisms of the CYP1A2 gene influence endurance performance after ingesting caffeine (Womack et al., 2012). In this study 35 male cyclists completed two cycle time trials one hour following caffeine (6 $mg \cdot kg^{-1}$) or placebo ingestion in a double-blind fashion. The time trial consisted of 8 laps of a 5-km loop. Participants were able to self-select the resistance by changing gears and were able to see their distance completed, but were not aware of their speed, power output or exercise time. Participants were classified as either AA homozygous (n = 16) or C allele carriers (n = 19). Oxygen uptake and RER were obtained and averaged over the last 2 min of each lap. Heart rate and RPE were measured at the end of each lap. Caffeine ingestion affected the 40-km time trial performance of individuals homozygous for the A variant to a greater degree than those with the C allele. Caffeine ingestion decreased the average time to complete the 40-km time trial by 3.8 min in the A/A group compared to 1.3 min in the C allele carriers compared to placebo. Womack et al. (2012) proposed that the difference in performance between the homozygous A and C allele carriers was due to a more rapid production of paraxanthine and/or theophylline which enhanced the ergogenic effect.

Another study (Klein et al., 2012) grouped collegiate tennis players into two groups, AA homozygous (n=7) and C allele carriers (n=9). Participants were given caffeine (6 $g \cdot kg^{-1}$) or placebo 1 h before performing a 45-min treadmill run intended to mimic the intensities of a tennis game, followed by a tennis skill test which assessed stroke accuracy. It was found that caffeine improved stroke accuracy by 2.1% but was not influenced by genotype. There was an increase in HR during exercise in the AA homozygous group but not in the C allele carriers. Klein et al. (2012) suggested that the results indicated a preliminary finding of greater physiological responses to caffeine in the AA homozygotes.

To the author's knowledge no study has investigated the possible effects of ADORA1A/ADORA2A gene variants on exercise performance. Further research is needed to determine caffeine metabolism during exercise across both the CYP1A2 and ADORA genotypes to further elucidate the mechanisms of the ergogenic effect of caffeine.

2.9 Summary

The use of caffeine as an ergogenic aid has been well researched across multiple exercise modalities. It appears that caffeine has the greatest ergogenic effect on endurance performance, as studies investigating the effects of caffeine on strength and power activities show mixed results. The exact mechanisms of action of caffeine are not fully understood, due to its ability to affect multiple organs and tissues around the body, but its role as an adenosine receptor antagonist is likely the major cause of its effects. In addition to exercise performance, caffeine has been shown to affect sleep, cognition, perception and mood, all of which may impact on an athlete's performance. Despite the overall positive effect that caffeine consumption has on exercise performance, responses vary widely between individuals, with some not responding at all. Therefore, it is likely that genetics plays a major role in an individual's responses to caffeine supplementation during exercise. However, this area of research is still new and has not been fully explored. Future research should aim to clarify the mechanisms of action and determine the extent to which genetics plays a role in the ergogenic effects of caffeine.

CHAPTER 3 - METHODOLOGY

3.1 Participants

Seventeen male, non-smoking, recreationally trained athletes (age 26.9 ± 7.9 yrs.; weight 77.0 ± 9.0 kg) volunteered to participate in this study, which was approved by the Massey University Human Ethics Committee (Southern A, Application 15/12; Appendix 1). After being given information sheet with details about the study (Appendix 2), all participants gave written consent (Appendix 3). All participants were regular users of caffeine in one or more forms (coffee, tea, chocolate, soft drinks and/or energy drinks). Participants were screened for potential health issues and caffeine consumption (Appendix 4 and Appendix 5) and were excluded from the study if their caffeine consumption was greater than 4 cups of coffee per day, if they were caffeine naïve or actively refrained from consuming caffeine. Participants were informed about the requirements, benefits and potential risks of this study before providing written consent.

3.2 Participant control

Participants were asked to abstain from consuming any caffeine for the duration of the study (from the familiarisation session until two days following the second main trial). Participants would abstain for a minimum of 3 days prior to the first trial. Withdrawal effects usually last for approximately 3 days (Juliano and Griffiths, 2004), therefore most participants should minimal withdrawal effects at the start of the first trial. In addition, the participants were asked to keep a food record diary (Appendix 6) for two days before each of the main trials and were asked to replicate food intake as accurately as possible for the second main trial.

3.3 Description of physiological tests and measures

3.3.1 Height and weight measures

Height was measured during the familiarisation session using a stadiometer (Seca portable stadiometer, Amtech, New Zealand). Participants were asked to stand (without shoes) with their heels to the back board of the stadiometer and head angled in the Frankfurt plane. They removed shoes and any excess clothing that they would not be running in and body mass was measured using scales accurate to 0.1 kg (AND Weighing Hv 200-KGL, Australia). Body mass was used to determine the quantity of anhydrous caffeine they would be given during the trials.

3.3.2 Heart rate

Heart rate (HR) was measured prior to caffeine intake up until one hour post exercise, as well as 24 and 48 h post caffeine ingestion. Heart rate was recorded using a short range telemetry chest strap (T31 Polar heart rate monitor, Kempele, Finland). Participants remained standing for all heart rate measures.

3.3.3 Urine collection and storage

Urine was collected in a pottle in a private bathroom. Urine specific gravity (USG) was determined using a handheld refractometer (Sur-Ne, Atago. Co. Ltd, Japan). A 1.5-ml sample of urine was stored in a -80°C freezer for later analysis of caffeine and caffeine metabolites (theobromine, theophylline and paraxanthine).

3.3.4 Saliva collection and storage

Saliva was collected from participants using a bud method (Nunes et al., 2012; Appendix 7). Two large cotton buds (Jumbo cotton applicators 18 cm, Livingston) were placed in the participant's

mouth to absorb saliva (Fig. 3.1); one was placed under the tongue and one placed on the inside of the cheek. Buds were left in the participant's mouth for 3 min to allow for adequate absorption of saliva. After 3 min, buds were placed in a test tube and centrifuged (MF-50, Hanil Science Industrial, Korea) at 3500 rpm (1330 x g) for 2 min to extract the saliva from the cotton buds. A 1.5-ml aliquot of saliva was pipetted into Eppendorf vials and stored at -80°C for later analysis of immunoglobulin-A and cortisol. A saliva sample was also collected during the familiarisation session to be used for the genetic tests. DNA extraction from saliva was done at the Molecular Ecology laboratory at Massey University (Auckland, New Zealand; Appendix 8). Extracted DNA samples were then sent to the Liggins Institute (Auckland University, New Zealand) for genotyping.

Figure 3.1 – Saliva collection method: A) Equipment: 2 x large cotton buds, 1 x 15 ml screw top test tube, 1 x 2.5 ml Eppendorf vial. B) Bud placement: One bud placed against inside cheek, the other placed under the tongue. C) – Centrifuge configuration: Bud stalks are cut off to allow buds to be placed in the test tube ready for centrifugation.

3.3.5 Blood sampling and storage

Blood was collected from participants in two different ways. At the beginning of the trial, a cannula was inserted into either the basilic, cephalic or median vein located at the participant's antecubital fossa. An extension kit was attached to the cannula allowing blood to be drawn using a syringe and the cannula was secured using surgical tape and a bandage. A blood sample of 12 ml was drawn before and after caffeine ingestion, as well as before, after and 1 h after exercise. Blood samples were dispensed into a 6 ml EDTA vacutainer and a 6 ml lithium heparin vacutainer. Vacutainers were then centrifuged (MF-50, Hanil Science Industrial, Korea) at 3500 rpm (1330 x g)

for 10 min and the supernatant was pipetted into three 1.5-ml Eppendorf vials. Plasma was stored at -80°C for later analysis of plasma caffeine and caffeine metabolite concentration. Blood was During the two follow-up days, blood was drawn using venepuncture instead of a cannula and 6 ml of blood was drawn into an EDTA vacutainer and 6 ml of blood was drawn into a lithium heparin vacutainer.

3.3.6 Urine and plasma caffeine concentration analysis

A high-performance liquid chromatography (HPLC) method was used for the determination of urine and plasma caffeine and caffeine metabolites. Frozen samples were thawed and deproteinised by adding 400 µl of the sample to 400 µl of 0.8 M perchloric acid. Samples were then vortexed for 10 s to ensure thorough mixing and then centrifuged for 10 min at 10,000 rpm (9900 x g). Once centrifuged, a 400 µl aliquot of the supernatant was removed and placed in a glass HPLC vial. Samples were then analysed by reversed-phase HPLC (Appendix 9).

3.3.7 Vertical jump height

Squat (SJ) and countermovement (CMJ) jumps were measured using a jump mat (Just jump system 761, Perform Better, USA). Both jumps were performed with the participants' hands on their hips to minimise momentum generated by movements of the upper body. Participants kept their backs upright, legs straight and toes pointed when jumping. This ensured that all the participants exhibited the same jumping technique and decreased variation between athletes. During the squat jump, participants would hold the bottom of the squat for 2 s before jumping up. If the participant dipped or bounced before jumping, the jump would not be recorded. During the countermovement jump, participants would squat down and jump without any pause at the bottom of the squat.

3.3.8 Leg strength and power

Leg torque of the knee extensors of the dominant leg was assessed using an isokinetic dynamometer (Model 850-230, Biodex Medical Systems Inc., New York, USA). The participants sat in the Biodex chair while performing all of the tests. The researcher adjusted the position of the chair and attachments so that the axis of rotation about the dynamometer arm was in line with the participant's lateral femoral epicondyle of their dominant leg. The participant was secured using a strap across the thigh and two straps crossing over the chest as well as fastening the participant's leg, around the shin, into the knee attachment dynamometer arm. Securing the participant prevents movement in the rest of the body and isolates the quadriceps. The dynamometer was calibrated before each use and the participant's range of motion and limb weight was calculated for each use.

Participants completed 5 repetitions of concentric contraction of the quadriceps followed by a 2-min rest period. After the rest period, participants then completed 5 repetitions of eccentric contraction of the quadriceps. Each test was completed at $60°\cdot s^{-1}$ as numerous studies have used speeds of between $30°\cdot s^{-1}$ and $180°\cdot s^{-1}$ (Bond et al, 1986; Jacobson et al, 1991) Participants received verbal encouragement to contract as forcefully as possible, as well as receiving visual feedback via the computer monitor which showed the torque that they produced during the contraction. Peak torque was recorded as the highest value across the 5 contractions.

3.3.9 10-km time trial run

The 10-km run was performed on a treadmill (ELG70, Woodway, USA) set at a 1% incline to simulate running outside on flat terrain (Jones and Doust, 1996). Participants were not able to adjust the incline during the run, but were free to adjust the speed. They were instructed to run the 10 km as fast as possible, but did not receive any further encouragement during the run. Participants were not privy to their speeds during the run, but were told when they had completed each km. The speed, distance and time were recorded when the participant started running, stopped running, or changed speeds during the run.

3.4 Description of perceptual scales

Three different perceptual scales were used throughout the trials. The rating of perceived exertion scale (RPE; Borg, 1982; Appendix 10) was used to measure subjective exercise intensity at that specific point in time. The RPE scale ranges from 6 – 20 with anchors ranging from "very, very light" to "very, very hard". Participants were asked to estimate the overall feeling of exertion and fatigue that they were feeling at that point in time.

The feeling scale (FS; Hardy and Rejeski, 1989, Appendix 11) was used to assess the overall feeling of pleasure and displeasure during the trial. This is an 11-point scale ranging from +5 to -5. Anchor words were attached to every second value ranging from "very good" (+5) to "very bad" (-5) as well as "neutral" (0). Participants were asked how they felt overall physically and mentally. The felt arousal scale (FAS; Svebak and Murgatroyd, 1985; Appendix 12) was used to estimate the arousal/activation level during the trials. It is a 6-point scale with two anchors: "high arousal" (6) and "low arousal" (1). Participants were informed that high arousal constituted of emotions such as anger, anxiety, excitement and alertness, whereas low arousal constituted of feelings such as boredom, tiredness and calmness.

The profile of mood states (POMS; Grove and Prapavessis, 1992; Appendix 13) was used to assess changes in mood as a result of exercise and/or caffeine supplementation over the course of the trials. The POMS questionnaire presented participants with 40 mood-describing adjectives split into 7 groups (fatigue, anger, esteem, depression, vigour, tension and confusion). Participants would indicate how well each adjective described their mood at that point in time by selecting a number on a Likert scale ranging from 0-4 with anchor words ranging from "not at all" to "extremely" respectively.

3.5 Description of cognitive tests

Three cognitive tests from a software suite (Computerised Mental Performance Assessment System [COMPASS], Northumbria University, Newcastle, United Kingdom) were used for this study. These included digit vigilance, Corsi blocks and rapid visual information processing (RVIP) modules.

The digit vigilance module consisted of a 1-min test where two numbers appear on screen. The left-hand side number remains the same while the right-hand side number continuously changes at constant speed. The participant is instructed to hit the spacebar (on the keyboard) whenever the two numbers match up. Reaction time and errors were measured during this test. The Corsi blocks' test has a number of light blue blocks that appear on the screen in a random pattern. A certain number of blocks (beginning at 5) would light up for one second in a specific pattern. The participant has to remember in which order the blocks lit up and repeat the pattern by clicking on the blocks. This test measured time taken to complete each set of blocks, number of errors and number of correct sets. This test had no time limit but got progressively harder by increasing the number of blocks that are lit up in each series. The test was terminated when the participant got more than 3 (out of 5) sets incorrect for that level. The RVIP test involved a single random digit on the screen that would rapidly change for 1 minute. Participants pressed the spacebar every time three odd or three even numbers appeared consecutively. The RVIP test assessed reaction time and number of errors made.

3.6 Description of sleep quality measures

Participants were asked to keep a sleep diary and wear an actigraph (Actiwatch 2, Phillips Respironics, Netherlands) to record their sleep quality and patterns for three days post and prior to each of the two main trials. Subjects were instructed on how to wear the actigraph as well as how to complete the sleep diary during the familiarisation. The actigraph was worn on the non-dominant arm, and was worn at all times apart from during contact sports, showering or swimming. The sleep diary (LSEQ; Appendix 14) consisted of a time scale on which participants indicated with a line the time they attempted to go to sleep and the time they woke up, and a section for additional comments about their sleep. Participants were asked to record all sleep that lasted for longer than 10 min. In addition, participants completed several questions pertaining to the quality of their sleep over the duration of the trials (e.g. how did you find sleep compared to usual?). This was in the form of a linear scale and participants would indicate how they felt with a line on the scale. Each question has two anchor words

at the end of the scale and participants would use this to guide their decision on how they would assess their sleep such as “tired” or “alert”.

3.7 Familiarisation protocol

Participants were asked to attend a familiarisation trial at least one week before their first main trial. During this session participants were informed of all the procedures and measures taken during the main trials. Participants completed each of the three cognitive tests and could retry each of them until they felt competent. They were required to complete at least 5 squat jumps and counter movement jumps so as to accustom themselves to the correct jumping techniques. Participants performed at least 5 concentric and eccentric contractions of the quadriceps during leg extensions on the Biodex. A tutorial on the use of the treadmill controls was given and participants were required to run at least 1 km to ensure familiarity with the machine. During the familiarisation, a saliva sample was taken to serve as the genetic sample to determine the CYP1A2 and ADORA2A genotype of each participant. Participants were shown how the urine and blood samples were to be collected, however, these were not performed during the familiarisation. Perceptual scales (RPE, FS and FAS) along with the POMS questionnaire were explained to the participants in detail and they were then asked to complete and score each one. Participants were also briefed on how to use the actigraph, which they would wear for the 2-week duration of the trial, and how to fill in the sleep and food diaries.

3.8 Study Design

After an initial familiarisation session participants were required to perform their main trials one week apart with the follow-up assessments 22-26 h and 46-50 h after caffeine ingestion. The main trials were all conducted at the same time of the day per participant. To prevent any trial order effects, treatments were randomly assigned in a placebo-control, double-blind crossover design.

Figure 3.2 shows a schematic representation of each main trial (over 3-day period). Upon arrival, participants were equipped with a heart rate monitor which was worn for the remainder of the trial and provided a urine and saliva sample. Water (2 $ml \cdot kg^{-1}$ BM) was given to participants at regular intervals throughout the trial period. Cognitive testing, POMS and perceptual scales (excluding RPE) were then completed, followed by strength and power measures. Lastly, a blood sample was taken via cannula or venepuncture. Participants then ingested a gelatine capsule (Vegie capsules, Bioblanace, New Zealand) which contained either 6 $mg \cdot kg^{-1}$ BM caffeine (Fluka Sigma-Aldrich, MO, USA) or placebo (maltodextrin) and rested quietly for 50 min after which all measures were repeated. Following the rest period, participants then began the 10-km time trial. Heart rate measures were taken every 1 km completed, while perceptual measures were collected every 2.5 km. At 5 km the participant was stopped for no longer than 2 min while a blood and saliva sample were collected. At the completion of the 10-km time trial all measures were repeated and the participant was asked to wait quietly for one hour, after which all measures were repeated (excluding strength, power, and body weight). Participants returned ~24 and ~48 h after caffeine ingestion for post-exercise measures, which included a blood, urine and saliva sample, cognitive testing, POMS and perceptual scales, strength and power measures and resting heart rate. This protocol was then repeated the following week with the alternate treatment to that they received initially.

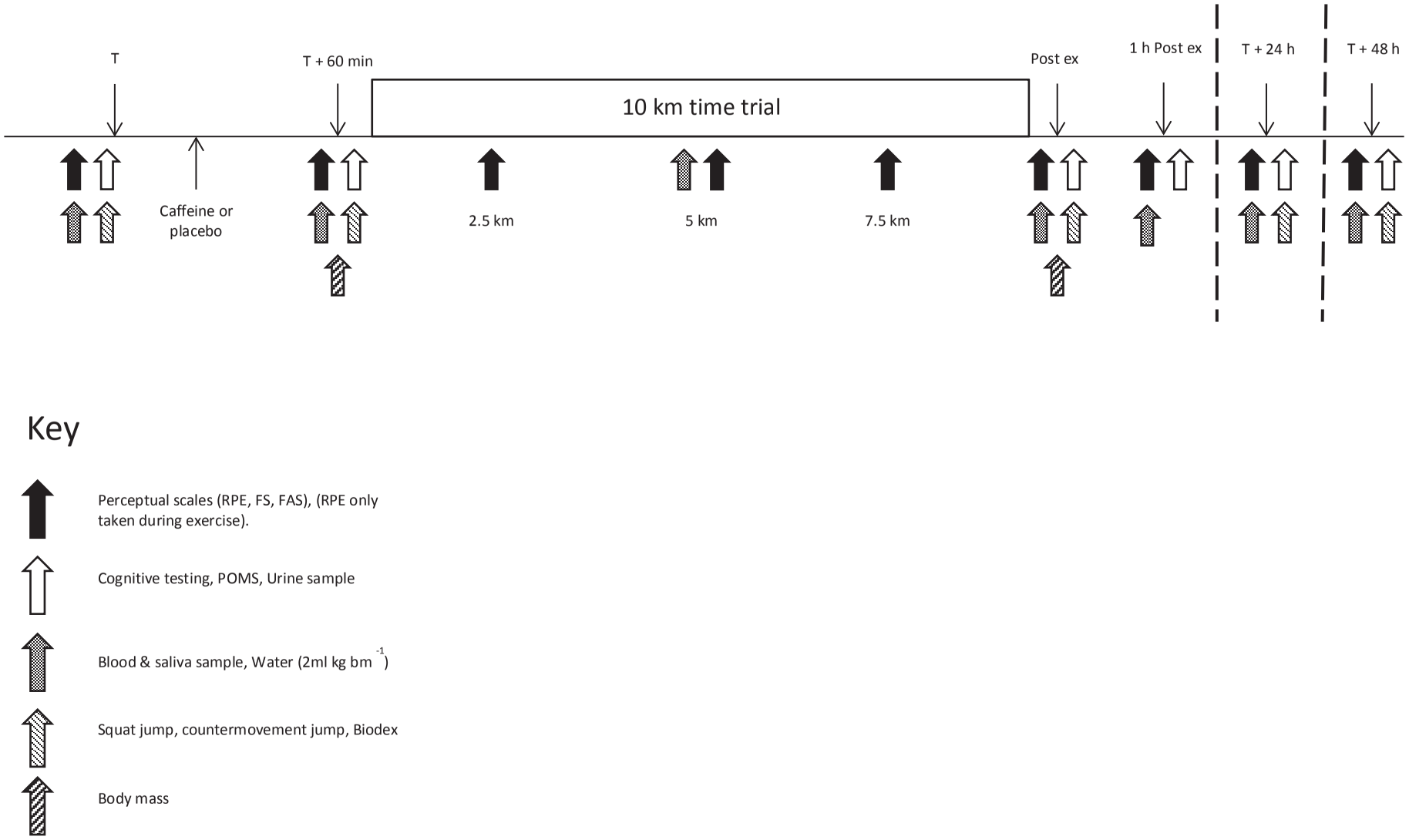

Figure 3.2. Detailed schematic diagram of main trials showing time of sample collection, physiological measures and cognitive tests. T represents initial measures and ingestion of placebo or caffeine; T + 60 min represents the time point 60 minutes post caffeine/placebo ingestion; Post ex represents the time point immediately following completion of the 10-km time trial; 1 h post ex represents the time point 1 hour following completion of the time trial; T + 24 h/T + 48 h represents the time points 24 and 48 hours post caffeine ingestion. RPE – rating of perceived exertion, FS – feeling scale, FAS – felt arousal scale, POMS - profile of mood states.

3.9 Data and statistical analysis

Statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS, Chicago, IL) Version 23.0. Data are presented as mean ± standard deviation (SD). A repeated measures analysis of variance (ANOVA) was used to calculate differences between caffeine (CAFF) and placebo (PLA) trials in perceptual responses, mood, strength, power, cognition, plasma caffeine metabolites, and heart rate. A t-test was used to measure differences between CAFF and PLA in Leeds sleep evaluation questionnaire (LSEQ) data, actigraph sleep data and body weight. Post-hoc analysis was completed using a paired t-test with a stepwise Holm-Bonferroni correction. Effect sizes (Cohen's d and eta squared) were used to show practical significance. Cohen's d values of 0.2 are considered a small effect size, 0.5 a moderate effect size and 0.8 a large effect size (Cohen, 1988). Likewise eta squared values of 0.01 represents a small effect size, 0.09 a medium effect size and 0.25 a large effect size. Pearson's correlation coefficient was used to indicate relationships between measured outcomes.

CHAPTER 4 - RESULTS

4.1 Participants

Of the 17 participants who completed the study, 14 were heterozygous A/C allele carriers (87.5%; 'slow' metabolisers of caffeine) and 2 were homozygous A/A allele carriers ('fast' metabolisers of caffeine) and 1 participant's genotype was undetermined. Therefore, the results presented in this section will not compare between genotypes but will describe responses to caffeine in participants with the heterozygous A/C genotype.

4.2 10 km time trial

There was no difference in run time for either the first 5 km (5 km CAFF 24.2 ± 3.5 min vs. PLA 25.4 ± 3.8 min; P = 0.589) or the total 10 km (10 km CAFF 51.12 ± 7.12 min vs PLA 53.77 ± 8.21 min; P = 0.187, Cohen's d = 0.218; Fig 4.1). However, 11 of 14 participants had faster run times following caffeine ingestion compared to placebo with a mean overall improvement of 3.8% (Fig. 4.1 and 4.2). The lines in Figure 4.1 show a trend downwards which indicates a decrease in most participants' run times in the CAFF trial compared to PLA.

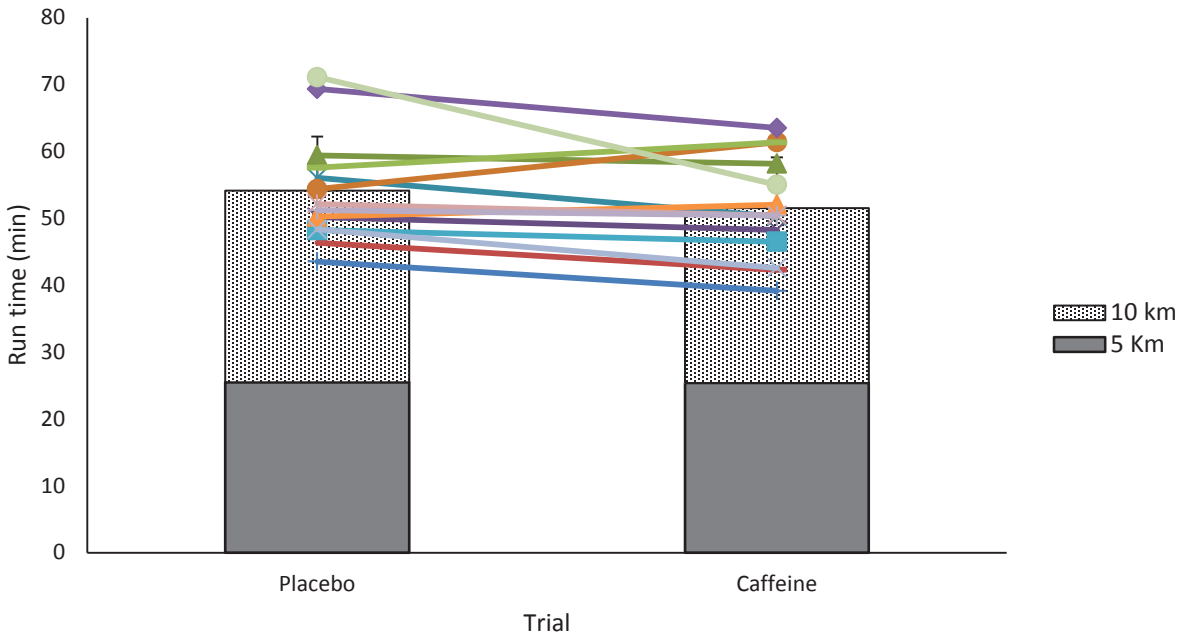

Figure 4.1 - Run times in CAFF and PLA split between the initial 5 km and last 5 km. Lines represent individual 10-km run times for CAFF and PLA trials.

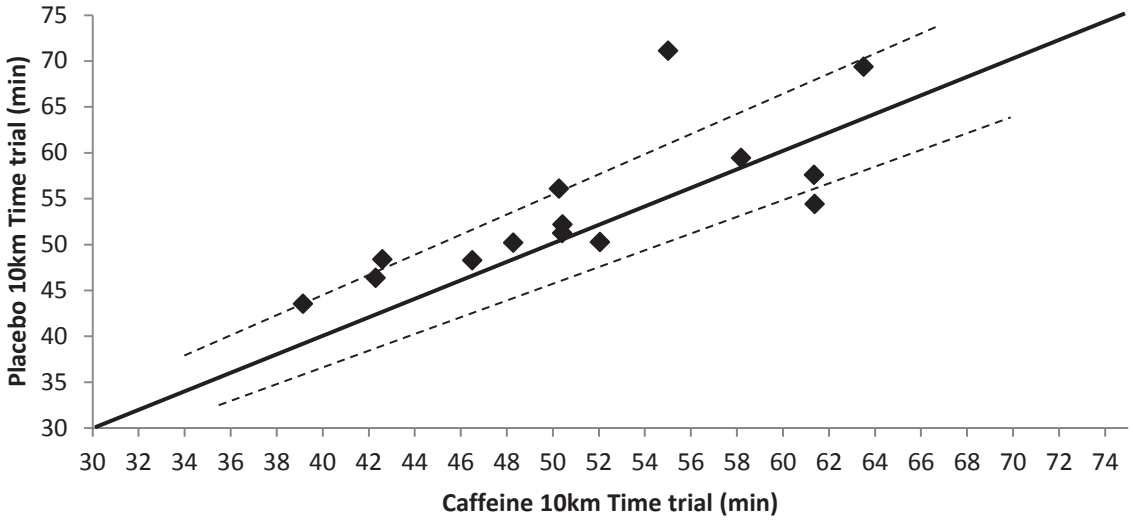

Figure 4.2 - 10 km run times in both the placebo trial (y-axis) and the caffeine trial (x-axis). The line of identity is plotted and represents no change between treatments, thus points which lie above the line indicate slower 10-km time in placebo trial for that individual. The dotted line represents a 10% improvement (above the solid line) and decrement (below the solid line) in run times between CAFF and PLA trials.

4.3 Heart rate

Caffeine ingestion did not have an effect on resting heart rate (HR; CAFF 70.2 ± 11.4 bpm vs. PLA 72.4 ± 10.5 bpm; P = 0.25). However, there was a trend for elevated HR during exercise in participants in the CAFF trial (CAFF 171.9 ± 9.6 bpm vs. PLA 167.1 ± 12.1 bpm; P = 0.062; $\eta^2 = 0.227$; Fig. 4.3). Heart rate of participants increased throughout exercise in both CAFF and PLA trials (P < 0.001; $\eta^2 = 0.754$) with heart rate differing more between the trials during the second half of the 10-km run (CAFF 178.3 ± 8.76 bpm vs. 171.8 ± 10.7 bpm; P = 0.016) compared to the initial 5 km (CAFF 165.6 ± 12.4 bpm vs. PLA 162.3 ± 14.2 bpm). However, there was no interaction of treatment x time on HR during exercise (P = 0.257; $\eta^2 = 0.088$).

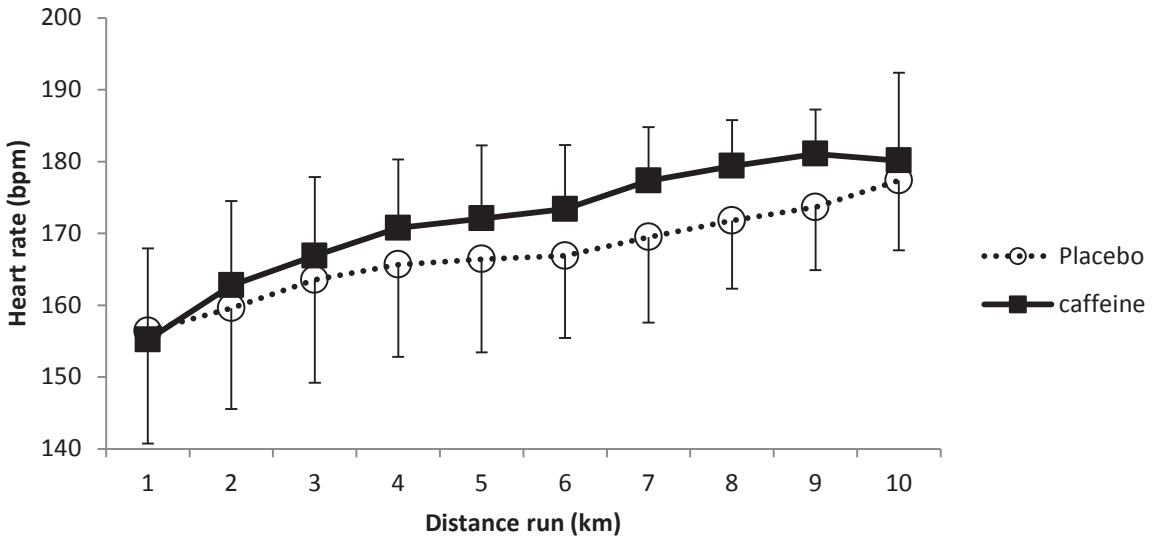

Figure 4.3 – Heart rate during the 10 km time trial in CAFF and PLA trials.

4.4 Leg strength and power

There was a trend for higher concentric knee extensor torque by participants in the CAFF trial compared to PLA (CAFF 198.5 N·m vs. PLA 183.3 N·m; P = 0.099; $\eta^2 = 0.248$; Fig. 4.4). Concentric knee extensor torque in both CAFF and PLA trials were not affected by time (P = 0.857; $\eta^2 = 0.022$). Similarly, no interaction of treatment x time was observed (P = 0.808; $\eta^2 = 0.030$).

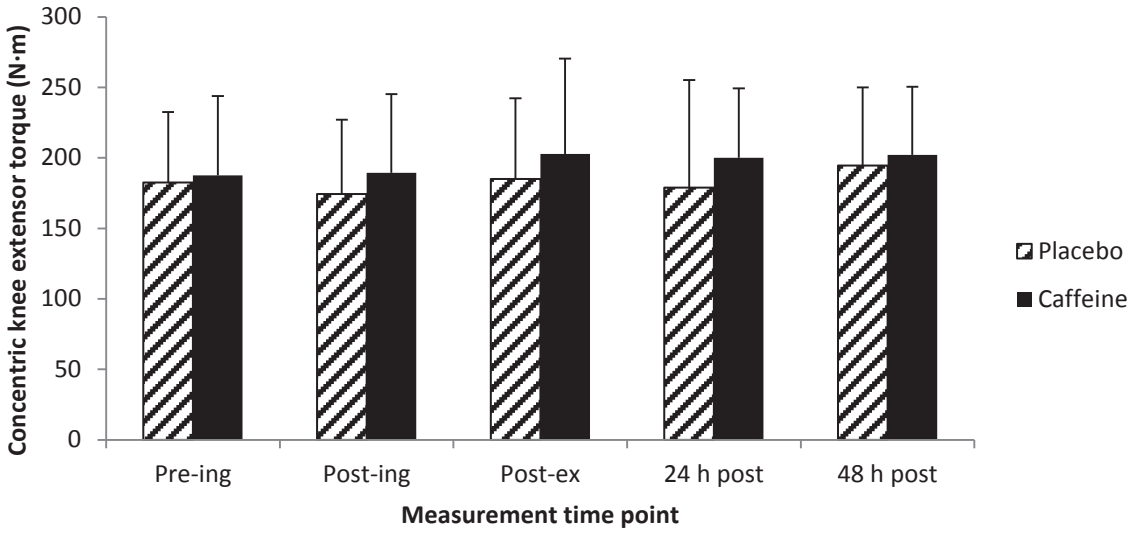

Figure 4.4 – Maximal concentric knee extensor torque by participants in the CAFF and PLA trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-ing' represents the time point immediately before exercise (1 h after caffeine/placebo ingestion); 'Post-ex' represents the time point immediately after completion of the 10-km time trial; '24 h post' and '48 h post' represents the time point 24 and 48 hours after caffeine/placebo ingestion.

Eccentric knee extensor torque was greater in the CAFF trial compared to PLA (CAFF 272.2 N·m vs. PLA 238.1 N·m; P = 0.015; η^2 = 0.461; Fig. 4.5). Furthermore, a trend of increased knee extensor torque was observed during the CAFF trial over time (interaction of treatment x time; P = 0.081; η^2 = 0.184). The largest differences were observed between CAFF and PLA trials post-ingestion (CAFF 273.9 vs. PLA 236.4) and post-exercise (CAFF 274.5 N·m vs. PLA 223.5 N·m). Eccentric knee extensor torque in both CAFF and PLA trials were not affected by time (P = 0.195; η^2 = 0.137).

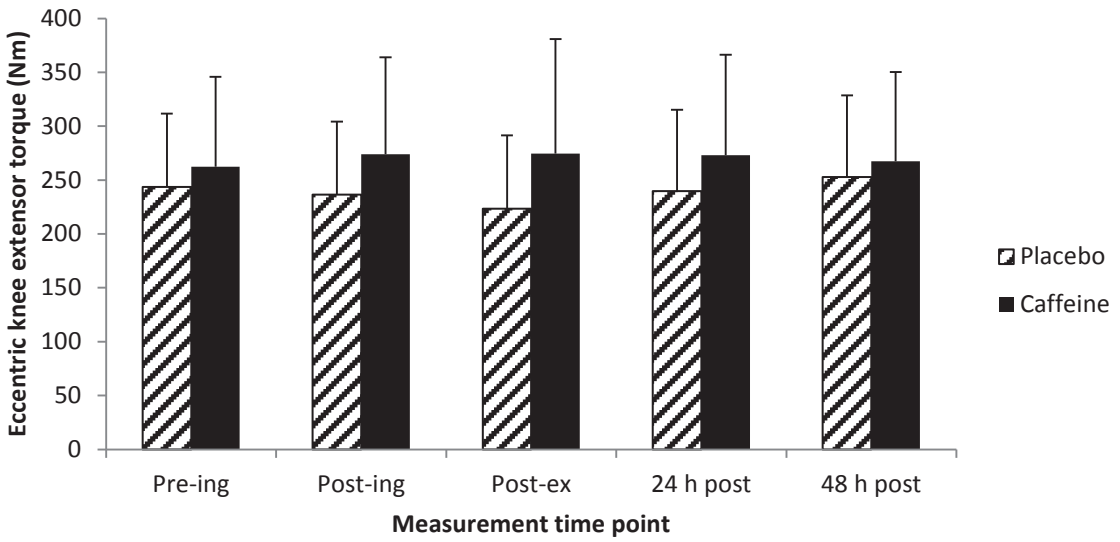

Figure 4.5 – Maximal eccentric knee extensor torque by participants in the CAFF and PLA trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-Ing' represents the time point immediately before exercise (1h after caffeine/placebo ingestion); 'Post-ex' represents the time point immediately after completion of the 10-km time trial; '24 h post' and '48 h post' represents the time point 24 and 48 hours after caffeine/placebo ingestion.

Squat jump (SJ) height increased following caffeine ingestion ($P = 0.017$; $\eta^2 = 0.45$; Fig. 4.6). In both CAFF and PLA trials squat jump height increased after the 10-km run time trial and decreased after rest post ingestion ($P = 0.035$; $\eta^2 = 0.336$). Therefore, the greatest SJ height was recorded immediately following the 10-km run and lowest post ingestion for both CAFF and PLA trials (post-ingestion 39.7 ± 5.26 cm vs. post-exercise 44.7 ± 7.05 cm). No time x treatment interaction effect was observed for SJ height ($P = 0.129$; $\eta^2 = 0.160$).

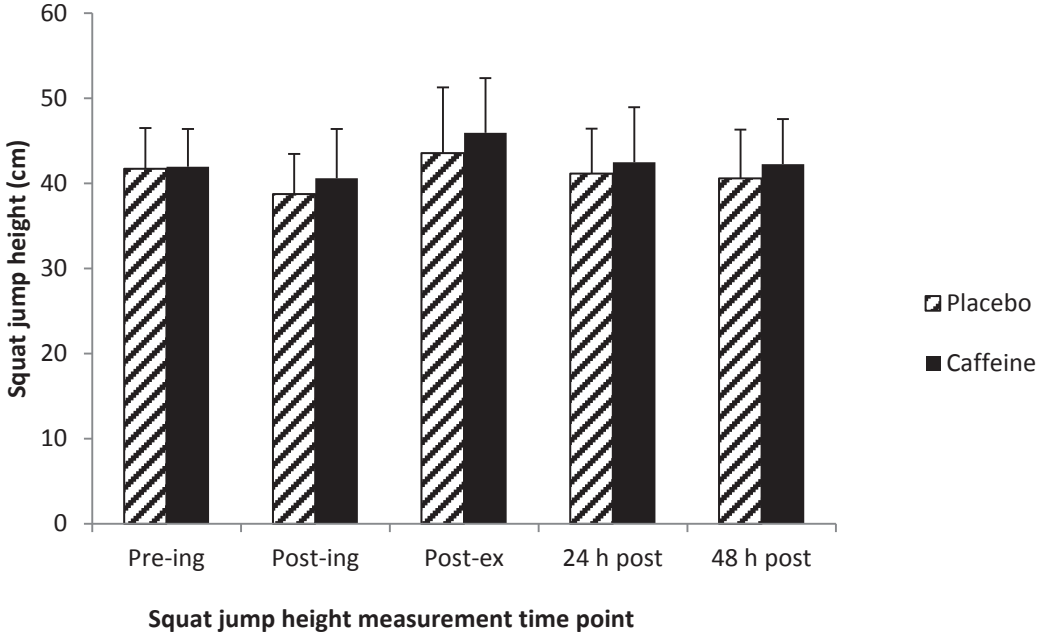

Figure 4.6 – Squat jump (SJ) height during CAFF and PLA trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-ing' represents the time point immediately before exercise (1h after caffeine/placebo ingestion); 'Post-ex' represents the time point immediately after completion of 10km time trial; '24 h post' and '48 h post' post treatment represents the time point 24 and 48 hours after caffeine/placebo ingestion.

In contrast, countermovement jump (CMJ) height was not affected by caffeine ingestion ($P = 0.325$; $\eta^2 = 0.097$, Fig. 4.7). However, similar to SJ, CMJ height increased after exercise and decreased after rest post ingestion ($P = 0.08$; $\eta^2 = 0.257$). CMJ height was greatest immediately following the 10 km run and lowest post ingestion (post-ingestion 41.7 ± 5.79 cm vs. post-exercise 45.5 ± 6.65 cm). No interaction effect of treatment x time was seen in CMJ height ($P = 0.209$; $\eta^2 = 0.136$).

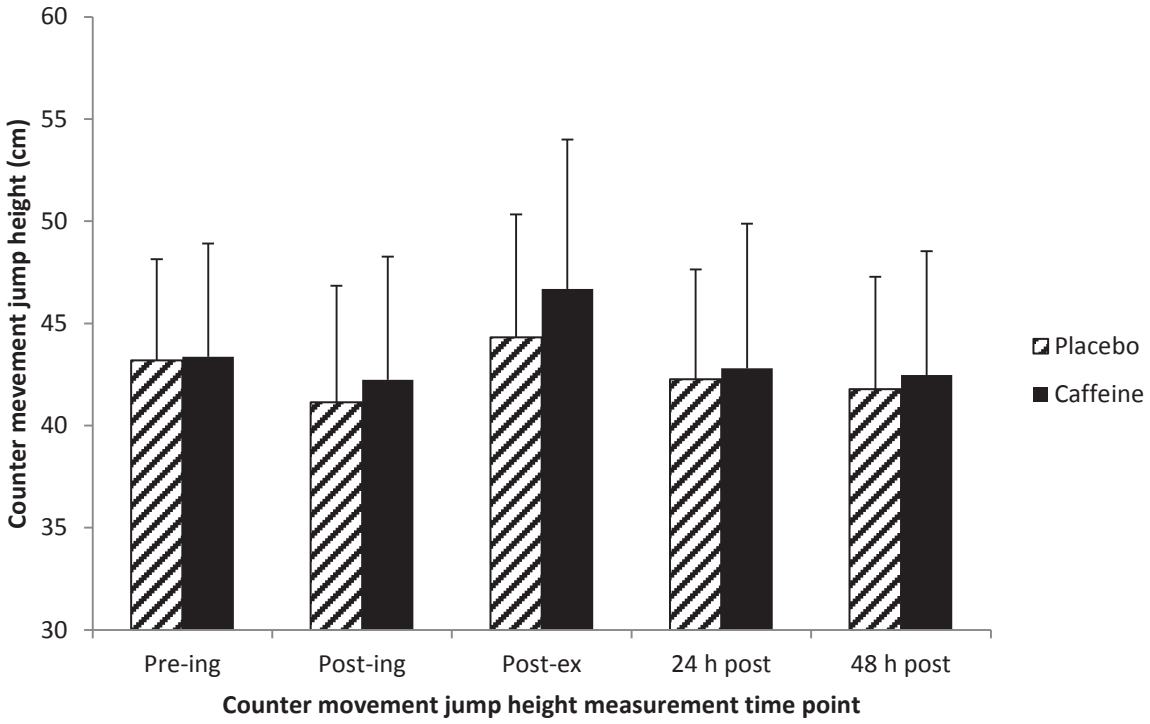

Figure 4.7 – Countermovement jump (CMJ) height during CAFF and PLA trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-ing' represents the time point immediately before exercise (1 h after caffeine/placebo ingestion); 'Post-ex' represents the time point immediately after completion of 10 km time trial; '24 h post' and '48 h post' post treatment represents the time point 24 and 48 hours after caffeine/placebo ingestion.

4.5 Perceptual responses

Ratings of perceived exertion (RPE) increased over the duration of the 10 km time trial ($P < 0.001$; $\eta^2 = 0.853$). However, RPE was not affected by caffeine ingestion ($P=0.309$; $\eta^2 = 0.079$; Table 4.1). There was no interaction of treatment x time for RPE ($P = 0.156$; $\eta^2 = 0.125$).

Table 4.1 – Mean ± standard deviation of ratings of perceived exertion (RPE) during the 10 km time trial for CAFF and PLA trials. RPE ranges from 6 "very light" to 20 "very, very hard" (Appendix 10).

Time	**RPE**	
	CAFF	**PLA**
2.5 km	12.2 ± 1.5	13.1 ± 1.3
5 km	13.5 ± 1.5	14.5 ± 1.6
7.5 km	15.7 ± 1.5	15.7 ± 1.5
10 km	17.2 ± 1.9	17.4 ± 1.5

There was no difference in feeling scale (FS) scores between CAFF and PLA trials (P = 0.35; $\eta^2 = 0.067$; Fig. 4.8). However, FS scores decreased during the 10 km time trial, and increased 1 hour post exercise (P < 0.001; $\eta^2 = 0.51$). Furthermore, FS was higher in the CAFF trial over time compared to PLA (interaction of treatment x time; P = 0.036; $\eta^2 = 0.176$). Post-hoc analyses indicated that at 2.5-km FS was increased in CAFF trial compared to PLA trials (P < 0.05).

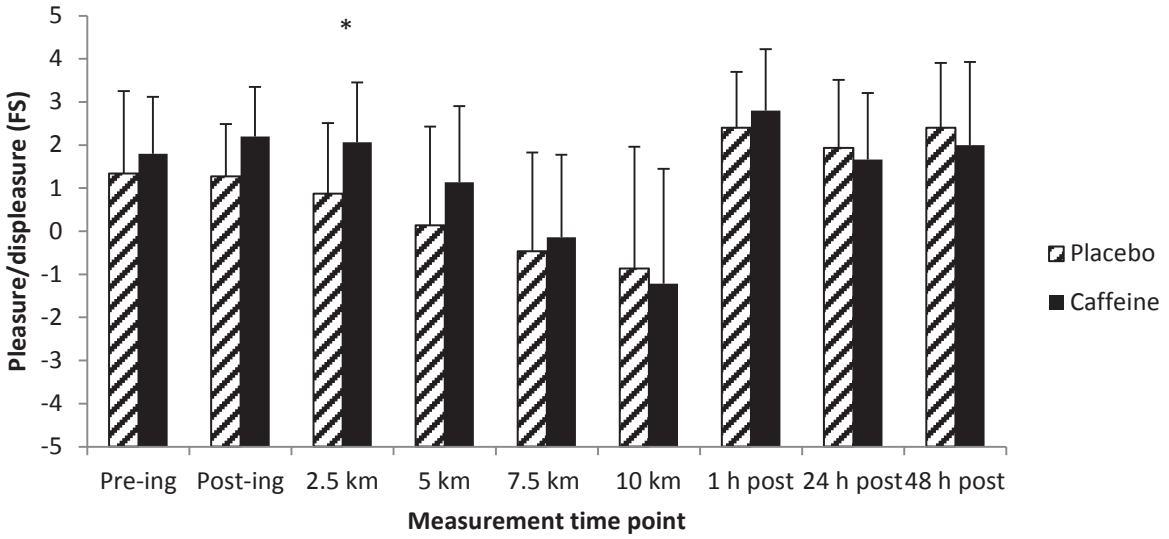

Figure 4.8 – Feeling scale (FS) scores of pleasure and displeasure during CAFF and PLA trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-ing' represents the time point immediately before exercise (1h after caffeine/placebo ingestion); 2.5, 5, 7.5, and 10 km represent distance run during the 10 km time trial, '1 h post' represents the time point 1 hour after completion of 10km time trial; '24 h post' and ' 48 h post' represents the time point 24 and 48 hours after caffeine/placebo ingestion. Asterisks (*) indicate significant differences in pleasure/displeasure between CAFF and PLA trials at specific time points (P < 0.05).

Felt arousal scale (FAS) ratings were not affected by caffeine supplementation ($P = 0.295$; $\eta^2 = 0.084$). However, FAS ratings increased during exercise and decreased after exercise ($P < 0.001$; $\eta^2 = 0.438$; Fig. 4.9). Furthermore, a trend was observed of greater FAS in the CAFF trial over time compared to PLA (interaction of treatment x time; $P = 0.081$; $\eta^2 = 0.135$; Fig. 4.9). However, post-hoc analysis showed no significant differences at any time points.

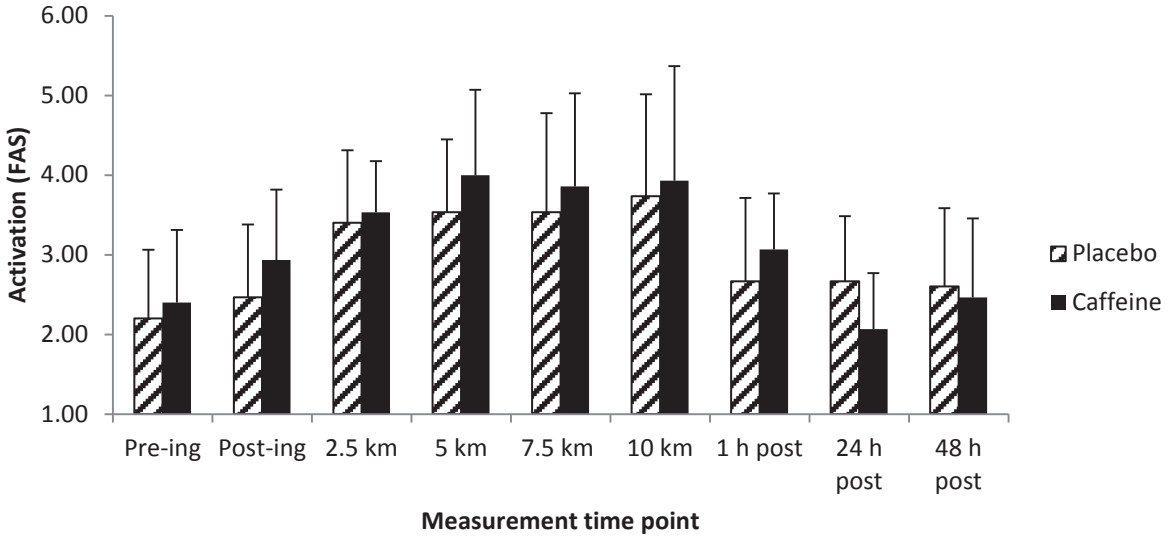

Figure 4.9 – Felt arousal scale scores of activation during CAFF and PLA trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-ing' represents the time point immediately before exercise (1h after caffeine/placebo ingestion); 2.5, 5, 7.5, and 10 km represent distance run during the 10 km time trial; '1 h post' represents the time point 1 hour after completion of the 10-km time trial; '24 h post' and '48 h post' represents the time point 24 and 48 hours after caffeine/placebo ingestion.

Additionally, FS and FAS values were plotted on the circumplex model (Fig. 4.10). During the first 5 km of the time trial it can be seen that participants are in the 'high arousal, pleasure' quadrant during the CAFF trial, whereas participants are in the 'low activation, pleasure' quadrant in the PLA trial. However, in both trials at the completion of the time trial participants were in the 'high activation, displeasure' quadrant.

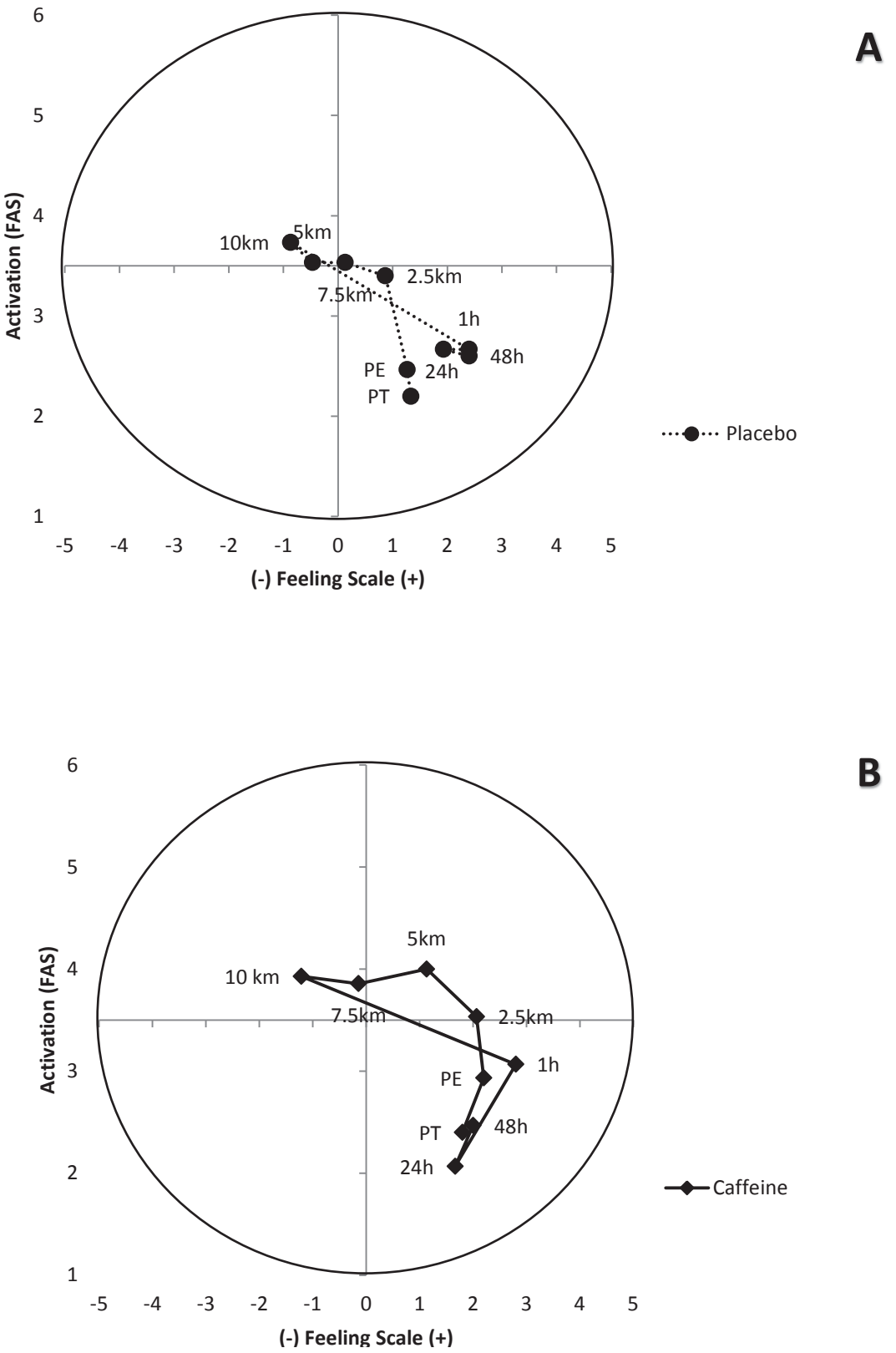

Figure 4.10 - Feeling scale (FS) and felt arousal scale (FAS) ratings plotted in a circumplex model. Fig. 4.10 A represents the PLA trial and Fig. 4.10 B represents the CAFF trial. Affective valence (pleasure and displeasure) is plotted along the x-axis, while activation (arousal) is plotted along the y-axis. This can be broken into four quadrants; 'high activation, pleasure' in the top right which reflects excitement and vigour; 'low activation, pleasure' in the bottom right side which represents calmness; 'low activation, displeasure' in the bottom left which represents boredom and fatigue; and 'high activation, displeasure' in the top left which represents anxiety or anger.

4.6 Mood

Overall profile of moods state (POMS) ratings were not different between trials (CAFF 141.2 ± 56.0 vs. PLA 135.9 ± 55.6; P = 0.42). Similarly, caffeine ingestion did not have a significant effect on the fatigue subset of the POMS questionnaire (P = 0.285; η^2 = 0.076). However, a main effect of time was observed with increased fatigue ratings post exercise and 1 hour post exercise in both CAFF and PLA trials (P < 0.001; η^2 = 0.53, Fig. 4.11). Furthermore, fatigue was lower in the CAFF trial over time compared to PLA (CAFF 5.31 ± 3.54 vs. PLA 6.94 ± 4.99; interaction of treatment x time; P = 0.022; η^2 = 0.18). However, post-hoc analysis revealed no significant pairwise differences at any time points.

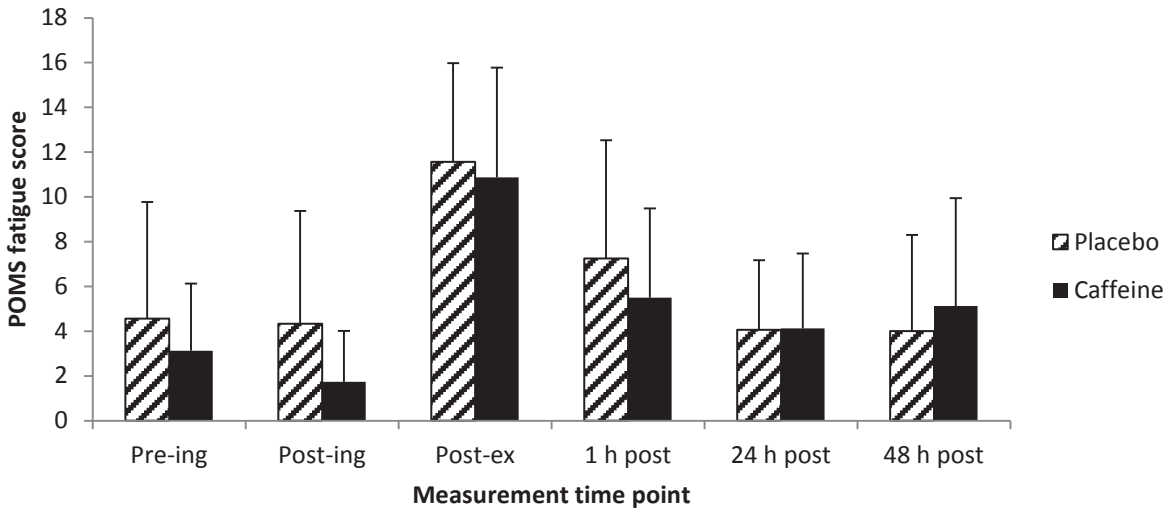

Figure 4.11 – Profile of mood states fatigue subset results during CAFF and PLA trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-ing' represents the time point immediately before exercise (1h after caffeine/placebo ingestion); 'Post-ex' represents the time point immediately after completion of 10 km time trial; '1 h post' represents the time point 1 hour after the completion of the 10 km run; '24 h post' and '48 h post' represents the time point 24 and 48 hours after caffeine/placebo ingestion.

The vigour subset of the POMS questionnaire was not significantly affected by caffeine ingestion (P = 0.197; η^2 = 0.11; Fig. 4.12). However, in both CAFF and PLA trials vigour increased during exercise and remained elevated 1 hour post exercise (P = 0.019; η^2 = 0.16). Caffeine ingestion resulted in increased vigour over time compared to placebo (CAFF 9.05 ± 4.64 vs. PLA 6.78 ± 3.89; interaction of treatment x time; P = 0.032; η^2 = 0.147). Post-hoc analysis did not show any significant pairwise differences between time points.

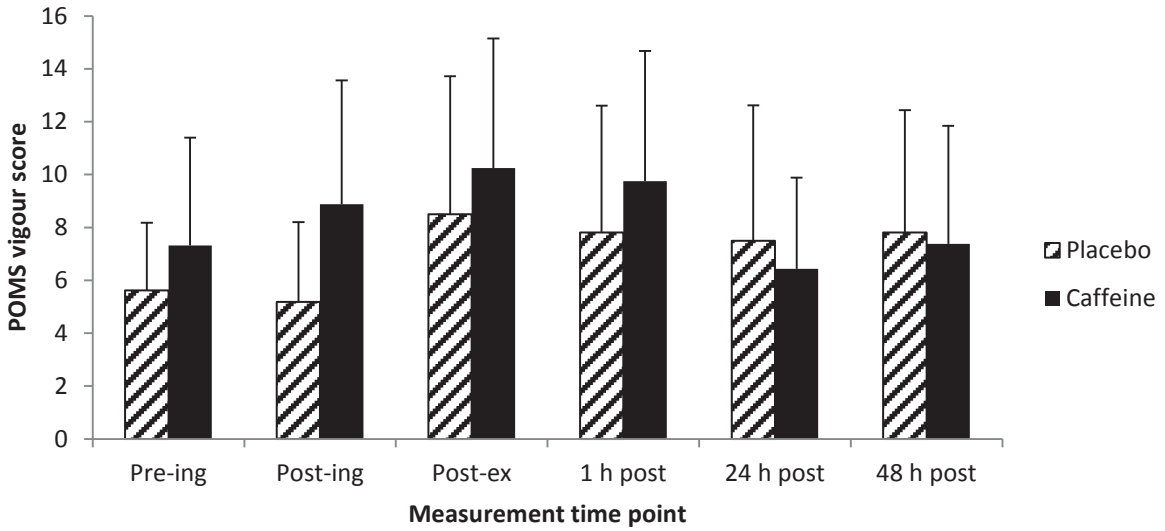

Figure 4.12 – Profile of mood states (POMS) vigour subset scores during CAFF and PLA trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-ing' represents the time point immediately before exercise (1h after caffeine/placebo ingestion); 'Post-ex' represents the time point immediately after completion of 10 km time trial; '1 h post' represents the time point 1 hour after the completion of the 10 km run; '24 h post' and '48 h post' represents the time point 24 and 48 hours after caffeine/placebo ingestion.

4.7 Cognition

Caffeine ingestion did not influence digit vigilance (DV) accuracy (P = 0.441; η^2 = 0.05). Additionally, DV accuracy did not change over time (P = 0.35; η^2 = 0.086; Table 4.2). However, DV reaction times decreased following caffeine ingestion compared to placebo (CAFF 404.1 ± 40.1 ms vs PLA 413.2 ± 39.5 ms; P = 0.007; η^2 = 0.467; Fig. 4.13). DV reaction time in both CAFF and PLA trials was decreased immediately after the 10-km run time trial (P < 0.001; η^2 = 0.467). No interaction effect of treatment x time was observed (P = 0.123; η^2 = 0.134).

Table 4.2 – Mean ± standard deviation of digit vigilance and rapid visual information processing (RVIP) accuracy in CAFF and PLA trials.

Time	Digit Vigilance % correct		RVIP % correct	
	CAFF	PLA	CAFF	PLA
Pre treatment	97.44 ± 4.34	98.97 ± 2.50	77.88 ± 24.02	81.73 ± 13.13
Pre exercise	96.92 ± 4.40	97.44 ± 4.34	80.77 ± 20.17	65.38 ± 27.55
Post exercise	97.43 ± 3.38	97.43 ± 3.38	86.54 ± 21.32	75.96 ± 27.72
1 h post exercise	95.38 ± 8.77	98.46 ± 2.92	84.62 ± 21.14	75.96 ± 20.70
24h post treatment	98.46 ± 3.99	97.95 ± 3.20	78.85 ± 23.04	77.88 ± 19.87
48h post treatment	99.49 ± 1.85	98.46 ± 2.92	78.85 ± 21.28	78.85 ± 22.47

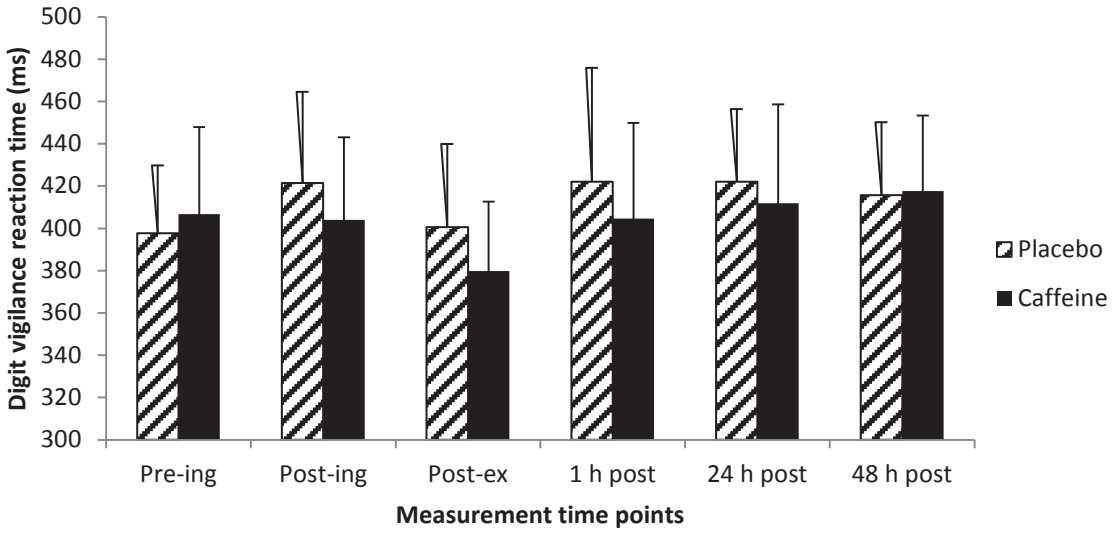

Figure 4.13 – Digit vigilance test reaction times during CAFF and PLA trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-ing' represents the time point immediately before exercise (1h after caffeine/placebo ingestion); 'Post-ex' represents the time point immediately after completion of 10 km time trial; '1 h post' represents the time point 1 hour after the completion of the 10 km run; '24 h post' and '48 h post' represents the time point 24 and 48 hours after caffeine/placebo ingestion.

There was a trend for increased rapid visual information processing RVIP accuracy following caffeine ingestion (P = 0.072; η^2 = 0.244). However, RVIP accuracy did not change over time (P = 0.286; η^2 = 0.096; Table 4.2). In contrast to DV reaction times, RVIP reaction times did not decrease following caffeine ingestion (P = 0.492; η^2 = 0.04; Fig. 4.14). Similarly, RVIP did not change over time (P = 0.491; η^2 = 0.069).

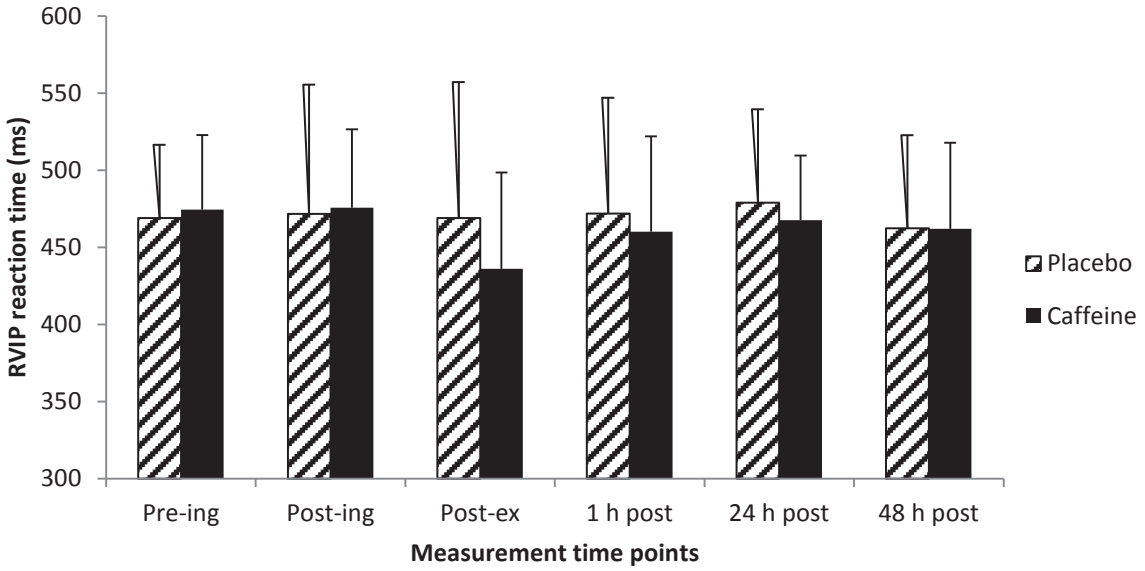

Figure 4.14 – Rapid visual information processing (RVIP) test reaction times during CAFF and PLA trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-ing' represents the time point immediately before exercise (1h after caffeine/placebo ingestion); 'Post-ex' represents the time point immediately after completion of 10km time trial; '1 h post' represents the time point 1 hour after the completion of the 10 km run; '24 h post' and '48 h post' represents the time point 24 and 48 hours after caffeine/placebo ingestion.

Corsi block scores were not affected by caffeine ingestion ($P = 0.647$; $\eta^2 = 0.018$). Similarly, Corsi block scores did not change over time ($P = 0.9$; $\eta^2 = 0.023$; Table 4.3). Furthermore, there was no treatment x time interaction for Corsi block scores ($P = 0.835$; $\eta^2 = 0.034$).

Table 4.3 – Mean ± standard deviation of Corsi span scores in CAFF and PLA trials. Corsi span is the maximum number of blocks remembered in the correct sequence.

Time	**Corsi span**	
	CAFF	**PLA**
Pre treatment	6.50 ± 0.65	6.50 ± 0.78
Pre exercise	6.64 ± 0.66	6.67 ± 1.16
Post exercise	6.54 ±1.00	6.48 ± 0.62
1 h post exercise	6.74 ± 1.30	6.36 ± 0.56
24h post treatment	6.60 ± 0.59	6.67 ± 0.84
48h post treatment	6.62 ± 0.94	6.69 ± 0.85

4.8 Sleep data

Actigraphy and Leeds sleep evaluation questionnaire (LSEQ) data was averaged for the 5 days following each specific treatment (CAFF or PLA). Ease of getting to sleep decreased during the CAFF trial compared to PLA (CAFF 49.4 ± 16.1 vs. PLA 54.5 ± 14.1; P = 0.014; Table 4.4). No effect of caffeine ingestion was found on the remainder of LSEQ measures; perceived quality of sleep (P = 0.358), ease of awakening following sleep (P = 0.790), and behaviour following sleep (P = 0.457) were similar between trials.

Table 4.4 – Mean ± standard deviation LSEQ questions; ease of getting to sleep (EGTS), perceived quality of sleep (PQS), ease of awakening from sleep (EAFS), behaviour following awakening (BFA). 50% represents normality. Therefore higher scores would imply that it is easier to fall asleep (EGTS), better quality of sleep (PQS), easier to wake after sleep (EAFS) and feel better after awakening (BFA).

Sleep measure	**CAFF**	**PLA**
Ease of getting to sleep (%)	49.4 ± 16.1	54.5 ± 14.1
Perceived quality of sleep (%)	48.7 ± 19.0	51.1 ± 16.7
Ease of awakening following sleep (%)	45.3 ± 16.4	44.7 ± 18.4
Behaviour following awakening (%)	45.0 ± 12.7	46.5 ± 12.7

Actigraph data showed a trend for decreased time spent in bed following caffeine ingestion compared to placebo (P = 0.085; Table 4.5). However, no other actigraph measure was significantly affected by caffeine ingestion; total sleep time (P = 0.119), onset latency (P = 0.689), and sleep efficiency (P = 0.992) were similar between trials.

Table 4.5 – Mean ± standard deviation of actigraphy data after caffeine and placebo ingestion. Time in bed is the number of hours likely spent in bed asleep or awake. Total sleep time is the numbers of hours the participants were estimated to be asleep. Onset latency represents the time taken to fall asleep. Sleep efficiency represents a percentage of the total time asleep to the total time in bed. Time in bed, total sleep time and onset latency are presented in decimal hours and minutes.

Actigraph measure	**CAFF**	**PLA**
Time in bed (h)	7.8 ± 1.3	8.2 ± 0.3
Total sleep time (h)	6.4 ± 1.2	6.6 ± 1.2
Onset Latency (min)	15.3 ± 27.4	13.8 ± 17.6
Sleep Efficiency (%)	81.9 ± 8.9	82.0 ± 8.2

4.9 Blood metabolites

There were minimal levels of caffeine in the blood prior to ingestion in both trials (CAFF 0.38 ± 0.74 $\mu g \cdot ml^{-1}$ vs. PLA 0.18 ± 0.46 $\mu g \cdot ml^{-1}$; P = 0.255). Plasma caffeine concentration increased following caffeine ingestion compared to placebo (P < 0.001; $\eta^2 = 0.98$; Fig. 4.15) and increased over time (treatment x time interaction; P < 0.001; $\eta^2 = 0.898$). Post-hoc analysis indicated significant differences in plasma caffeine concentration between CAFF and PLA trials at 5-km, immediately post-exercise and 1-h post-exercise time points.

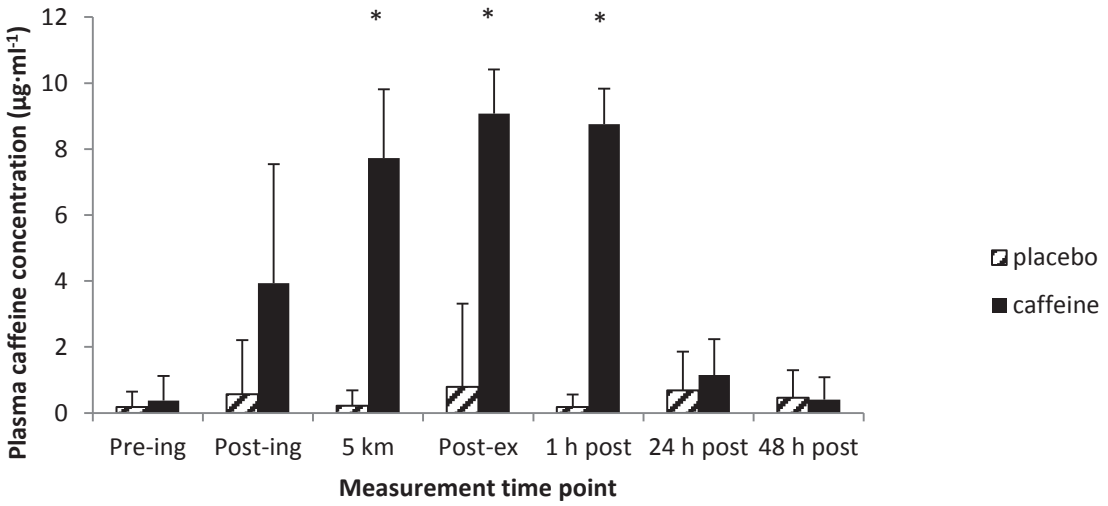

Figure 4.15 – Plasma caffeine concentration during CAFF and PLA ingestion trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-ing' represents the time point immediately before exercise (1h after caffeine/placebo ingestion); '5 km' represents the time point at the completion of the first 5 km of the 10 km time trial (half way); 'Post-ex' represents the time point immediately after completion of 10 km time trial; '1 h post' represents the time point 1 hour after the completion of the 10 km run; '24 h post' and '48 h post' represents the time point 24 and 48 hours after caffeine/placebo ingestion. Asterisks (*) indicate significant differences in plasma caffeine concentration between CAFF and PLA trials at specific time points (P < 0.05).

Plasma paraxanthine concentrations were not different at the start of the trial (P = 0.289). Paraxanthine concentration increased following caffeine ingestion compared to placebo (P < 0.001; η^2 = 0.896; Fig. 4.16) and increased over time (treatment x time; P = 0.016; η^2 = 0.462). Post-hoc analysis revealed significant differences in plasma paraxanthine concentration between CAFF and PLA trials at post-ingestion, 5 km, immediately post-exercise, and 1 h post-exercise time points (P < 0.05).

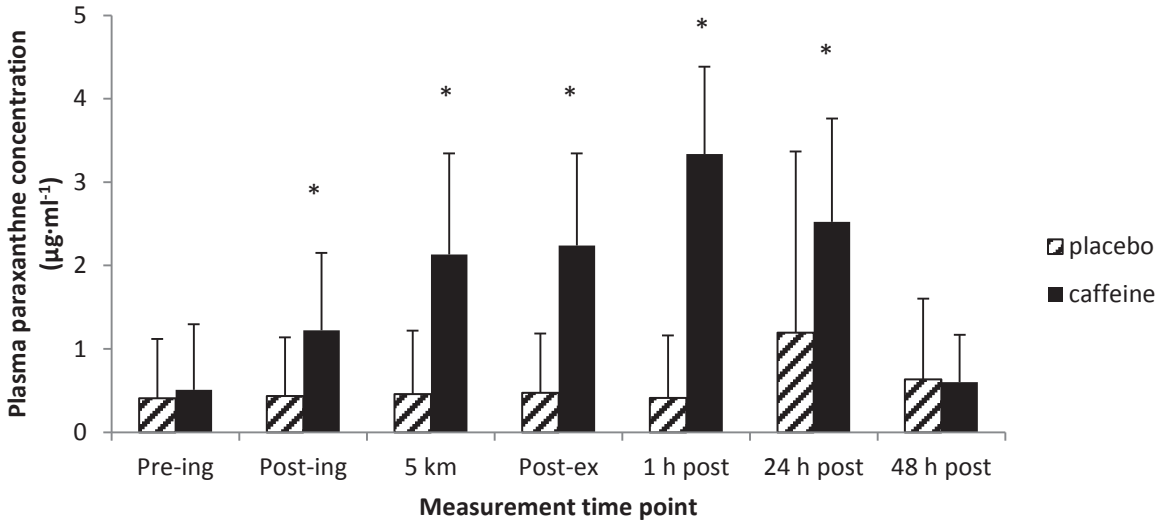

Figure 4.16 – Plasma paraxanthine concentration during CAFF and PLA ingestion trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-ing' represents the time point immediately before exercise (1h after caffeine/placebo ingestion); '5 km' represents the time point at the completion of the first 5 km of the 10 km time trial (half way); 'Post-ex' represents the time point immediately after completion of 10 km time trial; '1 h post' represents the time point 1 hour after the completion of the 10 km run; '24 h post' and '48 h post' represents the time point 24 and 48 hours after caffeine/placebo ingestion. Asterisks (*) indicate significant differences in plasma paraxanthine concentration between CAFF and PLA trials at specific time points (P < 0.05).

Plasma theobromine concentrations were not different at the start of the trial (P = 0.360). A trend was observed of higher plasma theobromine concentrations in the CAFF trial compared to PLA (P = 0.087; η^2 = 0.361; Fig. 4.17). However, plasma theobromine concentration did not change significantly over time (P = 0.478; η^2 = 0.107). No interaction effect of treatment x time was seen (P = 0.290; η^2 = 0.160).

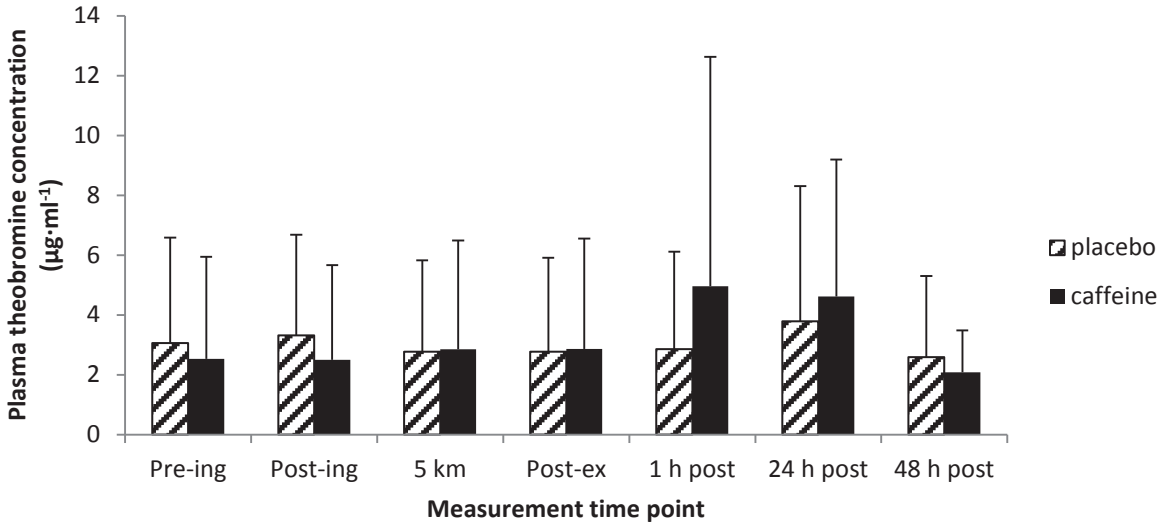

Figure 4.17 – Plasma theobromine concentration during CAFF and PLA trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-ing' represents the time point immediately before exercise (1h after caffeine/placebo ingestion); '5 km' represents the time point at the completion of the first 5 km of the 10 km time trial (half way); 'Post-ex' represents the time point immediately after completion of 10 km time trial; '1 h post' represents the time point 1 hour after the completion of the 10 km run; '24 h post' and '48 h post' represents the time point 24 and 48 hours after caffeine/placebo ingestion.

Plasma theophylline concentrations were not different at the start of the trial (P=0.246). Plasma theophylline concentration increased following caffeine ingestion ($P < 0.001$; $\eta^2 = 0.931$; Fig. 4.18) and increased over time (interaction of treatment x time; $P < 0.001$; $\eta^2 = 0.657$). Post-hoc analysis revealed significant differences in plasma theophylline concentration between CAFF and PLA trials at post-ingestion, 5-km, immediately post-exercise, 1 hour post-exercise and 24 hours post-ingestion time points ($P < 0.05$).

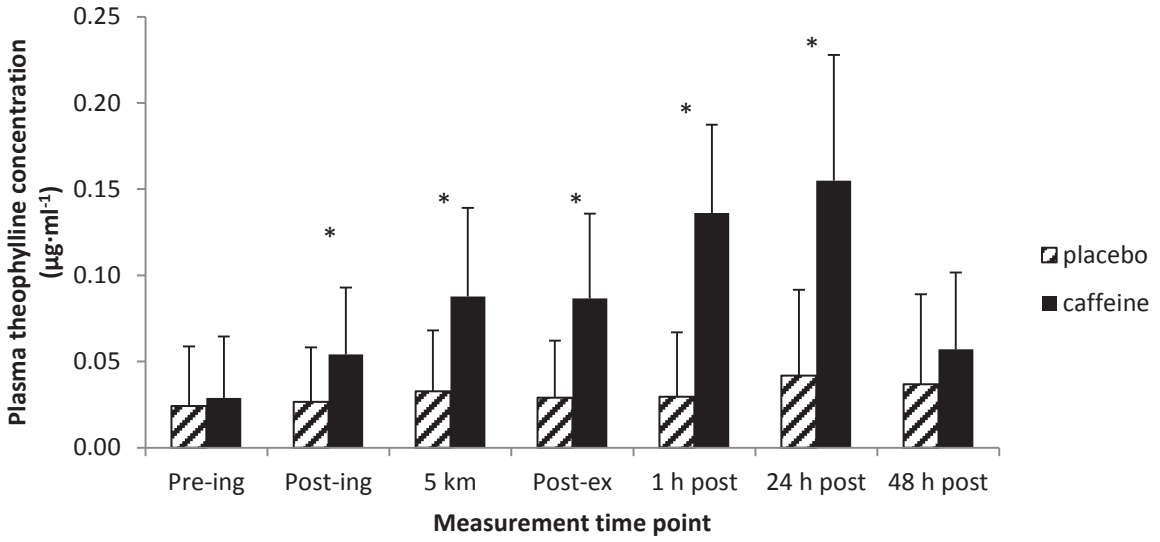

Figure 4.18 – Plasma theophylline concentration during CAFF and PLA trials. 'Pre-ing' represents the time point immediately before caffeine/placebo ingestion; 'Post-ing' represents the time point immediately before exercise (1h after caffeine/placebo ingestion); '5 km' represents the time point at the completion of the first 5 km of the 10 km time trial (half way); 'Post-ex' represents the time point immediately after completion of 10 km time trial; '1 h post' represents the time point 1 hour after the completion of the 10 km run; '24 h post' and '48 h post' represents the time point 24 and 48 hours after caffeine/placebo ingestion. Asterisks (*) indicate significant differences in plasma theophylline concentrations between CAFF and PLA trials at specific time points ($P < 0.05$).

Plasma caffeine, paraxanthine and theophylline concentrations for each individual are displayed in Figure 4.19. For most participants their plasma caffeine concentration peaked at the end of the 10-km run (93 - 121 min; Fig. 4.19 A). However, several participants peaked earlier during the 5-km range (67 - 82 min) and 2 participants peaked at 50 min (immediately before the time trial commenced). Plasma paraxanthine and theophylline concentrations (Fig. 4.19 B and C) show a slow increase trend over the first 175 min, and rapidly decline from 24 to 48 h post-ingestion.

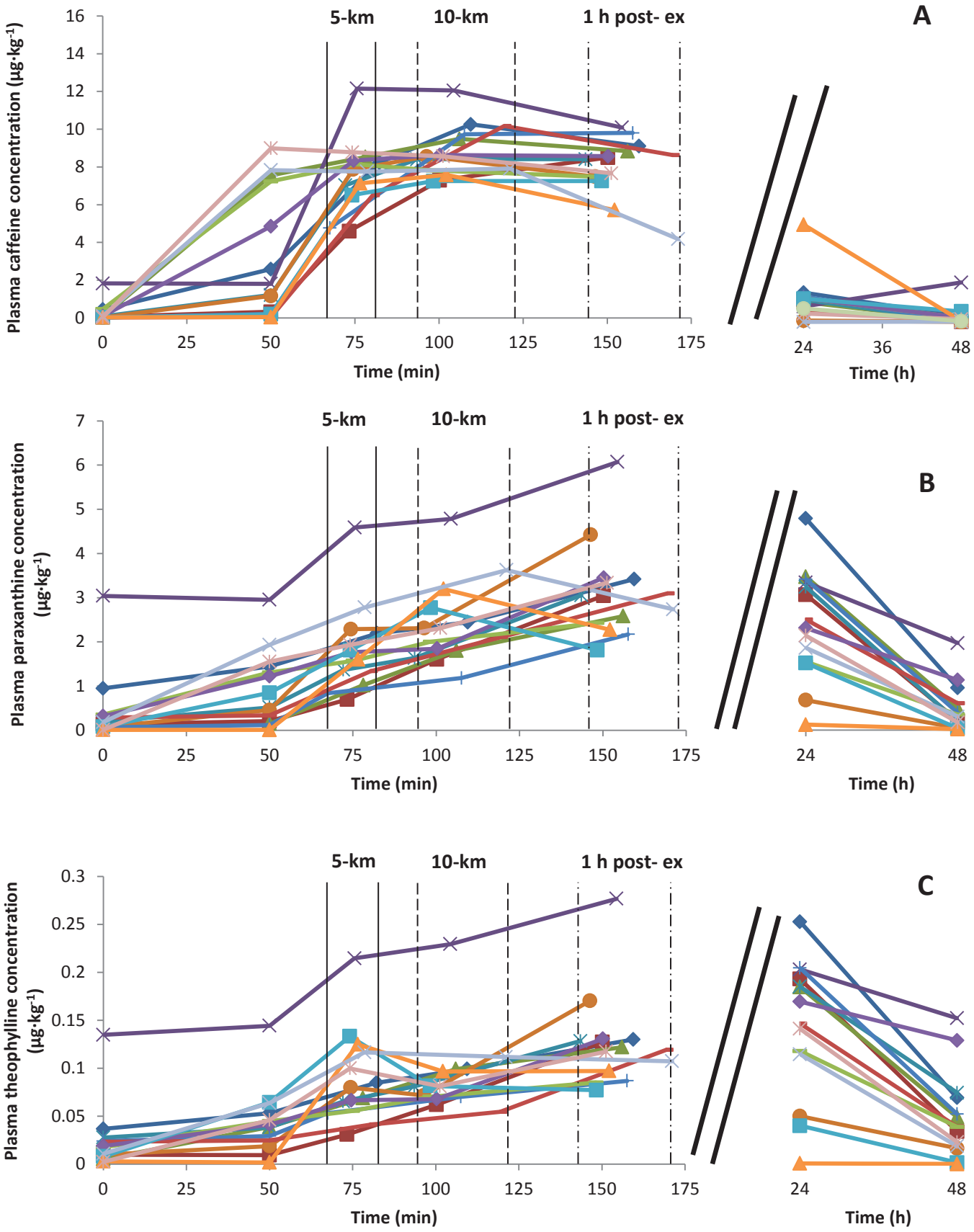

Figure 4.19 – Individual plasma A) caffeine, B) paraxanthine and C) theophylline concentration over the duration of the caffeine trial. The solid vertical lines represent the range of 5-km run times during the time trial, the vertical dashed lines represent the range of 10-km run times during the time trial, and the vertical dotted dashed lines represent the range of 1-h post-ex times. The x-axis of the graph represents time following caffeine ingestion and ranges from caffeine ingestion (0 min) to 1 h post-ex (175 min post caffeine ingestion), 24 and 48 h post caffeine ingestion.

CHAPTER 5 - DISCUSSION

The original aim of this study was to compare the ergogenic effects of caffeine on endurance performance between participants with different CYP1A2 genotypes. However, as insufficient numbers of homozygous A/A carriers volunteered for this study, comparisons between genotypes could not be made. Therefore, the results of this study describe responses to caffeine in slow metabolisers (heterozygous A/C genotype) only.

5.1 Kinetics of caffeine and caffeine metabolites

Caffeine ingestion led to significant increases in plasma caffeine, paraxanthine and theophylline concentrations, but not plasma theobromine concentration. Plasma caffeine levels were minimal prior to ingestion of caffeine/placebo, reflecting abstention of caffeine by the participants for 3 days prior to the trial. Plasma caffeine significantly increased during the caffeine trial compared to the placebo which also indicates that the treatment (caffeine ingestion) was successful. A single dose oral ingestion of caffeine typically causes a peak in plasma caffeine concentration approximately 1 h after ingestion (Bonati et al., 1982). However, for most participants in the present study a peak in plasma caffeine concentration occurred between 110 - 150 min after caffeine ingestion (Fig. 4.19 A). This indicates that caffeine would have peaked for many participants near the completion of the 10-km run or possibly even after completion of the run. This difference in plasma caffeine concentration peak time may be due to all the participants possessing the A/C CYP1A2 genotype, which has been shown to elicit a slower metabolism of caffeine compared to the faster A/A configuration of the CYP1A2 gene (Yang et al., 2010). However, a few of the participants had peak plasma caffeine concentrations between 50 and 90 min, which shows the large variation in caffeine metabolism despite having the same CYP1A2 genotype. Other factors may have influenced the rate of caffeine absorption such as gastric emptying, as the volume of food ingested, and the time at which it was ingested prior to the trial may impact the rate of caffeine absorption (Arnuad, 1987). To the author's knowledge, no study has clearly demonstrated kinetic profiles of caffeine's breakdown into

its metabolites across multiple genotypes. Therefore, more evidence from the individuals with various genotypes (CYP1A2 and ADOR2A) is needed to clearly identify the influence genetic variation has on caffeine metabolism.

Paraxanthine and theophylline concentrations increased following caffeine ingestion in a linear fashion reflecting the breakdown of caffeine into its metabolites. It is likely that plasma paraxanthine and theophylline concentrations would have peaked later than 1 h post-ex given the elevated levels of these metabolites observed 24 h post caffeine ingestion. A trend of increased plasma theobromine concentration was observed in the caffeine trial compared to placebo ($P = 0.087$) and peaked ~3 h post-ingestion (1 h post-exercise). Plasma caffeine, paraxanthine and theobromine concentrations all returned to baseline by 48 h post ingestion, however, theophylline still remained elevated 48 h post ingestion ($P = 0.034$). Therefore, any performance improvements in 10-km run time, leg strength (eccentric torque), leg power (squat jump height) and reaction time (DV reaction time) would likely be due to the effects of caffeine and not its metabolites as the times at which the metabolites peaked were well after the post-exercise measures had been completed. There were no improvements in any measures performed at 24 and 48 h post caffeine ingestion, which indicates that the caffeine metabolites were not present in sufficient quantities to elicit any significant improvements in performance, cognition, and mood or alter perceptual responses. This is supported by a statement which suggests that the quantities of theophylline and theobromine in commercially available products are unlikely to have an ergogenic effect (Pearce, 2012), and paraxanthine has yet to be shown to be ergogenic.

Variations in the CYP1A2 gene would likely have a profound effect on the rate of caffeine metabolite appearance. A faster metabolism of caffeine such as that seen in individuals with the homozygous A/A CYP1A2 polymorphism would likely lead to a faster rate of metabolite appearance and ultimately a faster decline in plasma caffeine concentration. However, it is not yet known how much the variation in the CYP1A2 gene will affect the rate of caffeine metabolism. Therefore future studies should seek to create a more comprehensive pharmacokinetic profile of caffeine and its metabolites paraxanthine, theophylline and theobromine over several days, with measures performed

at regular intervals after an acute single dose of caffeine in individuals with different CYP1A2 genotypes. The results of such a study would provide important information on the most relevant caffeine dose timings relative to performance in individuals with different CYP1A2 genotypes, potentially leading to genotype specific caffeine dosage timings. Faster metabolisers of caffeine would ingest the caffeine dose closer to the time of the exercise activity in order to elicit the greatest ergogenic effect during exercise, while slow metabolisers of caffeine would ingest caffeine earlier from the time of the exercise activity. Little work exists on the effect of caffeine's metabolites on exercise performance, and this should be further investigated, to determine at what levels paraxanthine, theophylline and theobromine become ergogenic and whether those levels can be achieved through a reasonable dose of caffeine.

5.2 10-km time trial run

Run times after the first 5-km were not different between CAFF and PLA trials (P = 0.589, Fig. 4.1), which is not unexpected given that plasma caffeine concentrations had not yet peaked in most participants (Fig. 4.19 A). In addition, as 5-km was not the end goal of the trial, participants may have remained running at a comfortable pace during the first half of the 10-km run in anticipation of speeding up over the last 5 km (i.e. a 'negative split'). Using Cohen's d, it can be seen that caffeine is likely to have a larger effect on run times during the second half of the 10-km time trial compared to the first 5-km (0-5 km d = 0.023 vs. 5-10 km d = 0.41). There was no significant improvement in 10-km run times in participants following caffeine ingestion, however, this may in part have been due to the large variation in run times (39.15 – 71.11 min). In support of this, analysis of the run times of the 8 fastest participants between both CAFF and PLA trials showed a significant improvement in performance following caffeine ingestion (P = 0.002). It was observed that 11 of the 14 participants had faster run times during the CAFF trial compared to PLA, and an overall improvement of 3.8% on endurance performance in the CAFF trial compared to PLA. In order to maximise the opportunity to observe the ergogenic effects of caffeine future trials should seek to use athletes who are capable of

completing a 10-km time trial within a consistent narrow time frame (i.e. 40 – 50 min), recruit a larger sample size and possibly ingest caffeine earlier relative to the start of exercise.

Heart rate increased over the duration of the 10-km time trial, but showed a trend of further elevation in the CAFF trial compared to PLA (P = 0.062). Additionally, there was no correlation between heart rate and run times (r = -0.169, P =0.56). This may suggest that the effect of caffeine, rather than an increase in work rate, is the likely cause of the additional increase in heart rate during exercise. The trend of higher heart rate in the CAFF trial compared to PLA may have been more pronounced if caffeine was ingested earlier relative to the start of exercise and thus plasma caffeine concentration would have likely peaked during the 10-km run. It is generally accepted that caffeine will lead to increases in heart rate during endurance performance (Bell and McLellan, 2002). However, few studies have directly investigated the effects of caffeine on heart rate (Graham, 2001). Womack et al. (2012) found a significant increase in heart rate during endurance exercise 1 h after caffeine consumption compared to placebo in trained cyclists, but the difference was not CYP1A2 genotype dependent. Heart rate before and after exercise in the present study were not significantly different (P = 0.198) and most studies have not seen any changes to heart rate during rest in healthy individuals following caffeine ingestion (McClaran and Wetter, 2007; Ruah et al., 2006). Currently there is little evidence to suggest that CYP1A2 genotype influences heart rate following caffeine ingestion, therefore, more thorough investigation of the effects of caffeine on heart rate should be conducted which also takes into account caffeine habituation of individuals as well as potential genes which may influence heart rate responses to caffeine such as CYP1A2 and ADORA2A.

5.3 Leg strength

Evidence for the ergogenic effects of caffeine on strength and power activities or short-term high-intensity exercise is currently equivocal (Astorino and Roberson, 2008). In the present study, eccentric leg strength was increased following caffeine ingestion compared to placebo (P = 0.015; Fig. 4.5). However, only a trend was observed of increased concentric leg strength (P = 0.099;

Fig. 4.4). This is supported by a meta-analysis which concluded that caffeine ingestion improves maximal voluntary contraction strength in knee extensor muscles (Warren et al., 2010). Astorino et al. (2010) found an improvement in knee flexion but not knee extension following (5 $mg \cdot kg^{-1}$) caffeine ingestion. It would be expected that the knee extensors would show an improvement if caffeine has a direct effect on the muscles, as has been previously suggested (Graham, 2001). In the present study, peak torque during eccentric contractions was maintained following exercise in the caffeine trial (Figure 4.5) which may be partly due to attenuation of pain perception (Hudson et al., 2008; Duncan et al., 2013) as it has been shown that muscle pain reduces torque (Graven-Nielsen et al., 2002). However, studies such as that by Graven and Nielsen used experimentally induced pain which may have more profound effects on muscle force production compared to naturally occurring pain as a result of exercise. Hudson et al. (2008) and Duncan et al. (2013) reported that caffeine intake attenuates muscle pain perception and suggested that this may lead to improvements in peak torque and average torque during resistance exercise. These studies used higher repetitions of resistance exercise to induce muscular pain, whereas in the present study incidental pain may have occurred as a result of the 10-km run, but pain was not specifically induced.

To the author's knowledge no study has investigated the effects of genotype of caffeine related genes on strength in individuals following caffeine ingestion. It has been shown that anxiety exacerbates pain perception (Tang and Gibson, 2005) and polymorphisms of ADORA2A have been linked to increased anxiety following caffeine ingestion (Childs et al., 2008), therefore individuals with a particular ADORA2A polymorphism may experience increased pain during exercise following caffeine ingestion, potentially decreasing exercise performance. Future studies should therefore investigate the effect of genotype on pain attenuation during exercise following caffeine ingestion.

5.4 Leg power

Squat jump height and countermovement jump height increased by an average of 4.1% and 2.8%, respectively across all time points, following caffeine ingestion (Fig. 4.6 and Fig 4.7). This is

similar to previous studies that found increases of 2.7% (Foskett et al., 2009) and 2.3% (Gant et al., 2010) in countermovement jump height. The largest differences in countermovement jump height between CAFF and PLA trials were found immediately after the 10-km run, which coincides with the peak in plasma caffeine concentration. However, as there was no warm up before any of the other jumps it is possible that there was a warm up effect on jump height following the 10-km run. Vertical jump height has been shown to strongly correlate with sprint speed and leg power (Wisloff et al., 2004). This suggests that caffeine-induced improvements in vertical jump height could potentially lead to improvements in sprint ability during team sports. A recent study (Del Coso et al., 2012) reported increased repeated sprint ability, increased distance covered during a high intensity simulated football game, and improved vertical jump height following caffeine ingestion. Therefore, the present study supports current literature which suggests that caffeine ingestion may lead to improvements in vertical jump height which is likely to translate into improved sprint ability and leg power. It should be noted that the studies mentioned (Foskett et al., 2009; Gant et al., 2010) did not undertake genetic testing for genes mediating caffeine metabolism and sensitivity such as CYP1A2 and ADORA2A. Therefore, the genetics of their population sample may have influenced the results as plasma caffeine concentration may not have peaked in slow metabolisers as caffeine was ingested 1 h prior to testing, or plasma caffeine concentration may have declined by the end of the 90 min testing protocol in fast metabolisers. The extent to which these genes affect short-term high-intensity performance is not yet fully understood and should be a focus of future research.

5.5 Perceptual responses

While there was no significant effect of caffeine ingestion on feeling scale (FS), there was a significant treatment x time interaction ($P = 0.036$). Post-hoc analysis indicated that a significant difference between CAFF and PLA trials was found at the 2.5-km mark ($P < 0.05$; Fig 4.8). A treatment x time trend was also seen in felt arousal scale (FAS; $P = 0.081$; Fig. 4.9) but post-hoc analysis revealed no significantly different time points. However, mean FAS in the CAFF trial was greater than the PLA trial at all-time points apart from 24 and 48 h post ingestion. Plasma caffeine

concentration peaked at the completion of the 10-km time trial which coincided with a peak in FAS and a strong correlation between plasma caffeine concentration and FAS was observed ($r = 0.838$; $P = 0.018$). However, a non-significant moderate correlation was seen between caffeine concentration and FS ($r = 0.43$, $P = 0.529$). It is likely that part of the correlation between FAS and plasma caffeine concentration was confounded by the time trial run, as FAS has been shown to increase with an increase exercise duration and intensity and FS decreases with an increase in exercise duration and intensity (Ekkekakis et al., 2008).

Previous studies have mostly used constant-pace exercise protocols when investigating the effects of caffeine intake on perceptual responses (Backhouse et al., 2011). During submaximal constant-pace endurance, exercise ratings of pleasure/displeasure and activation are maintained throughout the activity (Backhouse et al., 2011). However, this is not the case in most real world sports as intensity varies throughout the duration of the event. Even though constant-pace exercise makes up the largest portion of endurance running, most long distance events are characterised by multiple changes of pace and intensity throughout the event (Garcin et al., 2008). The circumplex model (Russell, 1980) maps FS and FAS scores as Cartesian co-ordinates, and this was used to demonstrate the effect of caffeine on activation and pleasure-displeasure during exercise. Use of the circumplex model showed that participants remained in the 'high activation, pleasure' quadrant of the model for a greater duration during the caffeine trial compared to the placebo (Fig. 4.10). However, at the cessation of exercise, participants in both CAFF and PLA trials were in the 'high activation, displeasure' quadrant (Fig. 4.10). Therefore, results of the present study support the current literature which suggests that caffeine ingestion may lead to changes in affect, thus causing an ergogenic effect leading to enhanced endurance performance.

No difference in RPE was observed between trials ($P = 0.309$). This is similar to previous studies using a time trial protocol to investigate the ergogenic effects of caffeine (Irwin et al., 2011). A shorter overall run time by most participants in the caffeine trial, coupled with no change in RPE suggests that participants were able to work at a higher absolute work load for a given rate of exertion. This provides evidence for the theory that alterations in the perception of effort results in the

ergogenic effect of caffeine (Doherty and Smith, 2005). However, it is unclear whether genetics affect RPE, activation or pleasure/displeasure as only one study (Womack et al., 2012) has previously used RPE as a measure of perceived exertion following caffeine ingestion during endurance exercise and genotyped participants for their CYP1A2 gene. The study by Womack et al. (2012) found no differences in RPE during a 40-km cycle time trial between genotypes or between caffeine and placebo trials. Therefore, this should be a point of interest for future studies to investigate differences in affect and perceptual responses following caffeine ingestion amongst the various genotypes.

5.6 Mood

Overall POMS ratings were not different between trials (P = 0.42). However, caffeine decreased fatigue ratings immediately before and after exercise, but was similar 24 and 48 h post caffeine ingestion (Fig. 4.11). This supports previous findings that caffeine ingestion reduces feelings of fatigue and improves alertness (Lieberman et al, 2002). Similarly, caffeine ingestion also increased the vigour subset of POMS before and immediately after exercise (Fig. 4.12). However, similar ratings of fatigue and vigour during the subsequent days indicate that the effects of caffeine on mood lasted only during the day that it was ingested and did not carry over to the subsequent days (Fig. 4.11 and 4.12). The positive effect of caffeine on aspects of mood is supported by decreased fatigue ratings in the CAFF trial compared to PLA when plasma caffeine concentrations were high (post-ex) and similar fatigue ratings between CAFF and PLA when plasma caffeine levels were low (24 and 48 h post-ingestion). Most studies that have investigated mood state following caffeine ingestion have predominantly examined post-exercise mood state (Green et al., 2007). However, more recent studies (Duncan et al., 2012) have used both pre- and post-exercise mood state measures following caffeine ingestion. Similar to the present study, Duncan et al. (2012) found an increase in vigour and a decrease in fatigue subscales of the Brunel Mood States Inventory (BRUM) both before and after exercise. Duncan et al. (2012) concluded that an increase in vigour and a decrease in fatigue is most likely due to caffeine ingestion and not exercise per se, since vigour scores decreased after exercise for both caffeine and placebo trials, while vigour scores increased pre-exercise in the caffeine trial and

decreased in the placebo trial. These results support previous findings that caffeine intake leads to improvements in mood before and after exercise (Walsh et al., 2010).

It has been stated that mood responses to caffeine may be somewhat affected by habituation, as some individuals may feel worse or better depending on the amount of caffeine they ingest in comparison to their usual intake (Roache and Griffiths, 1987; Loke, 1988; Haskell et al., 2005). Some studies (Richardson et al., 1995; Juliano and Griffiths, 2004; Rogers, 2007) have reported caffeine habituation can lead to fatiguing effects due to acute caffeine withdrawal or 'hangover' effects and caffeine ingestion may only reverse the withdrawal but not lead to any improvements in cognition and mood. In the present study, 10 of the 14 participants were moderate or high consumers of caffeine (>1 caffeinated beverage per day). Therefore, some participants in the present study may have begun trials in a 'hungover' state due to caffeine abstinence before trials, and thus any improvements seen following caffeine ingestion may have been due to a reversal of this state. Future research is still needed to fully understand how mood-related changes occur as a result of caffeine ingestion and whether these mood responses are influenced by genetics or habituation and withdrawal. Future research should also investigate whether caffeine related genes influence the length and severity of caffeine withdrawal symptoms.

5.7 Cognition

Digit vigilance (DV) accuracy was not affected by caffeine ingestion (P = 0.441), but there was a trend of improved rapid visual information processing (RVIP) accuracy following caffeine ingestion compared to placebo (P = 0.072). Faster reaction times for both DV and RVIP tests were found following caffeine ingestion compared to placebo in the present study (Fig. 4.13 and Fig. 4.14). Haskell et al. (2005) also found decreases in reaction time in DV but not RVIP in healthy adults; however, they did report increases in DV accuracy which was not seen in the present study. Much smaller doses of caffeine (75 mg and 150 mg) were used by Haskell et al. (2005) which may explain some of the variation in results between the studies. A review (Nehlig, 2010) into caffeine and

cognition reported equivocal findings on cognitive ability following caffeine ingestion, and improvements in cognition and reaction times are most notable when caffeine is taken in sub-optimal alertness conditions. This has been observed previously in sleep-deprived or fatigued individuals who showed improvements in cognitive ability and vigilance following caffeine ingestion (Lieberman et al., 2002). Therefore, caffeine ingestion may lead to more noticeable improvements in cognition among fatigued individuals and thus may be of greater importance in long-duration events where mental fatigue is more prominent. In addition, no studies to the author's knowledge have yet investigated the effects of genetics on cognition following caffeine ingestion. Results of the present study are from 'slow metabolisers' of caffeine, and it is not known whether 'fast metabolisers' of caffeine would have a significantly different cognitive response to caffeine ingestion. Therefore, future research should investigate whether caffeine improves cognition in both non-fatigued and fatigued individuals, as well as determine if genetics related to caffeine metabolism influence cognition following caffeine ingestion.

5.8 Sleep data

Sleep data compiled from the Leeds sleep evaluation questionnaire (LSEQ) and actigraphs showed little effect of caffeine on sleep quality in heterozygous A/C individuals (Table 4.4 and Table 4.5). In the present study ease of getting to sleep was found to be more difficult following caffeine ingestion, but no other effects were found on perceived quality of sleep, the ease of awakening after sleep or behaviour following awakening (Table 4.4). A trend for less time spent in bed was observed in the CAFF trial compared to PLA ($P = 0.085$; Table 4.5). However, there were no other effects of caffeine on total sleep time, sleep efficiency or onset of sleep. This is in contrast to many studies which have shown disturbances to sleep following caffeine ingestion (Roehrs and Roth, 2008; Snel and Lorist, 2011).

One reason for the contrasting results may be the time at which caffeine was consumed by participants, as there was no control for when a participant took part in the current trial. Therefore

some participants would have ingested caffeine earlier during the day compared to others, who would have consumed it in the late afternoon. It has been shown that timing of caffeine ingestion may change the degree to which caffeine disturbs sleep. One study (Nicholson and Stone, 1980) found caffeine did not affect onset to sleep when taken at 'lights out', however, others have shown that caffeine taken before sleep reduces total sleep time and increases onset to sleep (Karacan et al., 1976; Okuma et al., 1982). This has implications if athletes use caffeine as an ergogenic aid to improve physical and mental performance during a training session, but then may experience sleep disturbances which could potentially lead to a worse performance during subsequent days (Sokmen et al., 2008). Sleep disturbances have also been shown to decrease technical skill and decision-making during sports (Pilcher and Huffcutt, 1996).

One of the few studies (Retey et al., 2007) which has taken genetics into account when investigating the effects of caffeine on sleep quality, found that a common variation in ADORA2A (1083 T>C) contributed to subjective and objective responses to caffeine on sleep. Individuals with the C/C genotype are particularly susceptible to sleep disturbances following caffeine ingestion. This demonstrates the potential for variations in key genes to significantly affect responses to caffeine and sleep quality. Therefore, it may be recommended that caffeine should be used on the day of an event or important training session but should not be used the day before a key event because of the potential negative impacts on sleep. Further research should investigate whether CYP1A2 or ADORA2A genotypes attenuate or exacerbate the sleep disturbances caused by caffeine ingestion.

5.9 Conclusions

- Acute moderate (6 $mg \cdot kg^{-1}$) doses of caffeine did not lead to significant improvements in endurance performance in adult male athletes with the A/C CYP1A2 genotype. However, 11 of 14 participants did have faster run times following caffeine ingestion compared to placebo.

- A trend of increased concentric knee extensor torque and significantly increased eccentric knee extensor torque was observed following caffeine ingestion in adult male athletes with the A/C CYP1A2 genotype.
- An increase in squat jump height, but not countermovement jump height was found following caffeine ingestion in adult male athletes with the A/C CYP1A2 genotype.
- Perceptual responses were not significantly affected by caffeine ingestion before or after exercise in adult male athletes with the A/C CYP1A2 genotype.
- Mood was not significantly affected by caffeine ingestion before or after exercise in adult male athletes with the CYP1A2 genotype.
- Reaction times, but not accuracy of cognitive tasks was improved following caffeine ingestion compared to placebo in adult male athletes with the A/C CYP1A2 genotype.
- Caffeine ingestion did not significantly affect sleep quality in adult male athletes with the A/C CYP1A2 genotype.
- No 'hangover' effects on sleep, mood or perceptual responses were observed 24 and 48 h post-caffeine ingestion.

5.10 Limitations

- Due to low number of participants with the homozygous A/A genotype, comparisons could not be made between genotypes of the CYP1A2 genes (A/A and A/C).
- A larger sample size would have more likely resulted in statistically significant results in endurance performance, sleep quality and cognitive data.
- Training status was not controlled for and is likely to have increased variation in run times, and therefore may reduce the potential for significance of the endurance performance results.
- Time between caffeine ingestion and sleep was not controlled for, which could alter the effects of caffeine on sleep quality.

- Running speed had to be manually changed, which may have potentially dampened running speed compared to running free at a completely natural pace.

5.11 Future directions

- A similar protocol to the present study should be repeated with CYP1A2 homozygous A/A and homozygous C/C individuals to elucidate the role genotype plays on the ergogenic effects of caffeine during endurance exercise.
- Determine percentages of CYP1A2 and ADORA2A genotypes in recreational and elite athlete populations.
- Investigate the ergogenic effects of caffeine metabolism and caffeine sensitivity related genes such as CYP1A2 and ADORA2A and others during endurance exercise.
- Further research is needed to determine whether caffeine is ergogenic during short duration high intensity exercise.
- Further research is needed to clarify mechanisms of action of caffeine when used as an ergogenic aid for endurance exercise.

Based on the results of this study, adult male athletes with the A/C CYP1A2 genotype will likely receive an ergogenic effect from ingesting caffeine, particularly during endurance exercise. In this group, caffeine should be ingested approximately 2 hours before the start of exercise to maximise the potential ergogenic effect of caffeine.

CHAPTER 6 - REFERENCES

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CHAPTER 7 - APPENDICES

Appendix 1 – Ethical approval

Appendix 2 – Participant information sheet

Appendix 3 – Participant consent form

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Appendix 5 – Participant screening questionnaire

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APPENDIX 1

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA

COPY FOR YOUR INFORMATION

15 April 2015

Kyle Southward
10 Elsfield Place
Torbay
AUCKLAND 0630

Dear Kyle

Re: HEC: Southern A Application – 15/12
The effects of genetics and caffeine ingestion on exercise performance, mood, sleep and immune function in male athletes

Thank you for your letter dated 14 April 2015.

On behalf of the Massey University Human Ethics Committee: Southern A I am pleased to advise you that the ethics of your application are now approved. Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Mr Jeremy Hubbard, Acting Chair
Massey University Human Ethics Committee: Southern A

cc Dr Ajmol Ali
School of Sport & Exercise
ALBANY

Dr Kay Rutherfurd-Markwick
School of Food & Nutrition
ALBANY

Prof Steve LaGrow, HoS
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APPENDIX 2

The effects of genetics and caffeine ingestion on exercise performance, mood, sleep and immune function in male athletes.

INFORMATION SHEET

Invitation to participate in research study

I, Kyle Southward, am a postgraduate student currently undertaking a thesis to complete a Master's degree in Sport and Exercise Science at Massey University under the supervision of Dr Ajmol Ali from the School of Sport and Exercise and Dr Kay Rutherfurd-Markwick from the School of Food and Nutrition. Dr Austen Ganley and Dr Karyn O'Keeffe will conduct the genetic assays and analyse the sleep data, respectively. Megan Wraith, Deepna Nathu, Frances Martin and Angela Tyrrell from Auckland University of Technology will be assisting with data collection.

Caffeine is the most consumed psychoactive drug in the world and has been shown to have a performance-enhancing effect when taken in the correct doses. However, the effects of caffeine intake vary between individuals and have been attributed to factors such as age, gender and more recently genetics. A gene called CYP1A2 has been linked with caffeine metabolism. This gene can occur naturally in three variations which affects the rate of caffeine metabolism. These three variations can be grouped into fast, medium or slow metabolisers of caffeine. The differences in the rate of caffeine metabolism are likely to influence exercise performance. Little research has investigated the effects of genetics and caffeine ingestion on exercise performance. This study aims to investigate the effects of genotype and caffeine ingestion on exercise performance as well as mood, sleep and immune function.

As a participant in this study you will stand to benefit from gaining knowledge of your fitness levels as well as how caffeine ingestion affects performance – both positively and negatively. This will allow you to determine if caffeine can lead to an improved exercise performance and possibly could be incorporated into your future training sessions.

Participant recruitment and involvement

Approximately 36 participants will be recruited for this study to provide sufficient statistical power.

To participate in this study you must be:

- Male.
- Between the ages of 18 – 50 years old.
- Able to run 10 km comfortably without stopping.
- Able to consume caffeine. (if you don't consume any caffeine you cannot take part in this study)
- A non-smoker.
- Pass health screening questionnaire

Risks/discomforts as a result of this study may include:

- Physical discomfort or muscle soreness as a result of exercise.
- Mild soreness as a result of blood sampling procedures.
- Possible impact on sleep due to caffeine consumption.

Before commencing this study you will be asked to complete a health screen questionnaire and a caffeine consumption questionnaire. If you have any medical condition listed on the health screen questionnaire then you will have to be excluded from the study. All information collected on these questionnaires is strictly confidential and will only be used for the purpose of this study.

Project procedures

Familiarisation

Before the main trials begin you will be asked to complete a familiarisation session. This is a full familiarisation session and will accustom you to the main trials. During the familiarisation session a sterile cotton bud will be used to take a swab from the inside of your cheek; this will be used to determine your CYP1A2 genotype. Participants will be placed in three different groups for the main trials based on their genotype. Height and weight will also be measured during this initial session.

You will also be shown how to complete the two-day food record diary prior to each main trial. You will be asked to record your food and beverage intake for the day before and day of the main trial; you will need to replicate the intake for the second main trial. Please refrain from consuming caffeine-containing foods and beverages (e.g. tea, coffee, energy drinks, and chocolate) during this period. Also, we ask that you come in for the main trial after observing a two-hour fast (only water intake allowed).

Furthermore, we will show you how to use the sleep diary and how to wear the Actigraph sleep monitor. We will ask you to wear the Actigraph (like a watch on your wrist) and complete the sleep diary for three days leading up to the main trial as well as three days after the 10-km time trial.

For all trials you will be asked to wear comfortable running apparel such as trainers, shorts and a shirt. Please bring a towel as showering facilities will be available.

Main trials

The main trials will consist of 2 x 3-h sessions. Each session will consist of a 10 km time trial run on a treadmill. In one of the trials you will be given a caffeine capsule (6 mg per kg body mass) and in the other you will be given a placebo (flour capsule). Base measures will be taken at the beginning of each session, followed by caffeine or placebo ingestion and pre-exercise measures. Pre and post-exercise measures include a blood sample, saliva sample, urine sample and blood pressure. Before and approximately 45 minutes after caffeine ingestion leg strength and jump height will be measured using an isokinetic dynamometer and jump mat. One hour after caffeine or placebo ingestion, you will begin the 10 km time trial. Throughout the trial expired heart rate will be continuously measured. At 2.5 km, 5 km, 7.5 km and 10 km a number of perceptual measures including ratings of perceived exertion (RPE), Felt Arousal Scale (FAS) and Feeling Scale (FS) measures will be taken, along with another blood sample at 5 km. One hour after exercise has ended another set of measures will be taken.

At three stages during the session (before caffeine ingestion, approximately 1 hour after caffeine ingestion, and 1 hour after exercise), you will be asked to complete a profile of moods states (POMS) questionnaire and a set of cognitive tests at the beginning of each session. You will also be asked to return one and two days after the main session so that another set of measures can be taken (blood, urine, saliva, POMS, cognitive and perceptual tests).

Individuals trained in resuscitation (NZ Red Cross First Aid, Level 2) and the use of a defibrillator will be present for all exercise sessions. In addition, the researchers will be constantly monitoring physiological and perceptual variables that will aid in identifying any issues.

Participant's Rights

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- decline to answer any particular question;
- withdraw from the study up until two weeks following the data collection;
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded.

Note: As a participant you can agree to receive your genetic information. Before agreeing to this you should be aware that under New Zealand law an insurance company could ask you to disclose such information should you apply for life or health related insurance – such as medical cover. You could be obliged to disclose it even if the insurer does not ask for it expressly. Not disclosing it could result in the insurer not having to pay out under the policy.

Data Management:

All data and materials collected will be used only for this study. Hard copies will be kept in a locked filing cabinet on the Massey University Albany Campus accessible only to the researchers. Soft copies will be stored on password-protected magnetic media accessible only to the researchers. All participants will be assigned a code that will be used when collecting and presenting data. The raw results data will be kept for 10 years under the control of the researchers. Dr Ajmol Ali or another relevant member of staff from School of Sport and Exercise, and will dispose of the collected biological samples after 5 years.

The samples (blood, urine and saliva) will be collected in 2015 and, due to the number of assays being conducted, will be stored in the -80°C freezer in the Sport and Exercise Science Laboratory (B60, Oteha Rohe Campus). Samples will be analysed over a 5-year period. Any remaining samples will be destroyed in 2020 (5 years after initial collection).

Project Contacts:

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Committee Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 15/12. If you have any concerns about the conduct of this research, please contact Mr Jeremy Hubbard, Acting Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63487, email humanethicsoutha@massey.ac.nz.

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

APPENDIX 3

The effects of genetics and caffeine ingestion on exercise performance, mood, sleep and immune function in male athletes.

PARTICIPANT CONSENT FORM - INDIVIDUAL

- I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.
- I understand that I have the right to withdraw from the study at any time and to decline to answer any particular questions.
- I agree to provide information to the researcher on the understanding that my name will not be used without my permission. (The information will be used only for this research and publications arising from this research project).
- I understand that any samples (urine, saliva, blood) collected from me will only be used for this study, and that samples may be analysed over the next five (5) years.
- I agree to submit genetic material to the researcher for use only in this study. (Genetic material will not be deposited into a gene data bank.)
- I understand that if my genetic information obtained by the researcher is disclosed to me, I may have to pass this information to an insurance company should I seek life or health-related insurance cover in the future. I understand that failure to disclose the information could invalidate my insurance policy
- I agree to participate in this study under the conditions set out in the Information Sheet.

Signature:_____ Date:_____

Full name (printed):_____

Phone number:_____ Date of Birth:_____

APPENDIX 4

Pre exercise health screening questionnaire

Name:_____

Address:_____

Phone:_____

Age:_____

Please read the following questions carefully. If you have any difficulty, please advise the medical practitioner, nurse or exercise specialist who is conducting the exercise test.

Please answer all the following questions by ticking only one box per question.

This questionnaire has been designed to identify the small number of persons (15-69 years of age) for whom physical activity might be inappropriate. The questions are based on the Physical Activity Readiness Questionnaire (PAR-Q), originally devised by the British Columbia Dept. of Health (Canada), as revised by [1]Thomas *et al.* (1992) and [2]Cardinal *et al.* (1996), and with added requirements of the Massey University Human Ethics Committee. The information provided by you on this form will be treated with the strictest confidentiality.

Qu 1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

Yes ☐ No ☐

Qu 2. Do you feel pain in your chest when you do physical activity?

Yes ☐ No ☐

Qu 3. In the past month have you had any chest pain or palpitations when you were not doing physical activity?

Yes ☐ No ☐

Qu 4. Do you lose your balance because of dizziness or do you ever lose consciousness?

Yes ☐ No ☐

Qu 5. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?

Yes ☐ No ☐

Qu 6. Do you have a bone or joint problem that could be made worse through vigorous exercise?

Yes ☐ No ☐

Qu 7. Do you know of any other reason why you should not do physical activity?

☐

Yes ☐ No

Qu 8. Do you have any immediate family members that had heart problems prior to the age of 60?

Yes ☐ No ☐

Qu 9. Have you been hospitalized recently?

Yes ☐ No ☐

Qu 10. Do you have any infectious disease that may be transmitted in blood?

Yes ☐ No ☐

Qu 11. This test may include the taking of blood for testing of various markers. Do you have any objection to this?

Yes ☐ No ☐

Qu 12. Have you ever been diagnosed with or suffered from sleep disorders?

Yes ☐ No ☐

Qu 13. Have you ever had any adverse reactions from consuming caffeine?

Yes ☐ No ☐

Qu 14. Do you smoke, or have you ever smoked?

Yes ☐ No ☐

Qu 15. Do you use an inhaler or corticosteroid cream?

Yes ☐ No ☐

Qu 16. Do you have celiac disease or gluten intolerance?

Yes ☐ No ☐

You should be aware that even amongst healthy persons who undertake regular physical activity there is a risk of sudden death during exercise. Though extremely rare, such cases can occur in people with an undiagnosed heart condition. If you have any reason to suspect that you may have a heart condition that will put you at risk during exercise, you should seek advice from a medical practitioner before undertaking an exercise test.

I have read, understood and completed this questionnaire.

Signature:_____ Date:_____

References

1. Thomas S, Reading J, and Shepard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Sport Sci* 17(4): 338-345.
2. Cardinal BJ, Esters J and Cardinal MK. Evaluation of the revised physical activity readiness questionnaire in older adults. *Med SCI Sport Exerc* 28(4): 468-472.

APPENDIX 5

The effects of genetics and caffeine ingestion on exercise performance, mood, sleep and immune function in male athletes.

Participant screening questionnaire.

Name:_____

Gender:_____

Age:_____

Address:_____

_____

Phone:_____

Email:_____

Qu 1. How often do you exercise per week?

Less than1h ☐ 1h – 3h ☐

3h - 6h ☐ more than 6h ☐

Qu 2. Are you able to run 10km at a comfortable pace without stopping?

Yes ☐ No ☐

Qu 3. How often do you drink tea? (times per week/day)

_____

Qu 4. How often do you drink coffee? (times per week/day)

_____

Qu 5. What type of tea or coffee do you most often consume? (Espresso, decaf, earl grey, etc…)

_____

Qu 6. How often do you consume energy drinks? (Red bull, Monster, etc…)

_____

Qu 7. How often do you consume soft drinks? (Coca-cola, pepsi etc…)

_____

Qu 8. Describe how caffeinated products normally affect you.

_____

Qu 9. Do you normally eat chocolate or drink coffee, tea, soft drinks or energy drinks after 8pm?

APPENDIX 6

Caffeine Study Food Diary

What to do?

- Record all that you eat and drink on the following dates.

_____

_____

_____

_____

- If possible record food at the time of eating or just after – try to avoid doing it from memory at the end of the day.
- Include all meals, snacks, and drinks, even tap water.
- Include anything you have added to foods such as sauces, gravies, spreads, dressings, etc.
- Write down any information that might indicate size or weight of the food to identify the portion size eaten.
- Use a new line for each food and drink. You can use more than one line for a food or drink. See the examples given.
- Use as many pages of the booklet as you need.

Describing Food and Drink

- Provide as much detail as possible about the type of food eaten. For example **brand names and varieties / types** of food.

General description	Food record description
Breakfast example – cereal, milk, sugar	1 cup Sanitarium Natural Muesli 1 cup Pam's whole milk 1 tsp Chelsea white sugar
Coffee	1 tsp Gregg's instant coffee 1 x 200ml cup of water 2 Tbsp Meadow fresh light green milk
Pasta	1 cup San Remo whole grain pasta spirals (boiled)

Pie	Big Ben Classic Mince and Cheese Pie (170g)

- Give details of all the **cooking methods** used. For example, fried, grilled, baked, poached, boiled…

General description	**Food record description**
2 eggs	2 size 7 eggs fried in 2tsp canola oil 2 size 6 eggs (soft boiled)
Fish	100g salmon (no skin) poached in 1 cup of water for 10 minutes

- When using foods that are cooked (eg. pasta, rice, meat, vegetables, etc), please record the **cooked portion** of food.

General description	**Food record description**
Rice	1 cup cooked Jasmine rice (cooked on stove top)
Meat	90g lean T-bone steak (fat and bone removed)
Vegetables	½ cup cooked mixed vegetables (Wattie's peas, corn, carrots)

- Please specify the **actual amount of food eaten** (eg. for leftovers, foods where there is waste)

General description	**Food record description**
Apple	1 x 120g Granny Smith Apple (peeled, core not eaten – core equated to ¼ of the apple)
Fried chicken drumstick	100g chicken drumstick (100g includes skin and bone); fried in 3 Tbsp Fern leaf semi-soft butter

- **Record recipes** of home prepared dishes where possible and the proportion of the dish you ate. There are blank pages for you to add recipes or additional information.

Recording the amounts of food you eat

It is important to also record the quantity of each food and drink consumed. This can be done in several ways.

- By using household measures – for example, cups, teaspoons and tablespoons. Eg. 1 cup frozen peas, 1 heaped teaspoon of sugar.
- By weight marked on the packages – eg. a 425g tin of baked beans, a 32g cereal bar, 600ml Coke.
- Weighing the food – this is an ideal way to get an accurate idea of the quantity of food eaten, in particular for foods such as meat, fruits, vegetables and cheese.
- For bread – describe the size of the slices of bread (eg. sandwich, medium, toast) – also include brand and variety.
- Using comparisons – eg. Meat equal to the size of a pack of cards, a scoop of ice cream equal to the size of a hen's egg.
- Use the food record instructions provided to help describe portion sizes.

General description	**Food record description**
Cheese	1 heaped tablespoon of grated cheese 1 slice cheese (8.5 x 2.5 x 2mm) 1 cube cheese, match box size Grated cheese, size 10B

- If you go out for meals, describe the food eaten in as much detail as possible.

- ***Please eat as normally as possible - don't adjust what you would normally eat just because you are keeping a diet record and be honest! Your food record will be identified with a number rather than your name.***

Example day

Time food was eaten	**Complete description of food (food and beverage name, brand, variety, preparation method)**	**Amount consumed (units, measures, weight)**
Example 7:55am	Sanitarium weetbix	2 weetbix
" "	Anchor Blue Top milk	150ml

" "	Chelsea white sugar	2 heaped teaspoons
" "	Orange juice (Citrus Tree with added calcium – nutrition label attached)	1 glass (275 ml)
10.00am	Raw Apple (gala)	Ate all of apple except the core, whole apple was 125g (core was ¼ of whole apple)
12.00pm	Homemade pizza (recipe attached)	1 slice (similar size to 1 slice of sandwich bread, 2 Tbsp tomato paste, 4 olives, 2 rashers bacon (fat removed), 1 Tbsp chopped spring onion, 3 Tbsp mozzarella cheese)
1.00pm	Water	500ml plain tap water
3.00pm	Biscuits	6 x chocolate covered Girl Guide biscuits (standard size)
6.00pm	Lasagne	½ cup cooked mince, 1 cup cooked Budget lasagne shaped pasta , ½ cup Wattie's creamy mushroom and herb pasta sauce, ½ cup mixed vegetables (Pam's carrots, peas and corn), 4 Tbsp grated Edam cheese
6.30pm	Banana cake with chocolate icing (homemade, recipe attached)	1/8 of a cake (22cm diameter, 8 cm high), 2 Tbsp chocolate icing

Date_____ DAY 1

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

APPENDIX 7

Saliva collection method and standard operating procedures

Equipment:

1 x 15 ml sterile, conical centrifuge tube

2 x large, sterile cotton bud applicator

1 x 1.5 ml Eppendorf

Gloves

Facial tissues

Centrifuge (MF-50, Hanil Science Industrial, Korea)

Scissors

Stop watch

Procedure:

- Prepare centrifuge tube and Eppendorf before-hand by cutting the Eppendorf tip and lid off using scissors.
- Place the trimmed Eppendorf tube inside the centrifuge tube.
- Research should wash hands and put gloves on
- Give cotton bud applicator to participant and have them place the buds in the mouth. (One under the tongue and one on the inside of the cheek.
- Start timer and wait for three minutes to allow for the buds to absorb adequate saliva.
- Asking the participant to rotate the buds may encourage better saliva absorption.
- After three minutes ask the participant to remove one of the buds at a time and pass it to the researcher. Tissues should be available to the participant in case of drool.
- The researcher then places the buds on top of the Eppendorf inside the centrifuge tube.
- The stalks of the cotton applicators are then cut off to allow the centrifuge tube to be sealed with a lid.
- Place centrifuge tubes in centrifuge (MF-50, Hanil Science Industrial, Korea) and centrifuge for 3 minutes at 3500 rpm (1328.46xg).
- Once centrifuged, remove lid and using tweezers remove both the buds and the Eppendorf from the centrifuge tube. Discard Eppendorf and cotton buds in yellow biohazard bags.
- Pipette 3 aliquots of 500 µl of saliva into Eppendorf tubes to be stored and analysed.
- Discard centrifuge tube and gloves in yellow biohazard bags.

APPENDIX 8

DNA extraction method from saliva

DNA extraction

- Add preservation buffer to whole saliva in 1:1 ratio (can be stored up to 28 days)
- Add 250 µl of saliva/buffer solution to 1.5 ml centrifuge tube
- Vortex sample for 10 min
- Incubate at 95 °C for 10 min
- Add 0.68 volume of 5M KAc, mix and incubate on ice for 10 minutes
- Centrifuge at room temperature for 10 minutes at 15000 x g
- Pipette supernatant into a fresh centrifuge tube and discard the pellet
- Add 1.2 volumes of room temperature 95-100% ethanol and mix by inverting the tube
- Allow the sample to stand at room temperature for 10 min
- Centrifuge the tube at 15000 x g for 2-5 min
- Carefully remove the supernatant with a pipette without disturbing the DNA pellet
- Carefully add 300 µl of 70% ethanol and vortex to mix
- Centrifuge for a further 2-5 min at 15000 x g
- Remove ethanol from centrifuge tube without disturbing DNA pellet
- Incubate tube at 90 °C for 1 min
- Add 50-100 µl of 1 x TE solution to DNA pellet and vortex for 10 sec
- Rehydrate DNA pellet by incubating at room temperature overnight followed by vortexing
- DNA can be stored at 4 °C for up to 2 months or -20 °C for long term storage

Preservation buffer solution (20ml; pH 8.7)

- 0.3M Tris HCl	6ml	Buffer
- 0.67M urea	0.804g	Denaturing agent
- 0.6% SDS	1.2ml	Denaturing agent
- 20nM EDTA	4ml	Chelating agent
- 0.67 NaOAc	4.5ml	Denaturing agent
- 0.1M Ascorbate	0.35g	Reducing agent
- H_2O	4.3ml	

APPENDIX 9

Plasma and urine caffeine and caffeine metabolite HPLC assay

Preparation of samples

1. Dispense 400 µl of 0.8 M perchloric acid into labelled Eppendorf tubes.
2. Add 400 µl of well mixed plasma/urine sample to perchloric acid.
3. Vortex for approximately 10 sec to ensure samples is properly mixed.
4. Centrifuge for 10 minutes at 10000 rcf (espresso microcentrifuge, Thermo Sceintific IEC, US)
5. Remove 400 µl aliquot of supernatant, careful not to suck up any precipitate, and place into glass HPLC vial.

HPLC analysis

Mobile phase A: 0.1% trifluoroacetic acid ($C_2HF_3O_2$) in water (H_2O)

Mobile phase B: 0.1% trifluoroacetic acid ($C_2HF_3O_2$) in 40% acetonitrile (C_2H_3N)

Stationary phase: Phenomenex luna

Flow rate: 0.75ml·min^{-1}

Oven temperature: 22°C

Wavelength: 274 nm

Injection volume: 20 µl

Standards: 0.0625 µg·ml^{-1}, 0.125 µg·ml^{-1}, 0.25 µg·ml^{-1}, 0.5 µg·ml^{-1}, 1 µg·ml^{-1}, 2.5 µg·ml^{-1}, 5 µg·ml^{-1}, 10 µg·ml^{-1}, 25 µg·ml^{-1}, 50 µg·ml^{-1}

Gradient method:

Table 13.1. HPLC gradient method of mobile phase concentrations A and B.Time (min)	A Concentration (%)	B Concentration (%)
0	100	0
15	65	35
20	65	35
28	50	50
30	0	100
35	0	100
41	100	0

APPENDIX 10

"During the exercise bout we want you to pay close attention to how hard you feel the exercise work rate is. This feeling should reflect your total amount of exertion and fatigue, combining all sensations of physical stress, effort and fatigue. Don't concern yourself with any one factor (e.g. leg pain, shortness of breath) but try to concentrate on your total inner feeling of exertion. Try not to underestimate or overestimate your feeling of exertion; be as accurate as you can."

APPENDIX 11

FEELING SCALE

+5	Very good
+4	
+3	Good
+2	
+1	Fairly good
0	Neutral
-1	Fairly bad
-2	
-3	Bad
-4	
-5	Very bad

"While participating in exercise it is common to experience changes in mood. Some individuals find exercise pleasurable, whereas others find it to be displeasurable. Additionally, feeling may fluctuate across time. That is, one might feel good and bad a number of times during exercise. Scientists have developed this scale to measure such responses."

APPENDIX 12

FELT AROUSAL SCALE (FAS)
(Svebak & Murgatroyd, 1985)

Estimate here how aroused you actually feel. Do this by circling the appropriate number. By "arousal" we meant how "worked-up" you feel. You might experience high arousal in one of a variety of ways, for example as excitement or anxiety or anger. Low arousal might also be experienced by you in one of a number of different ways, for example as relaxation or boredom or calmness.

1 LOW AROUSAL

2

3

4

5

6 HIGH AROUSAL

APPENDIX 13

The genetic effects of caffeine ingestion on exercise performance, mood, sleep and immune function in male athletes.

Profile of Mood States-Short Form (POMS-40)

Refer to the definitions below. Consider how you are feeling right now, when circling the appropriate response. Please make sure you have responded to all items.

FATIGUE	Not at all	A little	Moderately	Quite a bit	Extremely
Worn out	0	1	2	3	4
Weary	0	1	2	3	4
Bushed	0	1	2	3	4
Fatigued	0	1	2	3	4
Exhausted	0	1	2	3	4

Anger	Not at all	A little	Moderately	Quite a bit	Extremely
Peeved	0	1	2	3	4
Bitter	0	1	2	3	4
Resentful	0	1	2	3	4
Grouchy	0	1	2	3	4
Angry	0	1	2	3	4
Furious	0	1	2	3	4
Annoyed	0	1	2	3	4

Vigour	Not at all	A little	Moderately	Quite a bit	Extremely
Cheerful	0	1	2	3	4
Powerful	0	1	2	3	4
Full of Pep	0	1	2	3	4
Active	0	1	2	3	4
Energetic	0	1	2	3	4
Lively	0	1	2	3	4

TENSION	Not at all	A little	Moderately	Quite a bit	Extremely

Restless	0	1	2	3	4
Nervous	0	1	2	3	4
On-Edge	0	1	2	3	4
Tense	0	1	2	3	4
Uneasy	0	1	2	3	4
Anxious	0	1	2	3	4

ESTEEM	Not at all	A little	Moderately	Quite a bit	Extremely
Embarrassed	0	1	2	3	4
Ashamed	0	1	2	3	4
Proud	0	1	2	3	4
Competent	0	1	2	3	4
Satisfied	0	1	2	3	4

CONFUSION	Not at all	A little	Moderately	Quite a bit	Extremely
Bewildered	0	1	2	3	4
Forgetful	0	1	2	3	4
Confused	0	1	2	3	4
Unable to Concentrate	0	1	2	3	4
Uncertain About things	0	1	2	3	4

DEPRESSION	Not at all	A little	Moderately	Quite a bit	Extremely
Hopeless	0	1	2	3	4
Helpless	0	1	2	3	4
Sad	0	1	2	3	4
Worthless	0	1	2	3	4
Miserable	0	1	2	3	4
Discouraged	0	1	2	3	4

INFORMATION ABOUT WEARING THE ACTIGRAPH AND COMPLETING THE SLEEP DIARY

INFORMATION ABOUT WEARING THE ACTIGRAPH AND COMPLETING THE SLEEP DIARY

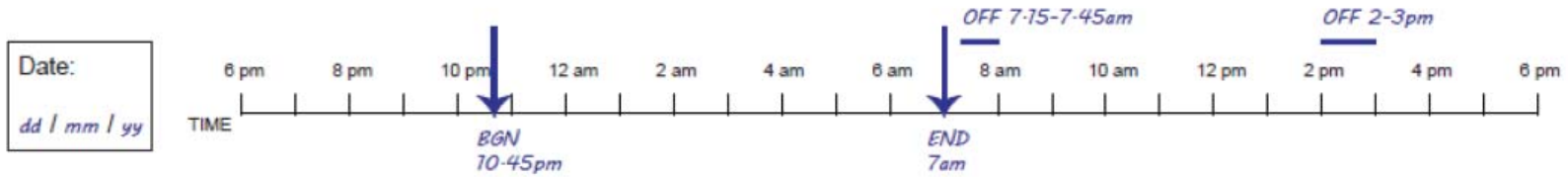
The small watch-sized object you will be wearing on your wrist is an actigraph. It contains an accelerometer and memory chip and records movement. The data from the actigraph is analysed along with the information from the sleep diary to determine sleep length and sleep quality.

Information about wearing the actigraph:

1. Wear the actigraph on your non-dominant wrist (the hand you don't write with). It is important that you do not change wrists as this may significantly change the information that we get from the actigraph.
2. The actigraph should be attached reasonably firmly so that it does not move about on your wrist. If it does move about, tighten the strap slightly.
3. The actigraph must be removed for any contact with water (e.g. showering, swimming) or any contact sport, but it is important that you put it back on again afterwards.
4. If you take the actigraph off for any reason (for showering, to take a swim, sport etc.) then please note this in your sleep diary.
5. If you forget to put the actigraph back on at any stage then put it on as soon as you remember. Please write in the diary the time when you put the actigraph back on.
6. **We cannot tell what you are doing from the actigraphy data. We can only tell whether you are moving or not.**
7. On the side of the actigraph there is a small grey button; this is the event marker. If you push this, a small mark will appear on the data output. It does not stop or start the actigraph. The actigraph will keep recording the entire time you are wearing it.
8. We would like you to push the event marker when you start trying to sleep and again when you stop trying to sleep. Please do this whenever you intend to sleep for **10 minutes or longer.**

Information about filling out the sleep diary

1. We are interested in any sleep that is 10 minutes or longer. It does not matter whether this is during the day or during the night.
2. The information that is important to us are the times that you **begin trying to sleep** and when you **finish trying to sleep** for any sleep that is **10 minutes or longer.**
3. When you are about to begin trying to sleep:
   a. Mark the time on the timeline with an arrow and record the time above or below it.
   b. **Beginning (BGN)** is the time when you begin trying to sleep. Some people may get into bed and read etc, but we do not need to know this, we only need to know when you begin trying to go to sleep.
4. When you have finished trying to sleep:
   a. Mark the time on the timeline with an arrow and record the time above or below it.
   b. **END** is when you wake up and are no longer trying to sleep. At this time you may either get out of bed or begin to read etc, but you are no longer trying to sleep.
5. Beginning and End are the times we would like you to **push the event marker** on the actigraph.
6. If you wake up during your sleep to get a drink, go to the toilet etc, you do not need to write anything in the sleep diary. **If you get up for more than 10 minutes then please treat any later sleep as a new sleep period.**
7. If you **take the watch off** for any reason, please mark this in your sleep diary by drawing a line and indicating the start and end times.



Did you wake in the morning with an alarm?

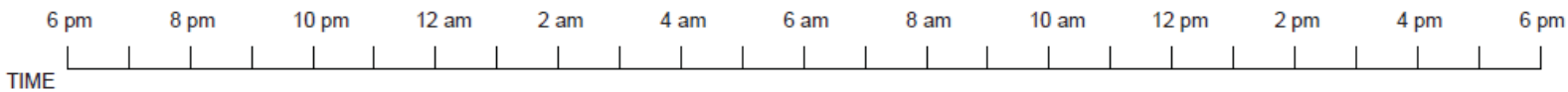
(YES) / NO

SLEEP DIARY

For any sleep that you have that is more than 10 mins, please indicate the beginning (BGN) and end (END) times of each sleep using arrows on the timeline and record the time above or below it. If you get up for more than 10 minutes then please treat any later sleep as a new sleep period. Please press the event marker when you begin or finish trying to sleep. Mark the times when you have removed the actiwatch (OFF).

Date:

-- / -- / --



Did you wake in the morning with an alarm?

YES / NO

Comments

Each question is answered by placing a vertical mark on the answer line. If no change was experienced then place your mark in the middle of the line. If a change was experienced then the position of your mark will indicate the nature and extent of the change, i.e. large changes near the ends of the line, small changes near the middle.

How would you describe the way you currently fall asleep in comparison to usual?

More difficult than usual _____ Easier than usual

Slower than usual _____ More quickly than usual

I feel less sleepy than usual _____ More sleepy than usual

How would you describe the quality of your sleep compared to normal sleep?

More restless than usual _____ Calmer than usual

With more wakeful periods than usual _____ With less wakeful periods than usual

How would you describe your awakening in comparison to usual?

More difficult than usual _____ Easier than usual

Longer than usual _____ Shorter than usual

How do you feel when you wake up?

Tired _____ Alert

How do you feel now?

Tired _____ Alert

How would you describe your balance and co-ordination upon awakening?

More disrupted than usual _____ Less disrupted than usual