EFFECT OF CAFFEINE SUPPLEMENTATION ON METABOLISM AND PHYSICAL AND COGNITIVE FUNCTION IN FEMALE INTERMITTENT GAMES PLAYERS

By

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ABSTRACT

Purpose: To investigate the effects of caffeine ingestion on metabolism and physical and cognitive performance in female team-sport players taking a monophasic oral contraceptive. ***Method:*** In a randomized, double-blind, placebo-controlled crossover design, 10 participants (age 23.6 ± 4.1 y; height 1.62 ± 0.06 m; body mass 59. O_2 max 50.0 ± 5.3 ml·kg-^{1}·min^{-1}) completed a 90 min intermittent treadmill-running protocol twice, during days 5-8 and 18-22 of their pill cycle. All participants were taking a monophasic oral contraceptive of the same hormonal composition (Levlen ED, Nordette, Monofeme, or Microgynon). During the familiarisation session participants completed a maximal oxygen uptake test, practiced the cognitive, strength, power testing, and underwent 30 min of the running protocol. Upon arrival at the laboratory for the main trials, hydration status was measured via urine specific gravity (USG) using a handheld refractometer and a heart rate (HR) monitor fitted. A capsule containing 6 mg·kg^{-1} body mass (BM) of anhydrous caffeine or placebo (Maltodextrin, 1.62 kJ·g^{-1}) was administered 45 min before commencing exercise with a 500 ml bolus of water. Further 3 ml·kg^{-1} BM boluses of water were provided every 15 min during exercise. Before, during and after the exercise protocol, venous blood samples were taken and cognitive (Choice Reaction Time, CRT; Digit Vigilance, DV; Stroop test), perceptual (Ratings of Perceived Exertion, RPE; Feeling Scale, FS; Felt Arousal Scale, FAS; Profile of Mood States, POMS), and physical tests (countermovement jump, CMJ; strength testing on the isokinetic dynamometer) were administered. These tests were then all performed again at a follow-up 12 h post session in the morning, including a venipuncture blood sample, and the addition of sleep quality assessment using the Leeds Sleep Evaluation Questionnaire (LSEQ). **Results:** There were no significant effects of caffeine supplementation on HR, USG, CMJ, isometric strength, RPE, cognitive performance or glucose and insulin concentrations. Caffeine supplementation improved levels of pleasure, activation and vigour compared to

placebo, and reduced levels of fatigue ($P < 0.05$). Caffeine supplementation also improved average power in eccentric contractions of the knee extensors and flexors, and peak torque in the eccentric contractions of the knee flexors ($P < 0.05$). Getting to sleep and subsequent quality of sleep was impaired following caffeine supplementation ($P < 0.05$). Free fatty acid (FFA) concentration increased over the duration of exercise ($P < 0.05$), and increased at a greater rate on the caffeine trial ($P < 0.05$). **Conclusions:** This is the first controlled study to examine caffeine supplementation in female games players who are taking a low-dose monophasic oral contraceptive, using an intermittent based running protocol. These athletes experienced an improved performance in various strength and power aspects, but no improvement in cognitive performance. Perceptual and mood responses were also improved as a result of caffeine supplementation. Metabolically, caffeine had an effect on markers of fat metabolism but not on carbohydrate metabolism.

Keywords: caffeine, female, metabolism, performance, intermittent exercise

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CHAPTER 1 – INTRODUCTION

Caffeine is one of the most widely consumed drugs in the world as it is present in tea, coffee, soft drinks, energy drinks, chocolate and in some diet aids (Graham, 2001). The National Nutrition Survey of New Zealand (1997) indicated that 53% of females between the ages of 19 and 24 consumed coffee, and 47% consumed tea (*NZ Food: NZ People. Key Results of the 1997 National Nutrition Survey*, 1999). Information regarding total caffeine consumption among women is scarce but considering the rise in popularity of caffeine-containing energy drinks since 1997, it is likely that total caffeine consumption will have increased in this age group.

Due to the popularity and widespread general use of caffeine, it was removed from the list of banned substances by the World Anti-Doping Agency (WADA) in 2004, although it remains on the monitoring list as much is still unknown about its effects throughout the body. Its ergogenic effects in relation to endurance and time-trial performance have been well demonstrated , including beneficial effects on exercise capacity (Cox, et al., 2002; Graham & Spriet, 1995), cognition (Lieberman, et al., 2002), repeated sprint ability (Glaister, et al., 2008), stimulation and release of adrenaline (Graham, et al., 1998), and adjustments in the central nervous system to alter perceived effort or fatigue (Motl, et al., 2006; Tarnopolsky, 2008).

Early research indicated that caffeine increased the mobilisation of free fatty acids (FFA) from adipose tissue, and consequently increased the use of fat as a fuel during exercise, therefore reducing dependence on muscle glycogen stores (Costill, 1978). More recent studies have shown this “glycogen sparing” effect during submaximal exercise to be

inconsistent and temporary (Chesley, et al., 1998). Rather, it is now thought that the alterations in the central nervous system are the most important factors in explaining the ergogenic, performance enhancing, effects of caffeine - in particular the ability of caffeine to act as an adenosine antagonist (Tarnopolsky, 2008).

Most of the studies regarding caffeine supplementation and its ergogenic benefits have involved male participants, and this area of research has been dominated by participants in endurance sports, with little research into team sport athletes. Thus the current recommendations are relevant to endurance trained men and may not apply to non-endurance trained, or intermittent games players. Furthermore, there are likely to be differences between males and females due to differing levels of sex hormones, which may then affect the way caffeine acts on the body, and overall performance (Graham & McLean, 1999). This means that current recommendations for caffeine supplementation (~3-6 $mg \cdot kg^{-1}$ in anhydrous form) (Goldstein, Ziegenfuss, et al., 2010) may not apply to female games players.

The effect of differing sex hormones between males and females has been investigated through consideration of the menstrual cycle on caffeine metabolism, although results have been inconclusive. Lane et al. (1992) reported that caffeine elimination was slowed in the late luteal phase due to the onset of menstruation and increased levels of progesterone. However, McLean et al. (2002) reported that the menstrual cycle had no effect on caffeine pharmacokinetics. Both of these studies used women who were not taking oral contraceptive steroids (OCS), but noted that OCS use was associated with decreased caffeine clearance (Abernathy & Todd, 1985; Patwardhan, et al., 1980).

One study that has examined caffeine supplementation in females using an exercise protocol was conducted by Anderson et al. (2000). Eight competitive rowers were required to perform three 2,000-m time-trials on a rowing ergometer after consuming either a placebo or different dosages (6 and 9 $mg \cdot kg^{-1}$ body mass) of caffeine. The authors reported that the higher dose of caffeine resulted in a significantly faster first 500 m of the 2,000-m row. Although subjects were tested in the follicular phase there was no mention of controlling for OCS use. Motl et al. (2006) examined the effect of caffeine supplementation on leg muscle pain during cycling in women. Eleven college-aged females were required to consume 5 or 10 $mg \cdot kg^{-1}$ anhydrous caffeine, and then complete 30 min cycling at approximately 60% oxygen uptake (O_2) peak. Caffeine was found to have a significant effect on leg muscle pain ratings, but the effect was not dose-dependent. The subjects were not taking OCS and menstrual cycle was attempted to be controlled for, as subjects performed in the self-reported follicular phase. Ahrens et al. (2007) examined the effects of caffeine supplementation on aerobic exercise in recreationally active women who were not habituated to caffeine. Subjects walked on a treadmill at 5.6 $km \cdot h^{-1}$ for 8 min after consuming either 3 or 6 $mg \cdot kg^{-1}$ of anhydrous caffeine with water. The 6 $mg \cdot kg^{-1}$ dose, compared to the 3 $mg \cdot kg^{-1}$ dose and placebo, resulted in a significant increase in the rate of energy expenditure; however the equivalent increase in O_2, while significant, is unlikely to be relevant in an athletic setting. This study can also be criticised as it did not control for menstrual cycle or OCS use.

A further aspect of caffeine supplementation is the possibility of an ergogenic effect for strength and power based activities. A recent study by Goldstein et al. (2010) assessed the effect of a 6 $mg \cdot kg^{-1}$ dose of caffeine on upper body strength in resistance trained women. It was found that caffeine improved a one- repetition max (1RM) barbell bench press test, but no improvement was seen performing repetitions to failure at 60% 1RM. However, while this

study reported a positive effect of caffeine, other research is still equivocal due to variation in participant training status, male versus female participants, differing protocols (intensity, upper versus lower body), caffeine habituation status, and differing doses. Future research is needed in this area, addressing the points outlined above as well as controlling for menstrual cycle and OCS use.

OCS use is widespread and common amongst young women who use it for contraception or cycle-length control (Constantini, et al., 2005), and therefore previous studies may not be relevant to women using OCS. Earlier research has demonstrated that low doses of estrogen impair caffeine clearance (Abernathy & Todd, 1985; Patwardhan, et al., 1980), but these studies have not been performed using an exercise protocol. Furthermore, the prevalence of OCS use in female athletes is estimated to be the same as the general population (Burrows & Peters, 2007), and it may be suggested that decreased caffeine clearance also occurs in female athletes using OCS. However this has not been verified as there is little research about caffeine pharmacokinetics in female athletes using OCS. As a result it remains to be seen whether OCS use in female athletes will affect the ergogenic potential of caffeine during exercise, and therefore there still remains the need for a well-designed study investigating caffeine supplementation in women, which controls for menstrual cycle and OCS use, in an exercise setting.

Further to the physical ergogenic benefits of caffeine during exercise, the central stimulant properties of caffeine have been widely researched in the literature, generally reporting enhanced cognitive performance, and increased arousal and vigilance. Lieberman et al. (2002) examined the effects of caffeine supplementation on mood and cognitive performance, following sleep deprivation and environmental and operational stress in US Navy Seals.

Seventy two hours of sleep deprivation and continuous exposure to other occupational stressors had severe detrimental effects on performance and mood. Caffeine was shown to attenuate many of these adverse outcomes and, in particular, improve vigilance, alertness, and reaction time (Lieberman, et al., 2002).

There is also evidence that higher doses of caffeine (>6 – 9 $mg \cdot kg^{-1}$) can cause negative side effects in some people. Graham and Spriet (1995) found that some subjects complained of "mental confusion", and Ahrens et al. (2007) initially included a 9 $mg \cdot kg^{-1}$ dose in their study on exercise performance on untrained women, but this elicited tremors, dizziness and vomiting in seven out of the ten women. Further to these ergolytic effects, research has demonstrated that caffeine can have an adverse effect on sleep quality. Drapeau et al. (2006) found that a caffeine dose of 200 mg given 3 h prior to bedtime, compared to a placebo, significantly decreased sleep duration and sleep efficiency, while lengthening the time it took to fall asleep. Therefore, because caffeine elimination has been shown to be impaired in women taking OCS, this may mean that women will be exposed to higher concentrations of caffeine for longer, which increases the risk of adverse effects occurring, not to mention the likelihood of reduced sleep quality.

Caffeine has also been linked with beneficial effects on perception during exercise. In fact, during aerobic, steady-state exercise there appears to be little doubt that caffeine reduces ratings of perceived exertion (RPE). More recent research is also exploring the effects of caffeine on pleasure and activation levels during exercise, using the Feeling Scale (FS) and the Felt Arousal Scale (FAS), respectively (Backhouse, et al., 2011). However, during exercise of a high-intensity or intermittent nature, the effects of caffeine on perception are less well understood. More research is needed in this area to fully understand the relationship

between exercise intensity, caffeine supplementation, and how this affects perception during exercise.

Previous research into caffeine supplementation has focussed on endurance exercise using male participants, while very few studies have used female participants. Female participants who take an OCS will experience delayed caffeine metabolism and, to the researcher's knowledge, no other study has examined caffeine supplementation using an exercise protocol in women who are taking the same OCS. Therefore the overall aim of this thesis was to examine the effect of caffeine supplementation on perception, strength and power, cognition, sleep quality, and metabolism during and following a 90-min intermittent treadmill-running protocol in female games players taking a monophasic oral contraceptive.

1.1 Hypotheses

The major null hypotheses (H0) for this research were:

$H0_1$: Caffeine supplementation will not affect strength and power measures in the knee extensors and flexors before, during or after intermittent exercise in females taking an OCS.

$H0_2$: Caffeine supplementation will not affect the perceptual or mood response to intermittent exercise in females taking an OCS.

$H0_3$: Caffeine supplementation will not affect cognitive performance before, during or after intermittent exercise in females taking an OCS.

$H0_4$: Caffeine supplementation will not affect metabolism during intermittent exercise in females taking an OCS.

$H0_5$: Caffeine supplementation will not affect sleep quality following intermittent exercise in females taking an OCS.

Overview

This thesis is presented in 5 chapters which portray the effect of caffeine supplementation on important aspects of intermittent exercise performance. Chapter 2 is a review of the literature which will examine the effects of caffeine on cognition, mood, perception, strength and power, and sleep quality. Chapter 3 describes the methodology of the study. Chapter 4 describes the effects of caffeine supplementation on cognition, mood, perception, strength and power, and sleep quality. Chapter 5 is a general discussion of the results from this study in relation to the current literature.

CHAPTER 2 – REVIEW OF THE LITERATURE

This review aims to provide a background to caffeine absorption, metabolism, and elimination, and to discuss possible mechanisms – whether central or peripheral in origin – for caffeine's ergogenic effect(s). More specifically, the review will examine the effects of caffeine on cognition, mood, perception, strength and power, and sleep quality. The review will also consider the use of caffeine supplementation by team sport athletes, and discuss the effects of the menstrual cycle on caffeine metabolism and performance in females.

2.1 Caffeine as intervention to overcome fatigue and improve performance

Caffeine is the most commonly consumed stimulant throughout the world as it is found not only in tea and coffee, but in chocolate and energy drinks (Graham, 2001; Harland, 2000). Its stimulant properties are well known, resulting in a diverse demographic of users – from shift workers, to soldiers, through to athletes – who wish to delay fatigue (Burke, 2008). Due to the popularity of its consumption and its increasing appearance in many foods and drinks, the World Anti-Doping Agency (WADA) removed caffeine from the list of banned substances in 2004, even though its ergogenic effects are not fully understood.

2.1.1 Absorption, metabolism, elimination

Caffeine is not essential for physiological function, yet is an important pharmacologically active substance that can affect the majority of organs and tissues in the human body (Benowitz, 1990). As such it is absorbed completely and quickly in the gastrointestinal tract, and directly absorbed from the stomach into the blood stream (Magkos & Kavouras, 2005). Complete absorption will have occurred an hour following ingestion, although the majority of

the caffeine will have already been absorbed 45 min after ingestion (Benowitz, 1990; Harland, 2000; Magkos & Kavouras, 2005).

Following absorption, caffeine is completely metabolised in the liver, by the cytochrome P450 system, to give three metabolites: paraxanthine (major metabolite), theophylline, and theobromine (Benowitz, 1990; Goldstein, Ziegenfuss, et al., 2010). The rate of metabolism is variable and affected by factors such as smoking, which shorten the half-life of caffeine (Magkos & Kavouras, 2005), or pregnancy and oral contraceptive use which increase the half-life (Abernathy & Todd, 1985; Patwardhan, et al., 1980). Generally the half-life is 4-6 hr (Benowitz, 1990; Kruskall & Miracle, 2009), although high doses or repeated-doses can prolong the half-life and reduce caffeine clearance due to saturation of the enzymes responsible for caffeine metabolism (Kaplan, et al., 1997; Magkos & Kavouras, 2005).

Graham and Spriet (1995) showed that while caffeine concentration increased concomitantly with dosage, the concentration of the primary metabolite, paraxanthine, did not increase between the 6 $mg \cdot kg^{-1}$ and 9 $mg \cdot kg^{-1}$ dosages indicating that the hepatic enzymes involved were saturated. The variability within the half-life and clearance rates of caffeine suggests that there are large inter-individual differences, and that these differences are largely involved with the metabolism and elimination of caffeine, rather than the absorption of caffeine (Magkos & Kavouras, 2005).

Caffeine is predominantly excreted via the kidneys, and approximately 3 – 10% of caffeine ingested will be excreted in the urine unchanged (Goldstein, Ziegenfuss, et al., 2010; Magkos & Kavouras, 2005). Therefore normal consumption of caffeine will give a low urinary caffeine concentration which is of advantage to many athletes who may have to be drug

tested in their sporting events. While caffeine was on the World Anti-Doping Agency's (WADA) banned list prior to 2004, the upper limit for urinary caffeine concentration was 12 $\mu g \cdot mL^{-1}$. To achieve this concentration most athletes would have to take a dose within the range of 9 – 13 $mg \cdot kg^{-1}$ one hour prior to exercise (Goldstein, Ziegenfuss, et al., 2010). Since its removal from the list of banned substances, further research has shown that performance benefits from caffeine do not occur in a dose-dependent manner (Graham & Spriet, 1995; Pasman, et al., 1995), and therefore dosages of 9 – 13 $mg \cdot kg^{-1}$ are unnecessarily high.

2.2 Caffeine and peripheral mechanisms of action

2.2.1 Fat oxidation and glycogen sparing

Intermittent sports such as soccer and hockey are played at ~70% O_2max (Bangsbo, 1994; Spencer, et al., 2004), and muscle glycogen is likely to be the most important substrate during the match. This was demonstrated by Saltin (1973) who found that muscle glycogen stores were almost depleted in players who had started with low muscle glycogen values. In those players that began the match with normal muscle glycogen stores, values were still comparatively high at half-time, but were significantly lowered at the end of the match. Recent research has also confirmed that after a soccer match, there is significant glycogen depletion in most muscle fibres (Krustrup, et al., 2006). As glycogen depletion occurs, there is a concomitant increase in the levels of free fatty acids (FFA) seen in the blood, particularly in the second half of intermittent sports (Bangsbo, 1994; Krustrup, et al., 2006). Therefore substrate metabolism during intermittent exercise is a complex and dynamic event, but is very important to performance.

The initial mechanism proposed to explain the ergogenic effect of caffeine was based on early research that indicated caffeine enhanced fat oxidation, and this led to a 'sparing effect'

of muscle glycogen (Costill, 1978; Essig, et al., 1980). Costill et al. (1978) assessed the effects of caffeine ingestion on metabolism and performance in nine competitive cyclists who cycled to volitional fatigue at 80% O_2max after either ingesting 330 mg of caffeine as coffee, or a decaffeinated coffee placebo. In the caffeine trial it was found that performance time was improved and that fat oxidation was significantly greater with caffeine ingestion. The ergogenic affect of caffeine was attributed to its positive effects on lipolysis that resulted in higher concentrations of circulating FFA. Following this study Essig et al. (1980) developed this theory and suggested the ergogenic effects of caffeine were not solely due to increased mobilisation, uptake and oxidation of FFA, but instead were likely to be due to changes in muscle metabolism. Muscle biopsies revealed a 42% decrease in muscle glycogen use and a 150% increase in muscle triglyceride use in the participants who took caffeine compared to the control decaffeinated trial (Essig et al 1980).

Aside from Essig (1980), early research often failed to directly measure muscle metabolism, instead relying on metabolite concentrations in venous blood, and respiratory measurements. This led to uncertainty about the actual ability of caffeine to alter fat or carbohydrate metabolism in active muscles. Graham et al. (2000) examined the effect of a 6 $mg \cdot kg^{-1}$ dose of caffeine on muscle carbohydrate and fat metabolism using arterial blood measures and muscle biopsies. They found an increase in serum fatty acids and glycerol at rest indicating an increase in lipolysis following caffeine ingestion. However there was no overall change in respiratory exchange ratio (RER) or net muscle glycogenolysis, therefore demonstrating that there was no change in substrate metabolism in the active muscle as a result of caffeine administration. An increase in adrenaline concentration was noted leading the researchers to conclude that caffeine stimulated the sympathetic nervous system but did not alter substrate

metabolism in the active muscle and that the effect on non-exercising tissues, such as the liver, may be more relevant to caffeine's ergogenic effects.

Therefore the mechanism explaining caffeine's ergogenic nature is unlikely to be based on changes in substrate metabolism or "glycogen-sparing". Rather, the mechanism is thought to be based on more centrally mediated factors as discussed later.

2.2.2 Blood glucose

Active muscle glucose uptake has been shown to increase with high-intensity exercise (Cooper, et al., 1989), most likely due to an increase in circulating catecholamine concentrations (Sigal, et al., 1996). Caffeine is known to increase adrenaline concentration (Graham & Spriet, 1995), therefore it could be expected that blood glucose levels would increase with caffeine supplementation (Davis & Green, 2009).

An increase in blood glucose as a potential mechanism to explain the ergogenic effect of caffeine has not been well studied. Some investigations have suggested that caffeine supplementation will increase levels of glucose in the blood (Graham, et al., 2000; Graham & Spriet, 1995; MacIntosh & Wright, 1995; Spriet, et al., 1992), but caffeine is generally thought to have little effect on glucose kinetics. Raguso et al. (1996) used 7 trained cyclists, in a 30 min protocol that consisted of cycling at 75% O_2max, to examine the effect of caffeine on glucose kinetics. It was found that the rate of glucose appearance was not affected by theophylline (a major metabolite of caffeine) but that the rate of disappearance was lower in the caffeine trial compared to the placebo trial. The authors attributed this to a decrease in the uptake of glucose by active muscles, and since no change was observed in respiratory

exchange ratio (RER) using indirect calorimetry, it was assumed that there was an increase in muscle glycogenolysis.

However, Graham et al. (2000) directly studied the effect of a caffeine supplement on glucose kinetics in active muscles using 10 males who performed 1 h cycling at 70% O_2max after ingesting either caffeine or placebo. Similar results to the Raguso et al (1996) study were seen: RER did not change, arterial glucose increased, insulin levels were low, and lactate was increased in the caffeine trials. However, Graham et al. (2000) gave a different explanation for their observations. As part of their study, Graham et al. (2000) had taken biopsies of the active muscle and therefore direct measures of muscle glycogen content and glucose uptake were possible; they also utilised indirect calorimetry. Their study failed to confirm an increase in muscle glycogenolysis as was suggested to have occurred by Raguso et al. (1996).

A more recent study by Hulston (2008) examined glucose kinetics in 10 endurance-trained male cyclists who completed 3 trials of 105 min steady-state cycling followed by a ~45 min time trial. Subjects consumed either a 6.4% glucose solution, a 6.4% glucose solution with 5.3 $mg \cdot kg^{-1}$ caffeine, or placebo (no caffeine or carbohydrate). The rate of appearance and disappearance of glucose was significantly higher in the glucose compared to placebo trial, however not significantly different between the glucose and glucose-caffeine trial. This study showed that caffeine did not influence glucose kinetics during steady-state exercise, and it was noted that there are likely to be large individual differences in glucose absorption which may account for the discrepancies in previous research findings.

Therefore it is likely that net glucose uptake by active muscles during exercise is unaffected by caffeine supplementation, and there is no increase in muscle glycogenolysis. Changes in

blood glucose as a result of caffeine administration are unlikely to provide the major mechanism for caffeine's ergogenic and performance effects.

2.2.3 Lactate

Lactic acid is the result of the anaerobic metabolism of glucose and glycogen, and at physiological pH it dissociates into lactate and H^+. Accumulation in muscle has long been thought to be a leading cause of muscle fatigue in high intensity exercise as reviewed by Fitts, (1994).

Higher blood lactate concentrations are observed following high-intensity intermittent exercise compared to continuous exercise, and there is a direct relationship with the amount of active muscle in use (Karlsson, 1971). During intermittent sports such as soccer, blood lactate values have been found to reach concentrations >10 $mmol \cdot L^{-1}$ (Bangsbo, 1994), meaning that it is likely that muscle concentrations will be even higher. This can negatively affect energy production and muscle contractions (Ekblom, 1986).

Many studies have shown an increase in blood lactate levels with caffeine supplementation (Graham, et al., 2000; Graham & Spriet, 1995; Raguso, et al., 1996; Spriet, et al., 1992), which is contradictory to the early theory of caffeine's ergogenic nature which was centred on a "carbohydrate-sparing" model. The underlying mechanism for this increase in blood lactate levels is not well understood.

One possibility is that caffeine supplementation causes a catecholamine-induced increase in muscle glycogenolysis, and this was shown in a study by Watt et al. (2001) who used an infusion of adrenaline protocol and reported increases in muscle glycogen breakdown and

lactate levels. However the adrenaline concentrations needed to achieve these increases in lactate levels and muscle glycogenolysis are higher than the levels of adrenaline seen as a result of caffeine ingestion (Chesley, et al., 1995).

Hulston et al. (2008) who, as mentioned before, investigated substrate metabolism under three different conditions (6.4% glucose solution; 6.4% glucose with 5.3 $mg \cdot kg^{-1}$ caffeine, or placebo), found that estimated rates of muscle glycogen breakdown were not altered between the three different conditions. Therefore, it is unlikely that the higher levels of blood lactate seen with caffeine ingestion are due to an increase in lactate production. However, Graham et al. (2000) found higher arterial lactate concentrations with caffeine supplementation but no increase in lactate clearance. Therefore, it is possible the increase in blood lactate levels seen with caffeine supplementation are due to the reduced clearance of lactate by non-exercising tissues such as the liver, rather than caffeine having a direct effect on active muscles.

2.2.4 Catecholamines

Caffeine has a well demonstrated positive effect on sympathetic nervous system activation, and therefore an increase in adrenaline concentration is witnessed with caffeine ingestion compared to placebo (Graham, et al., 2000; Graham & Spriet, 1995; Greer, et al., 2000; Jackman, et al., 1996; Spriet, et al., 1992; van Soeren & Graham, 1998). However the reported increase in adrenaline concentrations seen with caffeine supplementation may not be large enough to have significant physiological or performance effects (Graham, 2001).

Experiments have been performed on tetraplegic participants who have low levels of catecholamines, and no involvement of the brain in aspects of motor control and fatigue due to their injuries. Van Soeren et al. (1996) showed that in six tetraplegic participants there was

no change in adrenaline concentration following caffeine administration. This same group then sought to examine whether exercise performance would be improved in tetraplegic subjects following caffeine supplementation, when their previous research had shown no significant increase in catecholamine concentrations (Mohr, Van Soeren, Graham, & Kjaer, 1998). Nine tetraplegic males who were heavy caffeine users received either 6 $mg \cdot kg^{-1}$ caffeine or a placebo. The exercise protocol was performed on a modified cycle ergometer, where the participants' hamstrings, quadriceps, and gluteal muscles were electrically stimulated to perform involuntary pedalling. The test lasted approximately 25-30 min or until 35 $revolutions \cdot min^{-1}$ could not be maintained with full electrical stimulation. It was found that caffeine significantly improved time to fatigue compared to placebo, with no changes in circulating adrenaline concentration. Therefore this also supports the theory that caffeine has a direct effect on skeletal muscle, and the associated ergogenic effects, without a concomitant increase in catecholamines.

Additionally, Chesley et al. (1995) infused subjects with adrenaline to levels similar to those seen after caffeine ingestion. The corresponding increase in adrenaline did not affect muscle glycogenolysis, or levels of free fatty acids, lactate, or phosphocreatine. So it is unlikely that increased adrenaline levels seen with caffeine supplementation are the main mechanism for caffeine's ergogenic effects, as the increase is not likely to be large enough to translate into significant performance benefits.

2.2.5 Ionic balance

Fatigue can be caused by disruptions to electrolyte homeostasis i.e. a loss of K^+ or Ca^{2+} from the sarcoplasmic reticulum which would result in less motor unit activation or less force production. It is well known that after every depolarisation the concentration of intracellular

K^+ ions decreases while there is a concomitant increase in extracellular K^+ which is likely to affect the resting membrane potential (McKenna, 1992).

Caffeine has been shown to decrease the loss of intracellular K^+ ions as evidenced by an attenuation of the increase in extracellular K^+ ions (Lindinger, et al., 1993; MacIntosh & Wright, 1995). Lindinger et al. (1996) showed that caffeine stimulated resting muscle K^+ uptake, while Graham et al. (2000) showed that subjects had a smaller increase in arterial K^+ following caffeine ingestion, but that K^+ release was not altered. These studies support the possibility that caffeine may directly increase resting muscle reuptake of K^+, or indirectly through the increase in adrenaline concentrations seen with caffeine supplementation.

Further to this work van Soeren et al. (1996) sought to clarify whether the increased uptake of K^+ was due to caffeine or increased adrenaline levels. Six tetraplegic subjects, who have no sympathetic activation, were studied for 3 h after ingesting a 6 $mg \cdot kg^{-1}$ capsule of caffeine. It was found that caffeine attenuated the increase in extracellular K^+ with no change in adrenaline concentrations seen. Recently Mohr et al. (2011) found a similar result using a 6 $mg \cdot kg^{-1}$ caffeine dose and an intermittent exercise protocol. Caffeine compared to placebo reduced the accumulation of muscle interstitial potassium, but the concentrations seen were unlikely to cause fatigue.

Overall it is clear that caffeine directly improves K^+ clearance, however, whether this translates into a performance or ergogenic benefit is not clear. Studies have focused on single-fibre muscle preparations rather than more complex and practical *in vivo* experiments where other factors are likely to be of great importance such as hormones, cytokines, and

plasma proteins (Allen, et al., 2008; Tarnopolsky, 2008). Future research should seek to address these issues in more realistic *in vivo* experiments.

2.3 Central mechanisms – adenosine antagonism

Caffeine is known to stimulate the central nervous system (CNS) but more specifically it is an adenosine antagonist (Fredholm, 1995; Sawynok, 1998). Adenosine metabolism is primarily regulated through the breakdown of adenosine compounds: adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) (Costa, et al., 2001). Therefore during exercise the level of adenosine is increased in skeletal muscle (Latini & Pedata, 2001), circulation, and in the brain (Daly, 1982). Effects of adenosine include an increased pain perception (Abreu, et al., 1991), sleepiness (Porkka-Heiskanen, et al., 2002), and decreased arousal (Huston, et al., 1996).

Caffeine has a very similar structure to adenosine and is therefore able to compete with adenosine at the site of the receptor (Kalmar & Cafarelli, 1999a) thus allowing caffeine to inhibit the effects of adenosine in its role as an adenosine antagonist (Sawynok, 1998). There are four different subtypes of adenosine receptors - A_1, A_{2a}, A_{2b}, and A_3 – all producing differing effects with the binding of adenosine. A_1 and A_2 receptors have the highest affinity for adenosine. Binding of adenosine to A_1 receptors produces inhibitory effects, and binding to A_2 receptors produces an excitatory response (Dunwiddie & Masino, 2001; Sawynok, 1998). Due to caffeine's lipophilic structure, it can diffuse across the blood brain barrier and bind to any of the adenosine receptors thus inhibiting the effects of adenosine (Spriet, 1995), and this is currently the most accepted mechanism explaining caffeine's ergogenic effects (Goldstein, Jacobs, et al., 2010; Spriet, 1995; Tarnopolsky, 2008). Although future studies

may determine that other mechanisms (as discussed below) are more important depending on the type/duration of exercise being performed.

2.3.1 Pain perception

Research has shown that pain can influence motor unit recruitment proportional to the intensity of the pain (Farina, et al., 2004). The pain adaptation model states that pain will ultimately compromise the ability of the muscle to contract and generate force (Lund & Donga, 1991). Adenosine has been linked to muscle pain and several studies have shown that adenosine will actually bring about muscle pain (Slyven, et al., 1986), and therefore lower the pain threshold (Pappagallo, et al., 1993). Activation of the A_1 adenosine receptors is linked with pain suppression, while activation of the A_2 receptors is linked to an enhancement of pain (Sawynok, 1998).

Because caffeine is an adenosine antagonist and blocks the adenosine receptors, it has the ability to increase the pain threshold and reduce the perception of pain (Myers, et al., 1997). Therefore caffeine is commonly found in many over-the-counter medicines and, when combined with other pain-relievers, it is 40% more effective (Ribeiro & Sebastiao, 2010). In relation to exercise this means that caffeine should decrease the perception of pain and therefore muscle output could be maintained if not increased following caffeine supplementation.

Studies that have examined caffeine and pain perception have generally found that pain perception is decreased with caffeine administration. Motl et al. (2003) used a 10 $mg \cdot kg^{-1}$ dose on 16 male subjects who cycled for 30 min at 60% O_2max, which was based on a method for assessing pain during exercise from Cook et al. (1997). It was found that

measures of naturally occurring leg pain were significantly lower after the caffeine trial compared to placebo. The authors attributed this to the hypoalgesic effects of caffeine as an adenosine antagonist. Subsequently, O'Connor et al. (2004) reported a dose-dependent effect of caffeine on leg muscle pain using 5 $mg \cdot kg^{-1}$ and 10 $mg \cdot kg^{-1}$ doses. However a later study by Motl et al. (2006), using the same exercise protocol but with female participants, found that although caffeine decreased muscle leg pain this was not a dose-dependent effect. They noted that females had an overall lower muscle pain perception in response to caffeine compared to the males in their earlier study (Motl, et al., 2003). This is of interest as previous research has suggested that females actually have a higher muscle pain perception and a decreased pain threshold compared to males, when pain is experimentally induced (Dao & LeResche, 2000; Hong-You, et al., 2005). Therefore the difference in the impact of caffeine on performance, in men and women, remains uncertain as no studies have directly compared the effects of caffeine on performance in both men and women.

More recently a number of researchers have investigated the effect of caffeine and pain perception using resistance exercise protocols. Astorino et al. (2011) found no effect of two different dosages of caffeine (2 $mg \cdot kg^{-1}$ and 5 $mg \cdot kg^{-1}$) on pain perception in 15 males who completed 40 maximal contractions of the knee flexors and extensors. This contradicts studies by Bellar et al. (2011) and Duncan et al. (2011) who reported a decreased pain perception following caffeine administration using both male and female participants. This discrepancy may be due to the smaller amount of total work done in the Astorino et al. (2011) study compared to the Duncan et al. (2011) study; furthermore Bellar et al. (2011) used upper-body isometric exercise while Astorino (2011) used lower-body isokinetic exercise.

Therefore, it appears that the extent to which caffeine influences pain perception is dependent on the dose and form of exercise undertaken. It is likely that the effect of caffeine on pain perception through the CNS is extremely relevant to its ergogenic effects during exercise, however it remains to be seen whether caffeine triggers any local peripheral actions that may decrease the perception of pain.

2.3.2 Ratings of Perceived Exertion (RPE)

Further extension of the proposal that caffeine influences pain perception would suggest that caffeine should then lower the level of normal pain associated with exercise and decrease perceived exertion. Studies that have examined RPE in an aerobic exercise setting have tended to find that RPE values are lower during exercise following caffeine ingestion (Bell & McLellan, 2002; Cox, et al., 2002).

A meta-analysis conducted by Doherty and Smith (2005) investigated the effects of caffeine supplementation on ratings of perceived exertion. Caffeine was found to reduce RPE by 5.6% compared to placebo during exercise of a constant-load, and the authors proposed that this reduction in RPE may help explain the ergogenic effects of caffeine, although the mechanisms are not clear. In support of this Backhouse et al. (2011) recently examined the effect of a 6 $mg \cdot kg^{-1}$ dose in 12 endurance trained men who cycled for 90 min at 70% O_2max. RPE was found to be significantly lower in the caffeine trial compared to placebo.

Some studies have failed to find any effect of caffeine on RPE (Glaister, et al., 2008; Schneiker, et al., 2006). The reasons for this are unclear but are likely to be due to the nature of the exercise protocols employed. Glaister et al. (2008) and Schneiker et al. (2006) both used multiple sprints, and it is possible that at high exercise intensities the RPE scale may not

be sensitive enough to identify any changes in perceived exertion (Davis & Green, 2009). Of note is that the Schneiker et al. (2006) study, while finding no effect of caffeine on RPE, reported that participants exhibited a greater peak power and total sprint work after caffeine ingestion. Therefore caffeine improved aspects of performance, and it might be possible that caffeine reduced perceived exertion during high-intensity exercise.

During aerobic, steady-state exercise there appears to be little doubt that caffeine reduces RPE. During exercise of a high-intensity or intermittent nature, the effects of caffeine on RPE are less well understood. More research is needed in this direction to fully understand the relationship between caffeine and RPE.

2.3.3 β-endorphins

β-endorphins are endogenous opioid neurotransmitters that are mainly synthesised in the anterior pituitary gland (Goldfarb & Jamurtas, 1997). Previous research has shown that β-endorphin concentrations increase with exercise of an adequate intensity and duration (Goldfarb, et al., 1990; Shwarz & Kindermann, 1989). It has been suggested that caffeine supplementation may increase the secretion of β-endorphins during exercise and therefore improve endurance exercise performance due to the analgesic properties of β-endorphins which decrease the perception of pain (Hartwig, 1991).

Laurent et al. (2000) examined the effect of caffeine on the neuroendocrine axis in 20 trained men who received either a 6 $mg \cdot kg^{-1}$ of caffeine or placebo, and then 90 min later cycled for 2 h at 65% O_2 max followed by high intensity sprints. Plasma β-endorphins levels almost doubled in the caffeine trial compared to placebo, and it was proposed that caffeine lowered the threshold for the exercise-induced secretion of β-endorphins. Therefore this is a possible

mechanism that might explain caffeine's ergogenic effects, that warrants further investigation. Research suggests that the β-endorphin response to exercise is similar in men and women as long as exercise intensity is controlled (Goldfarb, et al., 1998). To the author's knowledge levels of β-endorphins have not been examined using an intermittent protocol.

2.4 The role of genetics and caffeine

Caffeine can have varying effects in individuals who receive the same relative caffeine dose, with some more likely to become anxious (Silverman & Griffiths, 1992) and others more likely to experience impaired sleep quality (Bchir, et al., 2006). It is to be expected that several factors contribute to these individual differences including environmental factors (age, other drug use, and circadian rhythms), demographic factors, and genetic variation (Yang, et al., 2010).

Caffeine is metabolised by the cytochrome P450 system which is coded for on the CYP1A2 gene (Graham, 2001; Magkos & Kavouras, 2005). Variation in this gene will have a large impact on caffeine pharmacokinetics as it affects the expression of the enzymes responsible for caffeine metabolism. This variation can directly affect the response to caffeine or indirectly by affecting other psychological and physiological processes related to the caffeine response (Yang, et al., 2010).

Variation in the CYP1A2 genotype has been shown to alter the risk for certain diseases that are associated with caffeine consumption such as Parkinson's and coronary heart disease (see Yang et al. (2010) for review). Additionally, Bebia et al. (2004) reported a lower activity of the cytochrome P450 enzyme, responsible for caffeine metabolism, in women compared to men, and Eugster et al. (1993) showed that progesterone and estradiol inhibit CYP1A2-

mediated caffeine metabolism. Therefore it is possible that sex differences exist within the CYP1A2 gene that has direct effects on caffeine metabolism.

2.5 Caffeine ingestion and effects on cognition, mood and perception

Caffeine has well recognized effects on cognition and mood (see reviews by Fredholm et al. 1999 and Lieberman, 2001). Improved cognition with caffeine supplementation has generally been attributed to increased alertness and vigilance (Lieberman, et al., 1987; Lorist, et al., 1994). In team sports, where there is a large skill component, it is likely that an important effect of caffeine will be on cognition and attenuating fatigue-related decreases in skills and concentration. Indeed, at the elite level, where there is often little difference in physical fitness, what becomes most important is the players' cognitive functioning and decision-making abilities (Williams & Reilly, 2000).

Team sports tend to last 60 – 90 min and, during the second half of a match, players will often experience fatigue-related decreases in concentration and a decline in skill performance (Mohr, Krustrup, & Bangsbo, 2005). Nevertheless, Foskett et al. (2009) found improved passing accuracy and control following a 6 $mg \cdot kg^{-1}$dose of caffeine during the Loughborough Soccer Passing Test (LSPT) – a reliable indicator of soccer skill performance (Ali, et al., 2007). The improvement in accuracy was attributed to an enhancement of motor skills and decision-making ability. Therefore caffeine has the potential to not only improve aspects of physical performance, but to improve cognitive performance.

2.5.1 Cognition

Decision-making time is a key component of successful skill performance during team sports. Many studies have used Choice Reaction Time (CRT; Teichner & Krebs, 1974) as a method

of examining the effect of caffeine on cognitive performance. Lieberman et al. (1987) administered four different doses of caffeine (32, 64, 128, and 256 mg) and found that even with a 32 mg dose CRT was improved. More recently Kruk et al. (2001) examined the effect of 5 $mg \cdot kg^{-1}$ dose of caffeine given in coffee on multiple CRT. Participants performed a graded incremental exercise test on a bicycle ergometer, and completed the CRT before, and during exercise. It was found that CRT improved with caffeine supplementation.

Other factors crucial to cognitive performance are reaction time and attention bias, and indeed players at a high level have improved selective attention (Fontani, et al., 2006), an improved ability to recognize patterns of play (Williams, et al., 2006), and will demonstrate a greater number of skilled tasks during a game (Williams & Reilly, 2000). The Stroop Test is a measure of attention bias and reaction time (Bench, et al., 1993). Hogervost et al. (1999) examined the effect of carbohydrate-electrolyte solutions with varying doses of caffeine on cognitive performance. Fifteen endurance-trained males received either water, a carbohydrate-electrolyte solution (68.8 $g \cdot L^{-1}$), or that carbohydrate-electrolyte solution plus 150 mg, 225 mg, or 320 mg of caffeine. Immediately before and after a 1 h time-trial on a cycle ergometer, cognitive performance was assessed. It was found that performance on the Stroop Test was improved with the 150 mg and 225 mg doses of caffeine in the carbohydrate-electrolyte, compared to placebo.

In a later study by Hogervorst et al. (2008), 24 well-trained cyclists performed 2.5 h cycling at 60% O_2max followed by a time to exhaustion trial at 75% O_2max. Immediately before commencing exercise, participants consumed a performance bar containing 100 mg of caffeine, an isocaloric noncaffeinated placebo bar, or 300 ml of a placebo beverage. Additional bars were given at 55 and 115 min of exercise. A Rapid Visual Information

Processing test and the Stroop Test were performed before, during, and after exercise. Caffeine resulted in significantly faster performance on the cognitive tests, and there was no speed-accuracy trade-off. The largest limitation of this study was that blinding of the caffeine-containing bar may have failed as pointed out by the authors.

As a result it appears that even small doses of caffeine will improve cognitive performance and in particular reaction time and decision-making ability.

2.5.2 Mood

The Profile of Mood States (POMS) has been extensively used to assess mood in exercise settings (Berger & Motl, 2000). It comprises seven separate sections: fatigue, anger, vigour, tension, esteem, confusion, and depression. Studies that have examined changes in mood as a result of caffeine supplementation have tended to find increased alertness, or reduced fatigue (Smith, 2002). Lieberman et al. (2002) randomly assigned 68 U.S. Navy SEAL trainees a 100, 200, or 300 mg caffeine or placebo capsule after 72 h of sleep deprivation and exposure to other continuous stressors. The POMS was administered 1 and 8 h after treatment; caffeine resulted in decreased levels of fatigue at both the 1 and 8 h time points. No other aspects of mood were affected by caffeine, including the anxiety section. From this study it is clear that a moderate dose of caffeine will improve measures of vigilance and reaction time in sleep-deprived participants. Moreover, caffeine continued to show improved performance 8 h after ingestion, even though the half-life is 4 – 6 h (Lieberman, et al., 2002). Individual reactions and habituation, which can vary extremely between participants, are likely to be responsible for this extended experience of ergogenic effects.

Penetar et al. (1993) also found similar results when they examined caffeine supplementation in 50 men who had been sleep deprived for 49 h. The participants received 0, 150, 300, or 600 mg per 70 kg body mass. Caffeine reversed the effects of sleep deprivation, and in particular in the fatigue, vigour, and confusion subsets of the POMS.

It is clear that caffeine does indeed improve mood, and in particular affects fatigue and vigour. However high doses of caffeine have also been associated with negative mood states such as increased anxiety (Loke, 1988; Roache & Griffiths, 1987). Nevertheless, these high doses are not normally consumed as part of the diet, and much of the effect of caffeine is dependent on the individual and their habituation levels (Smith, 2002). Low consumers of caffeine may find large doses induce anxiety, while moderate consumers are less likely to experience anxiety as a result of caffeine supplementation (Penetar, et al., 1993).

Therefore it is likely that participants will experience a change in mood as a result of caffeine supplementation. The extent of this change, and whether it is a positive or negative change, will depend on the individual's response and on the dosage.

2.5.3 Perception

Much of caffeine's effects are attributed to its role as an adenosine antagonist, and the subsequent effects this has on stimulation, vigilance, arousal, and pleasure-displeasure experienced during exercise. The Feeling Scale (FS; Hardy & Rejeski, 1989) and Felt Arousal Scale (FAS; Svebak & Murgatroyd, 1985) are simple scales that give an indication of the participant's subjective perceptual response to exercise. These scales are non-intrusive and easy to administer, however they are based on a single response and are more susceptible

to participant errors such as inattention, than multi-response assessments (Backhouse, et al., 2007).

Repeated administration of these scales allows a circumplex model (Russell, 1980) to be used to analyse the changes in pleasure and activation state throughout exercise. The circumplex model is an appropriate tool for analysis in nutritional intervention studies as it equally covers both pleasure, displeasure, low and high activation. As seen in Figure 2.1, the circumplex model enables an individual's perceptual response to be categorised into four quadrants.

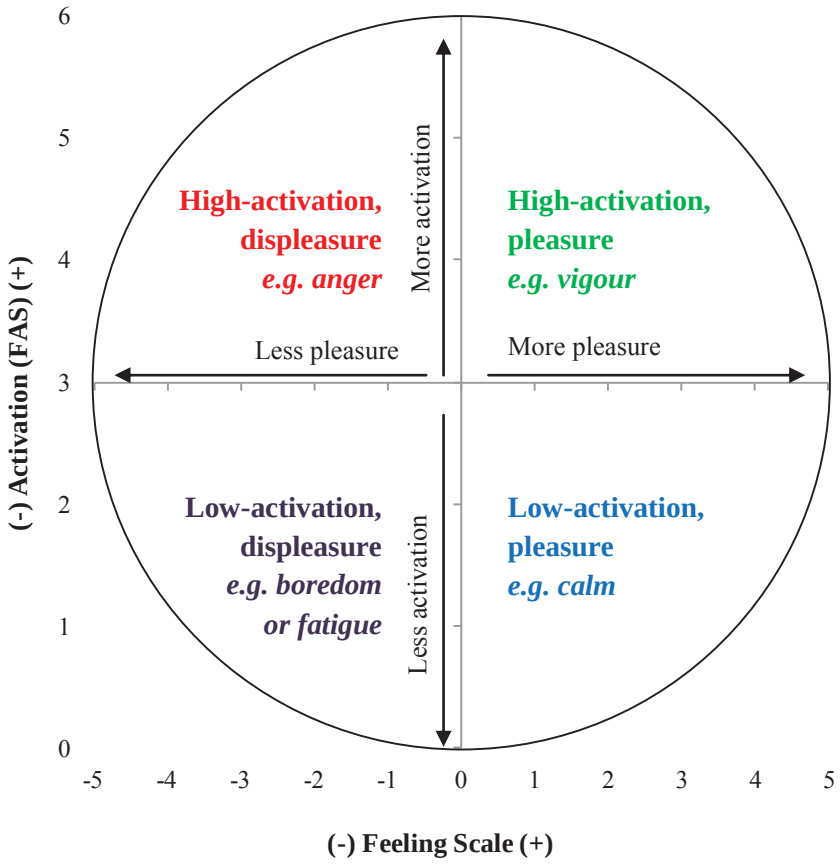

Figure 2.1 – A visual depiction of the circumplex model used for plotting the perceptual responses to exercise.

To the researcher's knowledge only one study has examined caffeine and its effects on levels of activation and pleasure during exercise. Backhouse et al. (2011) had 12 endurance-trained

male cyclists complete 1 h of cycling at 70% of O_2max after ingesting 6 mg·kg^{-1} caffeine or placebo. The RPE, FS, and FAS scales were administered frequently throughout exercise. Perceived exertion was significantly lower in the caffeine trial, and participants found the exercise more pleasurable on the caffeine compared to placebo trial. Levels of arousal did not differ significantly between trials. Therefore it appears that caffeine supplementation may result in a more pleasurable experience while exercising. However whether this occurs in females and for all forms of exercise, and not just steady-state exercise, remains to be seen.

2.6 Caffeine, strength and power

While the ergogenic effects of caffeine supplementation in endurance exercise have been well accepted, the effects on strength and power performance have only recently been investigated and the research remains equivocal.

Beck et al. (2006) investigated the effect of a caffeine-containing supplement on upper- and lower-body strength, muscular endurance, and anaerobic capacity. Thirty-seven resistance trained men took 201 mg of a caffeine-containing supplement (2.1 – 3.0 mg·kg^{-1}) one hour prior to testing. Testing involved bench press and leg extensions with repetitions to failure at 80% of one repetition max (1RM). Peak and average power were assessed in two Wingate tests separated by 4-min active rest period. Caffeine improved the 1RM of the bench press, but had no effect on lower body strength, muscular endurance or power. Considering that the participants were resistance-trained, the Wingate test was not a wholly relevant assessment protocol; instead a protocol that used resistant exercises the participants were familiar with may have been more applicable.

Woolf et al. (2008) completed a similar study where a 5 $mg \cdot kg^{-1}$ dose of caffeine was administered to a group of highly-trained team sport athletes to investigate the effects on leg press, chest press, and Wingate test. The study found that the caffeine trial resulted in a greater total mass lifted on the bench press, and a higher peak power output achieved on the Wingate test. There was no effect of caffeine on lower body strength.

In contrast, a study by Astorino et al. (2010) found that a caffeine dose of 5 $mg \cdot kg^{-1}$ improved peak flexion torque, knee flexion/extension total work, and knee extension/flexion power in the first of two maximal 40 repetition bouts. This study used recreationally active men, so these results cannot be applied to trained individuals as seen in Beck et al. (2006) and Woolf et al. (2008) which may account for the discrepancy in findings. Of interest another study by Astorino et al. (2011) also found a small but significant effect of caffeine on the number of repetitions for leg press in resistance-trained men who had relatively high habitual intakes of caffeine. However the magnitude of this improvement was small and in most cases the difference between caffeine and placebo was approximately one repetition. Therefore this increase in the number of repetitions after caffeine supplementation was of limited practical significance. The authors concluded that there was a large amount of inter-individual variation, and that further studies should try to explain the difference in effects of caffeine between individuals.

Another way to estimate leg power is to use vertical jump height which is often more practical as it can be more easily used in field settings (Harman, et al., 1991). It is accepted as a valid method for assessing leg power (Johnson & Bahamonde, 1996). Few researchers have examined vertical jump height and caffeine supplementation. Foskett et al. (2009) found that

in the caffeine trial, male team-sport athletes achieved a 2.7% higher vertical jump compared to placebo.

However examining all aspects of strength and power research, it seems unlikely that caffeine will have an effect on strength and power due to the short, high-intensity nature of anaerobic exercise (Sokmen, et al., 2008). However there are various factors that are likely to account for much of the discrepancy in the literature such as training status, caffeine habituation, and differing protocols. Future research is still needed in this area to quantify any effect of caffeine supplementation on strength and power performance, particularly in female athletes.

2.7 Caffeine, exercise, and sleep quality

As a stimulant, caffeine is likely to have detrimental effects on sleep. Brezinova (1974) administered a 300 mg dose of caffeine to six late-middle-age men 15 min before going to sleep. After caffeine ingestion, average sleep time was reduced by 2 h, the number of awakenings was increased, and time taken to fall asleep was increased. Similar findings were found in a study by Nicholson and Stone (1980) which used dosages of 100, 200, and 300 mg taken at "lights out", noting the reduced total sleep time but not finding any effect on time taken to fall asleep.

More recently Hindmarch et al. (2000) found similar results but using a protocol that more closely mimicked actual consumption throughout a 24-h period. Thirty healthy men received either one or two cups of tea (equivalent to 37.5 mg or 75 mg caffeine), one or two cups of coffee (75 mg or 150 mg of caffeine), or water in a randomised 5-way crossover design. Drinks were given four times throughout the day: 9 am, 1 pm, 5 pm, and 11 pm. Participants then completed the Leeds Sleep Evaluation Questionnaire (Parrott & Hindmarch, 1978b) the

following morning. Results indicated that the perceived ease of getting to sleep and perceived quality of sleep were reduced after consuming caffeinated beverages. Total sleep time was also reduced after consuming the caffeinated beverages. The largest effect on these variables was seen with the 150 mg dose of caffeine. Sleep assessment is very subjective and as these studies were not blinded it is possible that the participants' responses were not entirely reflective of the actual effect of caffeine, but the responses were what they thought the effect of caffeine on sleep quality should be. Therefore the results of these studies should be interpreted cautiously, and future studies should use a double-blinded methodology.

Drapeau et al. (2006) used a placebo-controlled study and found that a 200 mg dose of caffeine increased time taken to fall asleep, and shortened total time asleep in young and middle-aged moderate caffeine consumers. Youngstedt et al. (2000) used caffeine as a model for sleep disruption to see whether a vigorous acute bout of exercise would attenuate the negative effects of caffeine. Eight males of average aerobic fitness and with a moderate caffeine intake completed four protocols: cycling at 60% O_2max or quiet rest following placebo; and cycling at 60% O_2max, or quiet rest following caffeine. These protocols were performed between 4.15pm and 5.15pm. Participants received two 200 mg capsules of either placebo or caffeine on awakening, 4pm, and 2 h before bedtime. It was found that caffeine did not disturb the participants' sleep as much as originally thought and exercise only attenuated this to a small extent.

Overall it seems likely that caffeine has a negative effect on sleep quality and this must be taken into consideration when using caffeine as an ergogenic aid. Even if caffeine improves performance, if there is a negative impact on sleep, this is likely to affect subsequent performance.

2.8 Effect of caffeine on performance in team sport players

It is evident in situations of aerobic exercise, moderately to well-trained athletes will experience an ergogenic response to caffeine supplementation. Team sports are characterised by their intermittent nature as games are not continuous, but are stop-start performances. For situations that are more relevant to team sports, the effect of caffeine on high-intensity, intermittent exercise is not clear.

Table 1 provides a summary of caffeine studies that have used exercise protocols relevant to intermittent sports. These studies were selected on the basis that they all involve team sport players and therefore give a more truthful representation of the population, and a protocol relevant to intermittent sports was used. The protocols in these studies all required a task to be completed as fast as possible, instead of exercising until fatigue which is not an accurate reflection of real-life game situations (Currell & Jeukendrup, 2008).

Table 2.1 Summary of literature pertaining to caffeine supplementation and team sports

Study (year)	n	Dosage	Subjects	OCS	Protocol	Findings
Foskett et al. (2009)	12	6 mg·kg^{-1} (60 min pre-exercise)	Male soccer players	N/A	90 min Intermittent Shuttle Running (LIST) with the Loughborough Soccer passing test (LSPT)	Caffeine improved jump height by 2.7%, and passing accuracy.
Glaister et al. (2008)	21	5 mg·kg^{-1} (60 min pre-exercise)	Male sport science students (majority regularly participated in multiple sprint sports)	N/A	12 x 30 m sprints on interval of 35 s	Caffeine resulted in 1.4% reduction in fastest sprint time. No effect on RPE.
Mohr et al. (2011)	12	6 mg·kg^{-1} (70 min pre-exercise)	8 male and 4 female team sport players	N/S	Yo-yo intermittent recovery level 2	Caffeine resulted in 16% improvement compared to placebo.
Paton et al. (2001)	16	6 mg·kg^{-1} (60 min pre-exercise)	Male team sport athletes	N/A	10 x 20 m sprints on interval of 10 s	Negilible difference between caffeine and placebo trials on sprint speed
Schneiker et al. (2006)	10	6 mg·kg^{-1} (60 min pre-exercise)	Male team sport athletes	N/A	2 x 36 min protocol - 18 x 4 s sprint 2 min recovery cycle ergometer	Total work during sprints of first half was 8.5% greater with caffeine, and work in second half was also greater. Mean peak power scores in first and second halves were higher in caffeine trial.
Stuart et al. (2005)	9	6 mg·kg-1 (70 min pre-exercise)	Rugby players (male)	N/A	2 x 40 min circuits (simulating rugby game) - 20 m sprint speed - 30 m sprint speed - Offensive sprint - Defensive sprint - Drive 1 power - Drive 2 power - Tackle speed - Passing ability	Mean improvements of 0.5% - 3% in sprint task, with greater improvement seen in second half. 10% improvement of passing ability.

N/A - not applicable; N/S - not specified

Studies that have investigated repeated sprint ability have found conflicting results. Paton et al. (2001) found a negligible effect of caffeine on 10 x 20 m sprint performance. However, they did not measure resting and pre-exercise plasma caffeine concentrations to assess adherence to a 48 h washout period, and also did not measure caffeine concentrations during or after the protocol. The protocol used was not accurately representative of a game i.e. ten repeated sprints is a large underestimation of the demands of a game that will generally last longer than 60 min. Glaister et al. (2008) who also used a repeated sprint protocol, critiqued the study of Paton et al. (2001) for using single photocell beams to determine sprint time when a study by Yeadon et al. (1999) had recommended the use of a double beam system. Glaister et al. (2008) then used a double beam system for their sprint timing and found that caffeine improved sprint time compared to placebo by 1.4%.

Schneiker et al. (2006) used 10 male team sport athletes in a high-intensity exercise protocol performed on a cycle ergometer, lasting 72 min. Caffeine supplementation improved measures of total work and average peak power. However this exercise protocol, while involving repeated sprints, is not a valid test for team sport athletes as the exercise was performed on a cycle ergometer despite team sports being running-based activities.

Stuart et al. (2005) and Foskett et al. (2009) closely mimicked game-like situations in their efforts to assess the effect of caffeine supplementation on aspects of intermittent games performance. Stuart et al. (2005) reported an improvement in passing accuracy as a result of caffeine ingestion. However the passing test was measured as a closed skill instead of an open skill. Open skills are more representative of real-life game situations as the environment is not constant and this dictates factors influencing passing accuracy such as when, and how, to pass. Despite this Foskett et al. (2009) assessed soccer passing as an open skill and

confirmed the findings of Stuart et al. (2005). The participants' soccer passing accuracy, as assessed by the Loughborough Soccer Passing Test, was improved following caffeine administration. This was due to the decrease in penalty time accrued i.e. passing accuracy was improved in the caffeine trial compared to placebo.

From these studies the effect of caffeine on performance in team sport players appears to be ergogenic, but further research is still needed as there are many differing features of team sports that contribute to a successful performance. Future research needs to investigate these areas, and also look to use appropriate exercise protocols or field-studies where possible.

2.9 Caffeine and women/sex effects

While research into caffeine supplementation has been a popular subject, the majority of the studies have used male participants and the results then generalised to include females. Female-specific research is needed into caffeine supplementation as men are physiologically different from women as evidenced by differing concentrations of sex hormones and lean body mass, both factors which can affect metabolism (Graham & McLean, 1999).

2.9.1 Effect of menstrual cycle on caffeine metabolism

The effect of the female menstrual cycle on caffeine metabolism is not clear. Lane et al. (1992) investigated caffeine metabolism during the follicular and luteal phases of the menstrual cycle in 10 healthy women using repeated 24-h elimination studies. Systemic caffeine clearance was reduced in the late luteal phase prior to menstruation, although the half-life was not reportedly different. The authors attributed this reduced elimination to the increase in progesterone concentration associated with the beginning of menstruation,

however noted that this reduced elimination may not be of clinical significance to most women.

In contrast, Kamimori (1999) found no effect of the menstrual cycle on caffeine metabolism. Blood samples were collected throughout the menstrual cycle in the follicular, ovulatory, and luteal phases to establish concentrations of progesterone and estradiol. Ten healthy women were then given a 300 mg dose of caffeine in 100 ml of lemonade in each phase of the menstrual cycle. Following caffeine administration, blood samples were taken regularly over a 24-h period. Plasma caffeine concentrations were not different across the menstrual cycle phases. However this study can be criticised for its use of an absolute rather than relative dose as this would significantly affect the levels of caffeine each individual attained. Likewise, McLean & Graham (2002) found similar results in their study comparing the effects of exercise and thermal stress on caffeine pharmacokinetics. This study used a relative caffeine dose of 6 $mg \cdot kg^{-1}$ body mass rather than an absolute dose, therefore eliminating the inter-individual differences in size.

Therefore, it appears that there is no definite conclusion regarding the effect, if any, of the menstrual cycle on caffeine metabolism.

2.9.2 OCS use and the effect on caffeine metabolism

While the effect of the menstrual cycle on caffeine pharmacokinetics remains unclear, it is known that oral contraceptive steroid (OCS) use is associated with impaired clearance of caffeine (Abernathy & Todd, 1985; Patwardhan, et al., 1980; Rietveld, et al., 1984). An early study by Patwardhan et al. (1980) indicated that OCS use resulted in a longer half-life of caffeine and a decreased clearance rate after taking a 250 mg capsule of caffeine. This

resulted in overall impaired caffeine elimination in women taking OCS. This study used women who were taking a variety of OCS products all of differing hormonal compositions. Some of the OCS products could be classified as low-dose estrogen products, while other OCS products used by these women could be considered to have high doses of estrogen. As a result, Abernathy et al. (1985) performed a similar study examining caffeine pharmacokinetics but in women who were taking an OCS with an estrogen component of <50 μg. The women received a 162 mg dose of caffeine and regular blood samples were taken for the first 10 h, and then a single venipuncture 24 h after caffeine administration. Again an increased caffeine half-life and reduced clearance were seen in the OCS participants compared to the non-OCS users.

While these studies are older and can be criticised for using absolute doses of caffeine, and not always controlling for OCS type and composition, it is accepted that OCS use results in impaired caffeine clearance and increased half-life (Graham, 2001; Graham & McLean, 1999). However to the researcher's knowledge this has not been investigated in an exercise setting.

2.9.3 OCS use and intermittent exercise

Studies have also investigated the effect of taking an OCS on various aspects of athletic performance. It is beyond the scope of this literature review to address all aspects of athletic performance (see Constantini et al. (2005), Burrows & Peters (2007) for recent reviews), instead the effect of OCS on intermittent exercise shall be examined in more detail.

Lynch et al. (1998) used an intermittent treadmill protocol to examine the effect of the menstrual cycle and OCS on intermittent exercise and certain metabolic markers in 15

untrained women. Ten normally menstruating women and 5 women taking an OCS were required to complete repeated 20-s sprints with a 100-s passive recovery after each bout. The initial speed was set at 14.3 $km \cdot h^{-1}$, and increased 1.2 $km \cdot h^{-1}$ on every subsequent sprint. The non-OCS group were tested during the mid-follicular phase (MFP) and during the late luteal phase (LLP), while the OCS group were tested 1 week after taking the OCS (i.e. 1 week after cessation of the placebo pills) and then again 1 week after this. Performance times were identical between the non-OCS and OCS group, and there were no differences in lactate concentrations or heart rate. Plasma ammonia concentration was higher in the non-OCS group compared to the OCS group throughout recovery. Overall it appears that intermittent exercise performance is not affected by the number of days taking the OCS, and it does not vary between the MFP and LLP.

Lynch, Vito and Nimmo (2001) then did another study using the same intermittent exercise protocol in 9 untrained women who were all taking low-dose monophasic oral contraceptives. The two tests were performed during days 5-8 and 19-21 of one pill cycle. Performance time did not differ between the two trials; neither did heart rate or any metabolic markers (peak lactate, ammonia, glucose, glycerol, and resting FFA concentration).

It appears that there is little difference in intermittent exercise performance and energy metabolism between days 5-8 and 19-21 of one pill cycle, and this allows future researchers to use female participants and test at these time points without fear of confounding effects of timing during the pill cycle. However neither of these studies used trained female intermittent sports players and the actual intermittent exercise protocol may not have been of sufficient intensity to produce a significant metabolic challenge. Future studies should seek to use trained female athletes in a protocol that is more metabolically challenging.

2.9.4 Performance in female athletes with caffeine supplementation

Table 2 provides a summary of the few studies that have examined caffeine supplementation in females using an exercise protocol. These studies were selected due to their use of female participants and because caffeine was used as an ergogenic aid during exercise.

The most obvious critique is the failure of some of these studies to account for OCS use or to even report it. Another important factor to note is that none of these studies have accurately examined intermittent exercise, and this remains an area for further study. Furthermore, Ahrens et al. (2007) used a very short exercise protocol that would not be considered physically demanding even for recreationally active women, while in contrast Anderson et al. (2000) used an appropriately relevant test for oarswomen. Goldstein et al. (2010) was the first group to investigate the effect of caffeine on upper body strength in resistance-trained women. Caffeine had a positive effect on strength but not on muscular endurance. This is still a relatively new area of caffeine supplementation and as such requires further investigation.

Table 2.2 Summary of literature pertaining to caffeine supplementation and exercise performance in women

Study (year)	n	Dosage	Subjects	OCS	Protocol	Findings
Ahrens et al. (2007)	20	3 mg·kg^{-1} 6 mg·kg^{-1} (60 min pre-exercise)	Recreationally active	N/S	3 trials of 8 min walking at 5.6 km·h^{-1}	Increased REE and relative VO_2 only for 6 mg·kg^{-1} compared to placebo
Anderson et al. (2000)	8	6 mg·kg^{-1} 9 mg·kg^{-1} (60 min pre-exercise)	Competitive rowers	No	3 x 2000 m trials on a rowing ergometer	9 mg·kg^{-1} increased average power output by 2.7% and 1.3% improvement in TT performance. 6 mg·kg^{-1} increased average power output by 1.4% and 0.7% improvement in TT performance
Goldstein et al. (2010)	15	6 mg·kg^{-1} (60 min pre-exercise)	Reistance trained	N/S	1RM barbell bench press and repitions to failure at 60% of 1RM	Greater bench press maximum with caffeine, no effect on repititions to failure at 60% of 1RM
McLean & Graham (2002)	8 women 6 men	6 mg·kg^{-1} (60 min pre-exercise)	Active	No	4 x 8 h trials 90 min cycling at 65% VO_2max	No difference between trials in hematocrit, estradiol, progesterone, HR, temperature measurements, or O_2 consumption. No gender difference for urinary caffeine levels of O_2 consumption.
Motl et al. (2006)	11	5 mg·kg^{-1} 10 mg·kg^{-1} (60 min pre-exercise)	N/S	No	30 min cycling at 60% VO_2peak	No effect on HR, systolic BP or power output. Both doses decreased ratings of leg muscle pain

N/S - not specified

2.10 Summary

It has been well demonstrated that caffeine is an ergogenic aid in many exercise situations. The mechanisms for this ergogenic effect remains unclear due to caffeine's ability to affect a multitude of tissues and organs throughout the body, but it is widely believed that the major mechanism relates to caffeine's role as an adenosine antagonist. Caffeine is known to have effects on cognition, metabolism, sleep, perception, and mood. The majority of studies into caffeine supplementation have used male endurance athletes and, as a result, it is possible that the guidelines for caffeine supplementation are not applicable to either women, or team sport players. Men and women are likely to have varied responses to caffeine due to differing concentrations of sex hormones. Few studies have examined female athletes and caffeine supplementation in an exercise setting but they have tended to support an ergogenic effect of caffeine. OCS use is known to slow the metabolism of caffeine, and a growing number of female athletes use OCS to reduce cycle length variability. Future studies need to have more rigorous controls for menstrual cycle and OCS use, so that appropriate caffeine supplementation guidelines for females can be set.

CHAPTER 3 – METHODOLOGY

3.1 Participants

Ten female team sports players (mean ± SD; 24 ± 4 years; 59.7 ± 3.5 kg; maximal oxygen uptake (O_2 max) 50.0 ± 5.3 ml·kg^{-1}·min^{-1}) volunteered to participate in this study which was approved by the Massey University Ethics Committee. Participants were informed of the study requirements, benefits and risks before providing written consent. Participants played soccer, netball, hockey, basketball or rugby.

3.2 Subject control

3.2.1 Dietary controls

Participants varied in their daily caffeine intake (estimated range 0 – 300 mg·day^{-1}) based on self-reported daily consumption of common caffeine sources (not all participants consumed caffeine products every day; instead some indicated they consumed caffeine-containing products on a weekly basis). Participants were excluded from this study if they were considered to be caffeine naive and actively refrained from consuming caffeine-containing products, or if there was a high daily intake of caffeine (more than four cups a day).

To reduce the potential for diet-induced variability between trials all participants were asked to keep a 48-h food diary before their first main trial and to replicate this diet before their second main trial. During this 48-h period, participants were asked to avoid alcohol and foods containing caffeine (chocolate, tea, coffee, soft-drinks, and energy drinks). They were also asked to refrain from exercise for the final 24 h of this period and to fast for 3 h prior to the main with only water consumption allowed. Food diaries were analysed using Foodworks (version 6.0.2562, 2009, Xyris Software).

3.2.2 Oral contraceptive use and cycle control

All participants were taking a monophasic oral contraceptive of the same hormonal composition (Microgynon, Levlen ED, Nordette, or Monofeme: 30 µg Ethinyloestradiol and 150 µg Levonorgestrel; Your Guide to Oral Contraceptives, 2004) and had been taking it for at least 3 months prior to beginning the study.

To control for possible differences during the oral contraceptive cycle, all testing was performed during days 5-8 and 18-22 of the one pill-cycle. No changes in energy metabolism or high-intensity intermittent exercise performance have been reported between these periods of the menstrual cycle for participants taking a low dose monophasic oral contraceptive (Lynch & Nimmo, 1998; Lynch, et al., 2001). Participants were required to keep a daily record of their oral contraceptive use, recording the day and time administered. Participants were not required to take the oral contraceptive at the same time each day as the oral contraceptive pill has a 48-h leeway period (H. Roberts, personal communication, January 21, 2011). However, for the days of the main trial and the following morning session, participants were asked to keep the timing as consistent as possible to ensure similarity between trials.

3.3 Description of physiological tests and measures

3.3.1 Height and mass measurements

Height was measured using a stadiometer. Participants were instructed to place the backs of their heels in contact with the wall behind them and stand upright before the measurement was taken. Participants were asked to remove their shoes before body mass was measured using scales accurate to 0.1 kg (A & D Weighing, HV-200KGL, Australia).

3.3.2 Heart rate measurements

Heart rate (HR) was monitored and recorded throughout the O_2 max test and main trials, using short range telemetry (Polar RS400, Kempele, Finland). Data was downloaded using the Polar ProTrainer computer software (version 5.35.160, Finland).

3.3.3 Urine analysis

Hydration status was assessed before caffeine ingestion, and 12 h post-exercise, by measuring urine specific gravity using a hand-held refractometer (Sur-Ne, Atago Co. Ltd., Japan).

3.3.4 Expired air collection

Expiratory gas measures were taken to estimate rates of carbohydrate and fat oxidation using the respiratory exchange ratio (RER = CO_2/ O_2). Collection was performed using the PowerLab metabolic system (8M, Model ML870, AD Instruments, Australia) and analysis of the expired air for CO_2 and O_2 concentrations was performed using a gas analyser (Servomex, Model ML206, AD Instruments, Australia).

3.3.5 Strength and power testing

3.3.5.1 Isokinetic dynamometer

Leg strength and power were assessed using an isokinetic dynamometer (Model 850-230, Biodex Medical Systems Inc., New York). The participant sat upright in the Biodex chair and the researcher adjusted the position of the chair such that the axis of rotation about the dynamometer arm was in line with the lateral femoral epicondyle of the dominant leg. The participant was secured by straps across the thigh and torso to minimise unnecessary movement. Calibration was performed before each test according to the Biodex setup protocol, in which angles for joint range of motion were set individually.

Participants performed five reciprocal concentric contractions of the knee extensors and flexors at $30°\cdot s^{-1}$ which was repeated using eccentric contractions. The participant then performed isometric contractions using the knee flexors and extensors with the joint angle at the knee set at 75°. The four protocols were programmed to be performed one after the other, with little or no rest period due to time constraints. All participants used their dominant leg, received verbal encouragement, and visual feedback as displayed by the Biodex computer. Peak torque was measured and this was defined as the highest value achieved across the five contractions. Average power was reported for isokinetic concentric and eccentric contractions of the knee flexors and extensors as calculated by the Biodex software (Biodex Advantage Software, V.4X).

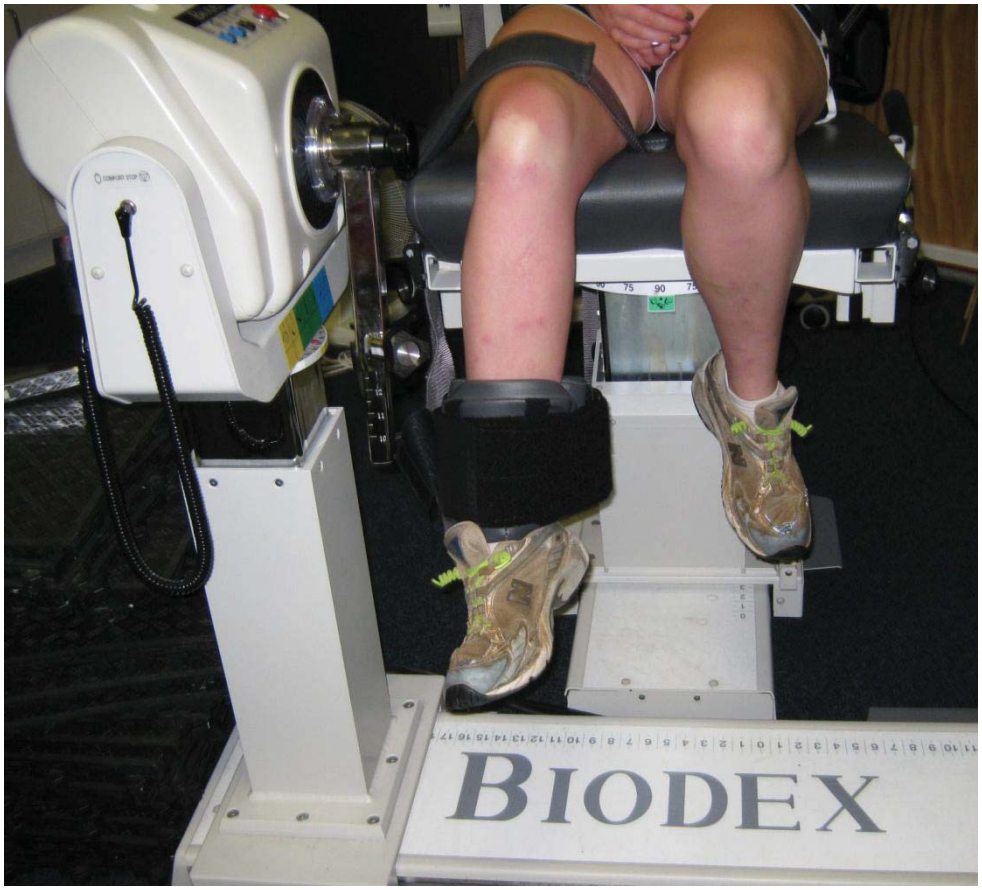

Figure 3.1 – Isokinetic dynamometer (Biodex) setup**.**

3.3.5.2 Countermovement jump

A countermovement jump (CMJ) was performed using a jump mat (Just Jump System 7610, Perform Better, USA) to estimate leg power as calculated from jump height. Power was determined as (Harman, et al., 1991):

Power (W) = 61.9 x jump height (cm) + 36.0 x body mass (kg) - 1822

During the CMJ participants kept their hands on their hips to minimise momentum generated from arm and upper body movements, which may have introduced uncertainty in measurements due to differing movements used by a participants.

3.3.6 Blood sampling and analysis

3.3.6.1 Sample collection

An 18-gauge, 1.3-mm intravenous cannula (reference 381244, Insyte, Becton Dickson, NJ, USA) was inserted into a medial antecubital vein, and 10-ml blood samples were taken at rest, every 15 min during exercise, immediately post-exercise and 12 ± 2 h post-exercise. The cannula was kept patent by regular flushing with saline (0.9% sodium chloride, Demo S.A. Pharmaceutical Industry, Athens, Greece).

3.3.6.2 Treatment, storage, and analysis of blood samples

Of the 10 ml collected, 6 ml was placed in EDTA tubes and 4 ml in heparin tubes and both centrifuged for 10 min at 3500 rpm (MF 50, Hanil Science Industrial Co., Ltd., Korea). Following this the plasma was stored at -70°C for later analysis of free fatty acids (FFA), glucose, and insulin from the EDTA tubes, and caffeine from the heparinised tubes.

A high-performance liquid chromatography (HPLC) method was used for determination of plasma caffeine concentrations (Holland, et al., 1998). Samples were thawed immediately before the assay, and were deproteinised by adding 350 µl of plasma to 350 µl of 0.8 *M* perchloric acid (BDH Poole, England). After thorough mixing (Vortex Mixer, Labnet International, Inc, Woodbridge, NJ), the deproteinised samples were centrifuged (Prominence model, Heraeus Labofuge 400R, Thermo Fisher Scientific New Zealand Ltd, North Shore City, New Zealand) at 11500 g for 4 min at room temperature. Two, 200 µl aliquots of the supernatant were removed and placed in an HPLC glass vial (Thermo Fisher Scientific New Zealand Ltd, North Shore City, New Zealand). To this a further 61.4 µl of 0.4 *M* sodium hydroxide (Mallinckrodt Baker Inc, Phillipsburg, NJ) were added to make the pH of the sample approximately 6 -7. Samples were then run through the HPLC and the caffeine concentration was read at a wavelength of 274 nm.

An assay based on the hexokinase method (see Appendix 15) was used to establish plasma glucose concentrations, and using a commercially available kit (Wako Pure Chemical Industries, Ltd. Osaka, Japan) FFA concentration was determined based on the ACS-ACOD method (see appendix 16). These analyses were carried out in a commercially run laboratory.

3.3.7 Maximal oxygen uptake (O_2 max) test

Participants performed a maximal oxygen uptake test (O_2 max) during the familiarisation session. This was carried out on a treadmill with a 1% incline, at an initial speed of 8 $km \cdot h^{-1}$ which increased by 1 $km \cdot h^{-1}$ every 2 min, until the participant signalled they could only continue for a further minute. Heart rate and expired air data was collected throughout the duration of the test. In addition, during the final minute, the expired air was collected into a Douglas bag, and analysed for CO_2 and O_2 concentrations using a gas analyser (Servomex,

Model ML206, AD Instruments, Australia), and the Harvard dry gas meter (Serial number K015078, Harvard Apparatus, MA, USA). Heart rate data and expired air were collected continuously throughout the duration of the test.

3.4 Description of cognitive measures

3.4.1 Cognitive testing

Cognitive function was assessed using a computer software program (Computerised Mental Performance Assessment System [COMPASS], Northumbria University, Newcastle, United Kingdom). Participants were required to perform two tasks: the Choice Reaction Time test (CRT) and the Stroop Test (St). While performing the cognitive testing the participants wore earmuffs, and the computer was positioned facing a blank wall, to minimise distractions to the participant that may have impeded performance.

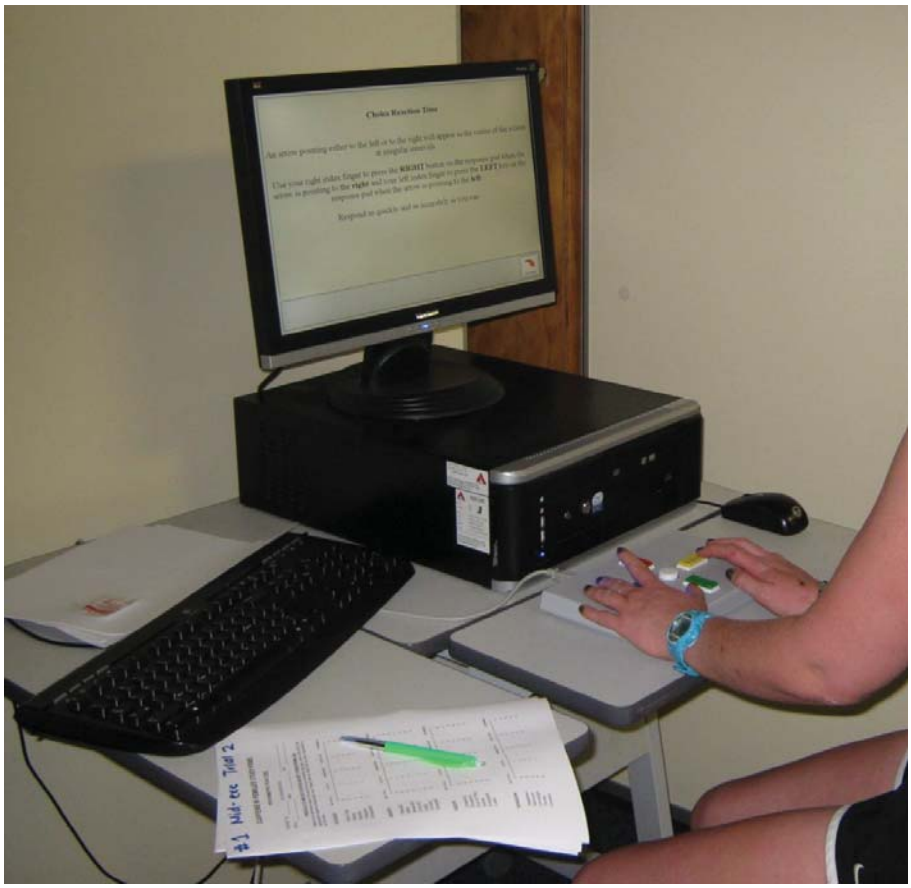

Figure 3.2 – COMPASS software for administering cognitive tests.

3.4.1.1 Choice Reaction Time test

The Choice Reaction Time (CRT) test was used as a measure of decision-making time (Teichner & Krebs, 1974). Participants were presented with more than one possible response for each test, which involved an arrow displayed on the screen facing either left or right. The participant had to select the correct corresponding left or right button as quickly as possible. The test involved the presentation of 35 stimuli.

3.4.1.2 Stroop Test

The Stroop Test was used as a measure of attention bias and reaction time (Bench, et al., 1993). For the Stroop Test participants were shown words for certain colours, though the actual colour of the word often differed from the colour the word represented, e.g. the word "blue" was displayed in a red font. The participants were required to respond with the colour of the font, and disregard the name of the colour. The duration of the task was 1.5 min.

3.4.2 Profile of Mood States questionnaire

The Profile of Mood States (POMS; Grove & Prapavessis, 1992) was used to assess changes in mood as a result of exercise and/or caffeine supplementation. The participant was presented with 40 adjectives that were grouped into seven subgroups (fatigue, anger, vigour, tension, esteem, confusion, and depression). Participants selected how well each adjective described the way they felt at that point in time using a 5-point Likert scale that ranged from "*Not at all*" to "*Extremely*".

3.5 Description of perceptual scales

3.5.1 Ratings of Perceived Exertion

The Ratings of Perceived Exertion scale (RPE; Borg, 1982) was used to measure the subjective intensity of each 15-min block of exercise. It is a 15-point scale ranging from 6 – 20 and corresponding verbal anchors that range from "*No Exertion at all*" to "*Maximal Exertion*". The standard instructions were read and shown to the participants (Appendix 11). Measures were taken throughout exercise, however this only indicates the level of exertion the participant is experiencing and does not give any indication of the participants other emotions during exercise, and it does not estimate the participant's perceived activation.

3.5.2 Feeling Scale

The Feeling Scale (FS; Hardy & Rejeski, 1989, Appendix 12) was used to assess pleasure or displeasure felt during exercise. It is an 11-point scale ranging from -5 "*Very Bad*" to +5 "*Very Good*".

3.5.3 Felt Arousal Scale

The Felt Arousal Scale (FAS) is a 6-point (0 – 5) scale that measures changes in perceived activation or arousal throughout exercise (Svebak & Murgatroyd, 1985; Appendix 13). This scale is based upon Apter's Reversal Theory (Apter, 1989), and is valid in an exercise setting as it requires a subjective assessment of the participants' perceived activation and arousal. High activation or arousal is characterised as anger or excitement, while low activation is representative of calmness or boredom.

3.6 Description of sleep quality measures

3.6.1 Leeds Sleep Evaluation Questionnaire

The Leeds Sleep Evaluation Questionnaire (LSEQ; Parrott & Hindmarch, 1980; Appendix 10) was used to examine the effect of caffeine on sleep quality the morning after both main trials. The LSEQ comprises 10 self-rating 100-mm line visual analogue scales. Each 100-mm horizontal line starts and finishes with opposite states, for example *'not alert'* and *'alert'*. The participants placed a vertical mark on the line to indicate how they felt the morning after that particular trial, and whether the intervention affected certain aspects of their sleep (Parrott & Hindmarch, 1980). Questions were asked regarding the speed of sleep onset, the quality of the sleep, and how alert the participant was on waking. The LSEQ has often been used to assess changes in sleep patterns due to pharmacological interventions such as the use of central nervous system (CNS) stimulants like caffeine (Zisapel & Laudon, 2003).

3.7 Description of intermittent running protocol

The intermittent exercise protocol consisted of six, 15-min blocks of treadmill running (Atkinson, et al., 2005). As seen in Figure 3.3, each block included running six 2 min periods at varying speeds, corresponding to percentages of O_2 max: 40%, 60%, 80%, 40%, 60%, and 80%. This was followed by 1 min at 60% O_2 max, and a 2-min walk (4 $km \cdot h^{-1}$).

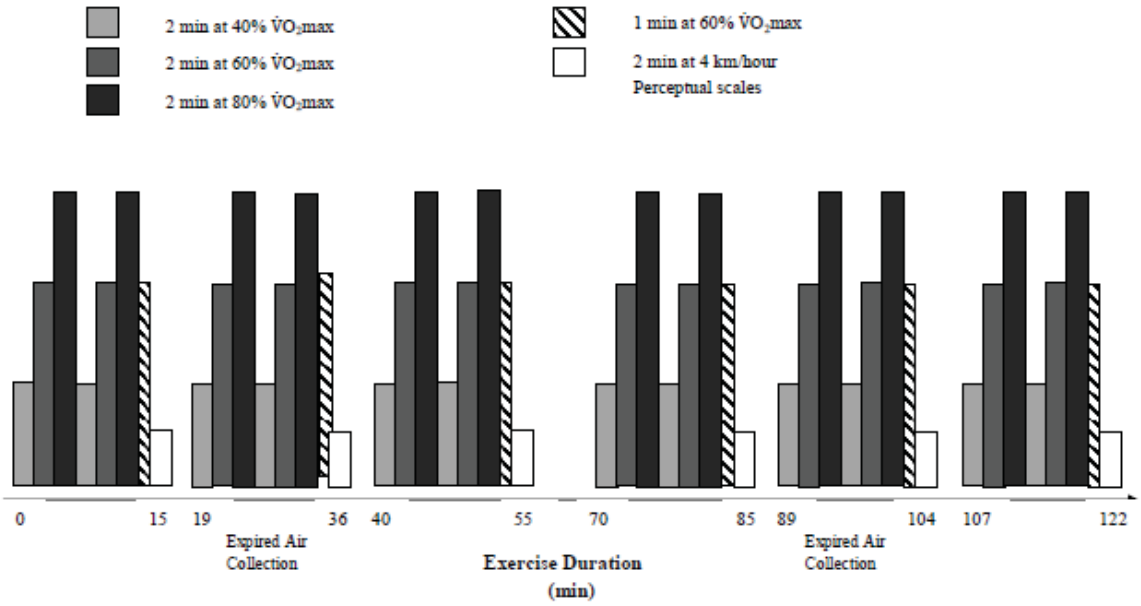

Figure 3.3 – Intermittent treadmill running protocol based on each participants O_2 max (Atkinson, et al., 2005).

3.8 Preliminary familiarisation testing

Participants were required to perform one familiarisation session before commencing the main trials. This required the participants to perform an incremental O_2 max test on a treadmill, undergo a minimum of three attempts at the cognitive testing (Choice Reaction Time and the Stroop Test), and a minimum of three attempts at the strength and power testing using the isokinetic dynamometer. The participants were encouraged to perform these tests until they felt comfortable with the procedures involved, or the results indicated that any variation due to a learning effect had decreased and their scores reached a plateau. Perceptual scales (RPE, FS, and FAS) and the POMS were also explained to the participant before the main trials. The preliminary session finished with the participant completing two 15-min blocks of the intermittent treadmill running protocol to familiarise themselves with the running patterns involved, and the overall experimental procedures.

At the familiarisation session participants were given four copies of the LSEQ to complete before they commenced main trials. Participants were asked to complete these for two nights' sleep where they had performed exercise after 6 pm, and two nights' sleep where no exercise was performed after 6 pm. Each LSEQ was completed upon awakening after the certain nights' sleep. The results from these questionnaires gave a baseline indication of sleep patterns for each participant.

3.9 Study design

After the familiarisation trial, participants returned during days 5-8 and 18-22 of their pill cycle to begin main trials which were completed in the evening at approximately 6 pm in order to investigate the effects of caffeine on sleep quality. To eliminate any trial order effect, treatments were randomly assigned using a placebo-controlled, double-blind crossover design.

Figure 3.4 shows a detailed schematic of the procedures involved in each main trial. On arrival participants provided a urine sample, body mass was measured, and a heart rate monitor fitted. Following this the participants completed the cognitive testing, strength and power testing (including CMJ), the POMS and perceptual scales (excluding RPE), before having a cannula inserted and a blood sample taken. Participants then ingested a whole gelatin capsule (Vegie Capsules, BioBalance, New Zealand) containing either 6 $mg \cdot kg^{-1}$ anhydrous caffeine (Fluka Sigma-Aldrich, St Louis, MO) or placebo (artificial sweetener, Equal). This was administered in a randomized double-blind manner with a 500-mL bolus of water. After consuming the capsule, the participant was required to quietly wait 45 min before beginning the intermittent running protocol which consisted of 90-min treadmill-running split into 6 x 15-min blocks (Figure 3.3.).

After the first, second, fourth and fifth blocks of running, the participant had a 4-min break in which a CMJ was performed, a blood sample taken, and water was administered (3 $ml \cdot kg^{-1}$). After the third block of running there was a 15-min break, where the above procedures were repeated but the participant also completed the POMS, the cognitive testing, and the physical testing on the Biodex. Further to this, in blocks 2 and 5, expired air was collected from the participant for the entire 15-min duration of each block.

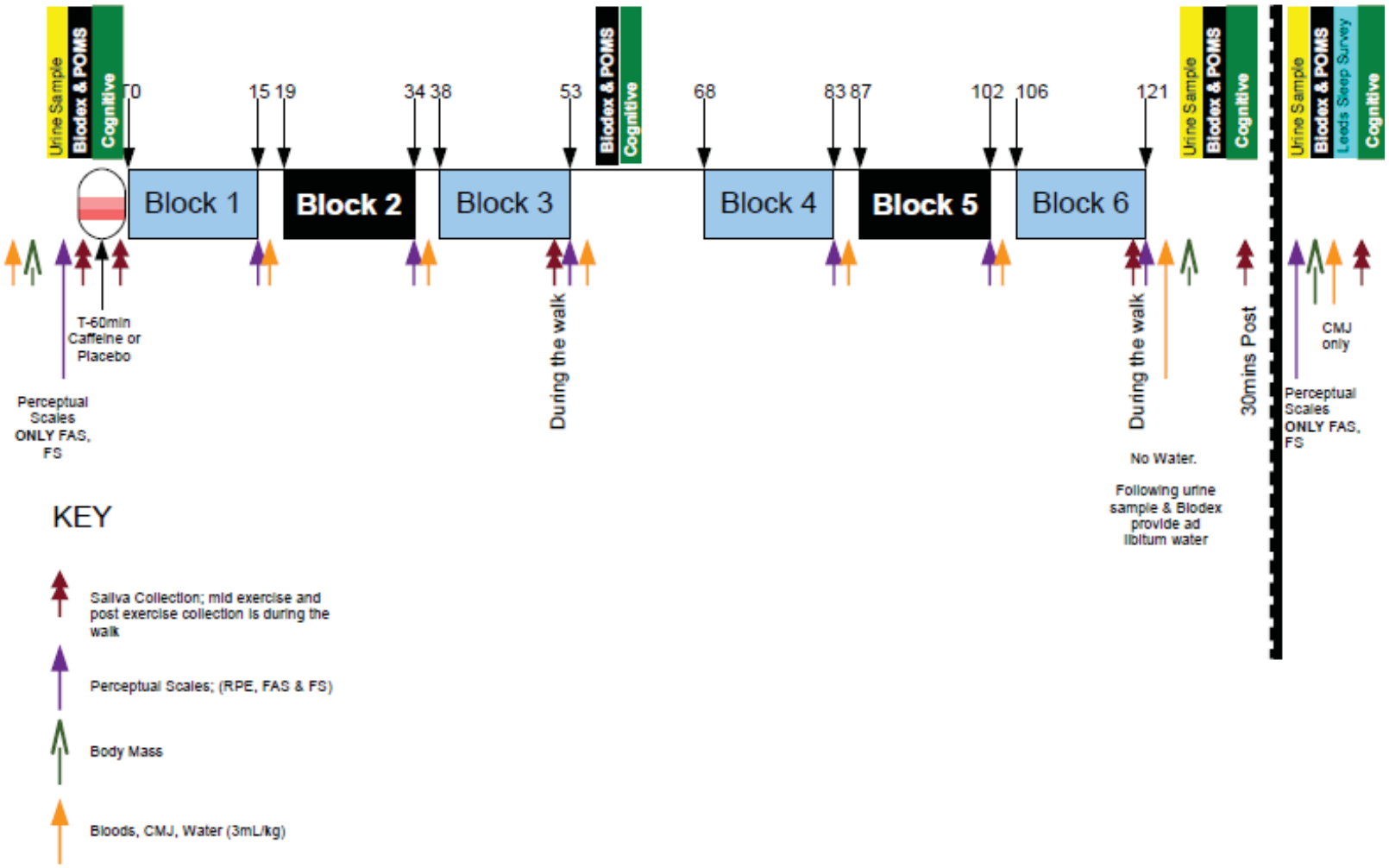

Figure 3.4 – Detailed schematic of main trials. RPE – Rate of perceived exertion. FS – Feeling scale. FAS – Felt arousal scale. CMJ – Countermovement jump. POMS – Profile of Mood States questionnaire

3.10 Statistical analysis

Sample size was estimated using data from Kalmar and Cafarelli (1999b), who investigated the effect of caffeine ingestion on muscle function, and an appropriate statistical package (GPower 3.1). A sample size of 10 was sufficient to detect 3.5% change in muscle strength performance between caffeine and placebo trials, with a power of 0.84 and at a *P*-value <0.05.

Statistical analyses were completed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL) Version 17.0. Data is expressed as mean ± standard deviation (SD). A two-way analysis of variance (ANOVA) was used to determine the difference between the caffeine and placebo conditions at each time point for perceptual and mood data. A mixed model analysis was used for strength/power and cognitive performance data. Post-hoc testing used paired *t*-tests with the stepwise Holm-Bonferroni method to determine where the differences lay. Pearson's correlation coefficient was used to examine relationships between outcome measures. An *r* value of 0.5 - 0.7 represents a weak correlation, 0.7 - 0.8 is a moderate correlation, and ≥ 0.8 is a strong correlation (Vincent, 2005). A *P*-value of <0.05 was considered indicative of statistical significance. Furthermore, effect sizes (Cohen's d) were calculated, where appropriate, to show practical significance. A value of 0.2 was considered a small effect, 0.5 moderate, and 0.8 a large effect (Cohen, 1988).

CHAPTER 4 – RESULTS

Participants completed approximately 12.1 km over the 90 min intermittent treadmill running. Environmental temperatures were not different between trials (20.3 ± 0.7°C and 20.4 ± 0.8°C). Following completion of each trial, participants were asked to speculate what treatment they had received: placebo or caffeine. Nine out of ten participants correctly identified the caffeine and placebo trials.

4.1. Strength and power data

There was no effect of caffeine on isometric strength in the knee extensors (CAFF vs. PLA, 155.0 ± 40.0 N m vs. 156.3 ± 37.0 N m; $F_{1, 45} = 0.047$, $P = 0.830$, Cohen's d = -0.03) or knee flexors (CAFF vs. PLA, 79.8 ± 19.7 N m vs. 76.9 ± 17.1 N m; $F_{1, 54} = 0.340$, $P = 0.562$, Cohen's d = 0.16).

During eccentric contractions, caffeine ingestion increased peak torque values for the knee flexors (CAFF vs. PLA, 179.3 ± 50.3 N m vs. 160.1 ± 44.0 N m; $F_{1, 45} = 11.5$, $P = 0.001$, Cohen's d = 0.41), and there was also a trend for caffeine ingestion to increase peak torque in the knee extensors (CAFF vs. PLA, 122.0 ± 39.3N m vs. 112.2 ± 32.5 N m; $F_{1, 45} = 2.93$, $P = 0.094$, Cohen's d = 0.29). However, during concentric contractions, peak torque values were not significantly different between caffeine and placebo trials for knee extensors (CAFF vs. PLA, 114.3 ± 36.2 N m vs. 107.1, 36.8 N m; $F_{1, 45} = 2.39$, $P = 0.129$, Cohen's d = 0.2), or knee flexors (CAFF vs. PLA, 78.9 ± 18.5 N m vs. 78.2 ± 21.1 N m; $F_{1, 45} = 0.117$, $P = 0.734$, Cohen's d = 0.03).

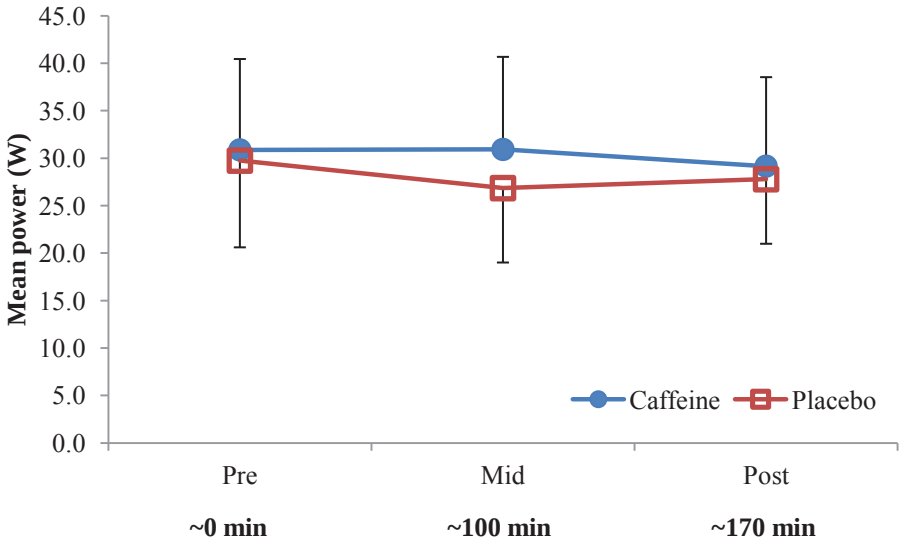

Figure 4.1 – Mean power output during eccentric contractions of knee extensors in caffeine and placebo trials. Values are mean ± SD. The 'pre' time point represents before caffeine/placebo administration; 'mid' is after block 3 during the 15 min break; and post following the final block of exercise.

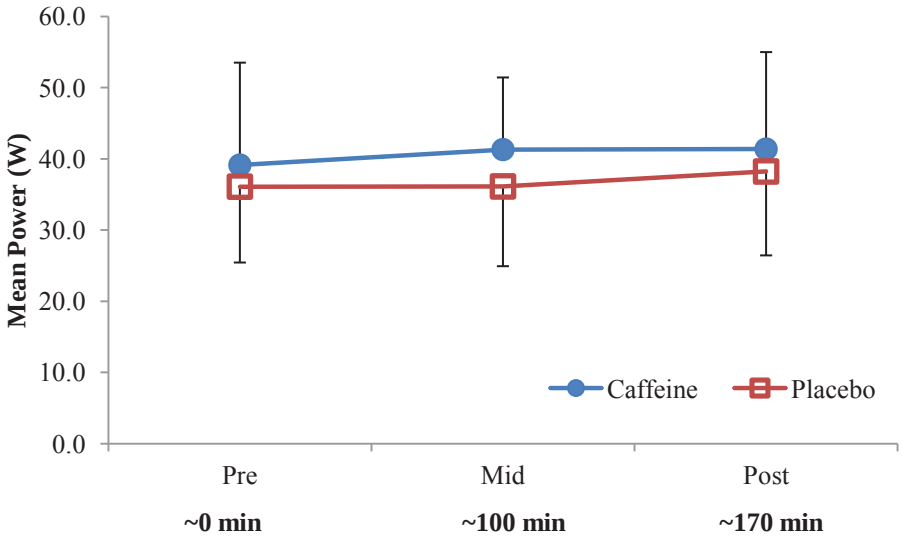

Figure 4.2 – Mean power output during eccentric contractions of the knee flexors in caffeine and placebo trials. Values are mean ± SD. The 'pre' time point represents before caffeine/placebo administration; 'mid' is after block 3 during the 15 min break; and post following the final block of exercise.

Mean power, as determined over the 5 contractions for each participant per sampling point, was significantly improved in the eccentric contractions of the knee extensors (Figure 4.1; CAFF vs. PLA, 30.3± 9.3 W vs. 28.1 ± 7.8 W; $F_{1, 45} = 8.51$, $P = 0.005$. Cohen's d = 0.26) and

in the eccentric contractions of the knee flexors (CAFF vs. PLA, 40.6 ± 12.4 W vs. 36.8 ± 10.9 W; $F_{1, 45}$ = 8.38, *P* = 0.006. Cohen's d = 0.32. Figure 4.2). There were no significant effects of caffeine on mean power in the knee extensors for concentric contractions (CAFF vs. PLA, 25.6 ± 8.0 W vs. 24.5 ± 8.7 W; $F_{1, 45}$ = 0.910, *P* = 0.345, Cohen's d = 0.13), or for concentric contractions in the knee flexors (CAFF vs. PLA, 24.6 ± 6.6 W vs. 24.4 ± 6.9 W; $F_{1, 45}$ = 0.064, *P* = 0.802, Cohen's d = 0.03).

There was no significant effect of caffeine on CMJ height (CAFF vs. PLA, 40.8 ± 4.3 cm vs. 40.5 ± 5.8 cm; $F_{2, 117}$ = 0.275, *P* = 0.760, Cohen's d = 0.06), or on the estimation of leg power from CMJ height (CAFF vs. PLA, 2813.6 ± 323.1 W vs. 2781.4 ± 392.7 W; $F_{2, 117}$ = 0.671, *P* = 0.513, Cohen's d = 0.09). However, CMJ height ($F_{6, 117}$ = 2.68, *P* = 0.018), and estimated leg power ($F_{6, 117}$ = 2.70, *P* = 0.017) decreased over time with post-hoc testing revealing that the differences lay between the pre-exercise value and the subsequent measures after each block of exercise.

4.2. Cognitive data

There was no effect of caffeine on percentage of correct responses in either the CRT (CAFF vs. PLA, 97.2 ± 2.65% vs. 96.7 ± 2.81%; $F_{1, 45}$ = 0.88, *P* = 0.354, Cohen's d = 0.18) or Stroop Test (CAFF vs. PLA, 97.7 ± 2.25% vs. 96.9 ± 3.01%; $F_{1, 54}$ = 1.27, *P* = 0.264, Cohen's d = 0.30). There was a trend towards faster reaction times in the caffeine trial for the CRT (CAFF vs. PLA, 381.4 ± 41.0 s vs. 396.6 ± 35.1 s; $F_{1, 45}$ = 3.37, *P* = 0.073), but not in the Stroop Test (CAFF vs. PLA, 616.2 ± 76.5 s vs. 620.1 ± 71.1 s; $F_{1, 54}$ = 0.044, *P* = 0.835).

4.3. Perceptual data

There was a trend towards a lower perceived exertion in the caffeine compared to placebo trial (CAFF vs. PLA, 11.9 ± 1.6 vs. 12.7 ± 1.3; $F_{1, 9} = 4.28$, $P = 0.068$; Figure 4.3). Perceived exertion increased over the duration of exercise ($F_{5, 45} = 12.86$, $P \leq 0.01$).

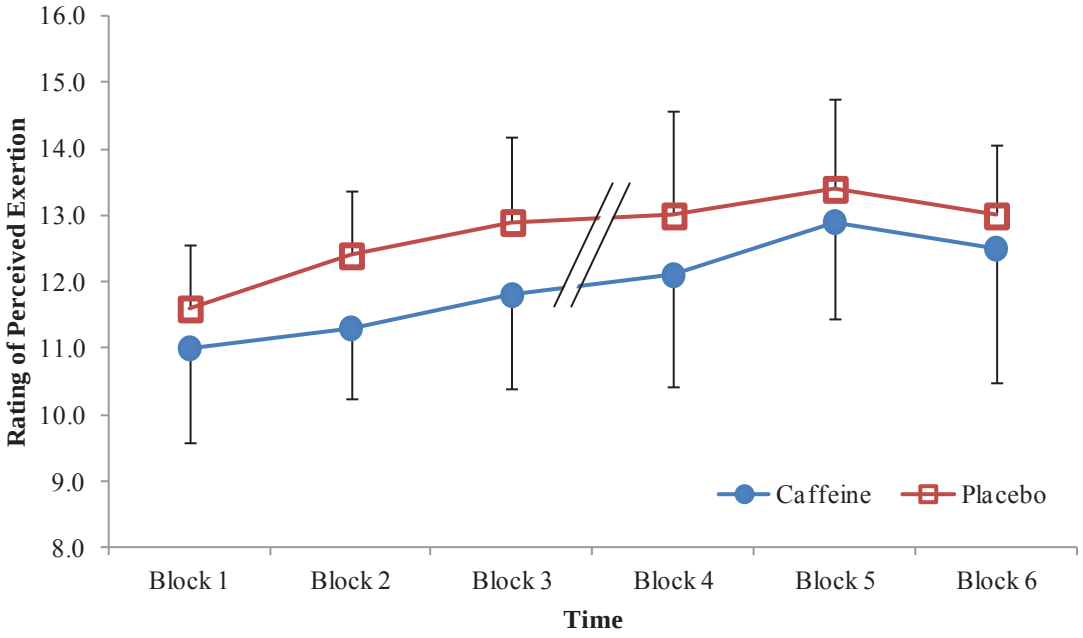

Figure 4.3 – Mean RPE scores for all participants in caffeine and placebo trials. Each block was 15 min long, and blocks 1, 2, 4, and 5 were separated by a 4 min break; after block 3 there was a 15 min break.

Caffeine ingestion showed a trend towards improved values for ratings of pleasure-displeasure (CAFF vs. PLA, 2.0 ± 1.2 vs. 1.0 ± 1.4; $F_{1, 9} = 4.73$, $P = 0.058$; Figure 4.4). Feelings of pleasure and displeasure on the placebo trial remained rather constant, while on the caffeine trial there was an increase indicated by an interaction effect ($F_{6, 54} = 2.59$; $P = 0.028$). Post-hoc analysis showed no significant differences at any of the time points.

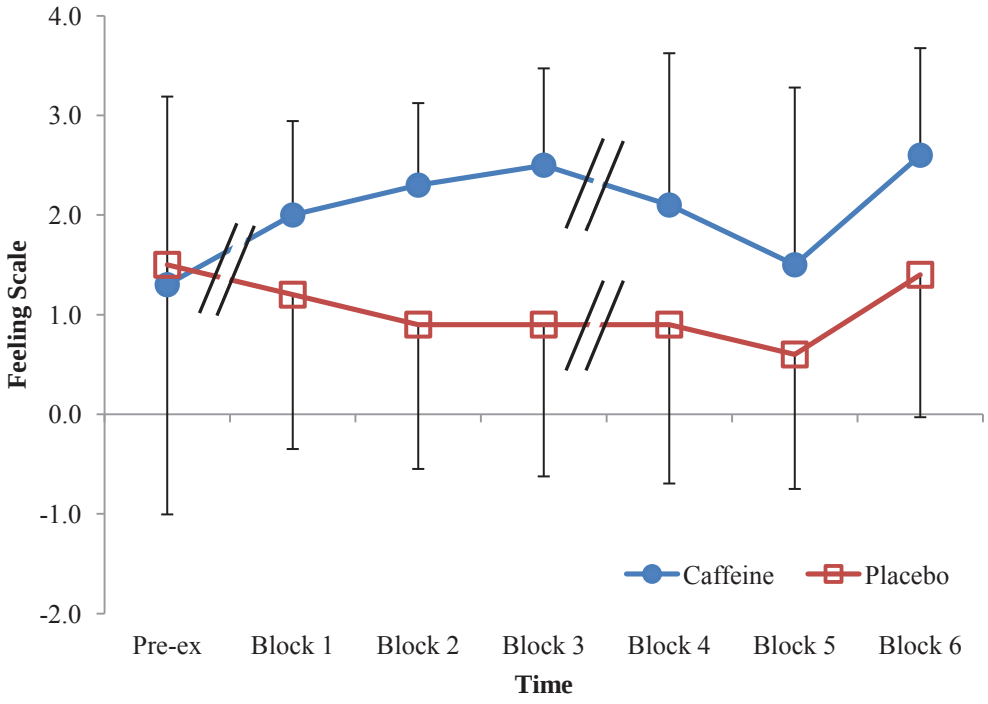

Figure 4.4 – Mean FS scores for all participants in caffeine and placebo trials. Each block was 15 min long, and blocks 1, 2, 4, and 5 were separated by a 4 min break; after block 3 there was a 15 min break.

There was a trend towards higher activation levels in the caffeine trial (CAFF vs. PLA, 3.6 ± 0.7 vs. 2.9 ± 0.8; $F_{1, 9} = 3.70$, $P = 0.087$; Figure 4.5). Activation levels also increased over time ($F_{6, 54} = 3.63$, $P = 0.04$) with higher levels in the latter stages of exercise. There was an interaction effect with activation levels increasing in the caffeine trial, but staying relatively constant throughout the placebo trial ($F_{6, 54} = 2.67$, $P = 0.031$). Post-hoc analysis revealed no specific differences at any of the time points.

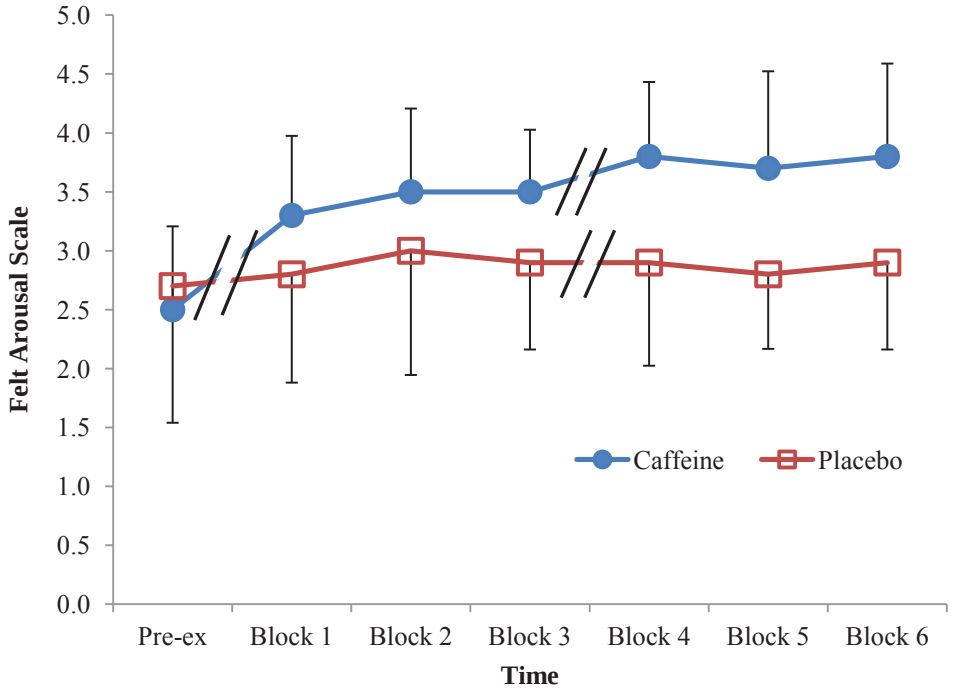

Figure 4.5 – Mean FAS scores for all participants in caffeine and placebo trials. Each block was 15 min long, and blocks 1, 2, 4, and 5 were separated by a 4 min break; after block 3 there was a 15 min break.

The FS and FAS values were additionally plotted in a circumplex model providing a visual description of how the participants' perception changed throughout the trial period (Figure 4.6). The vertical axis represents the arousal dimension (low - high), and the horizontal axis the data from the Feeling Scale (unpleasant – pleasant).

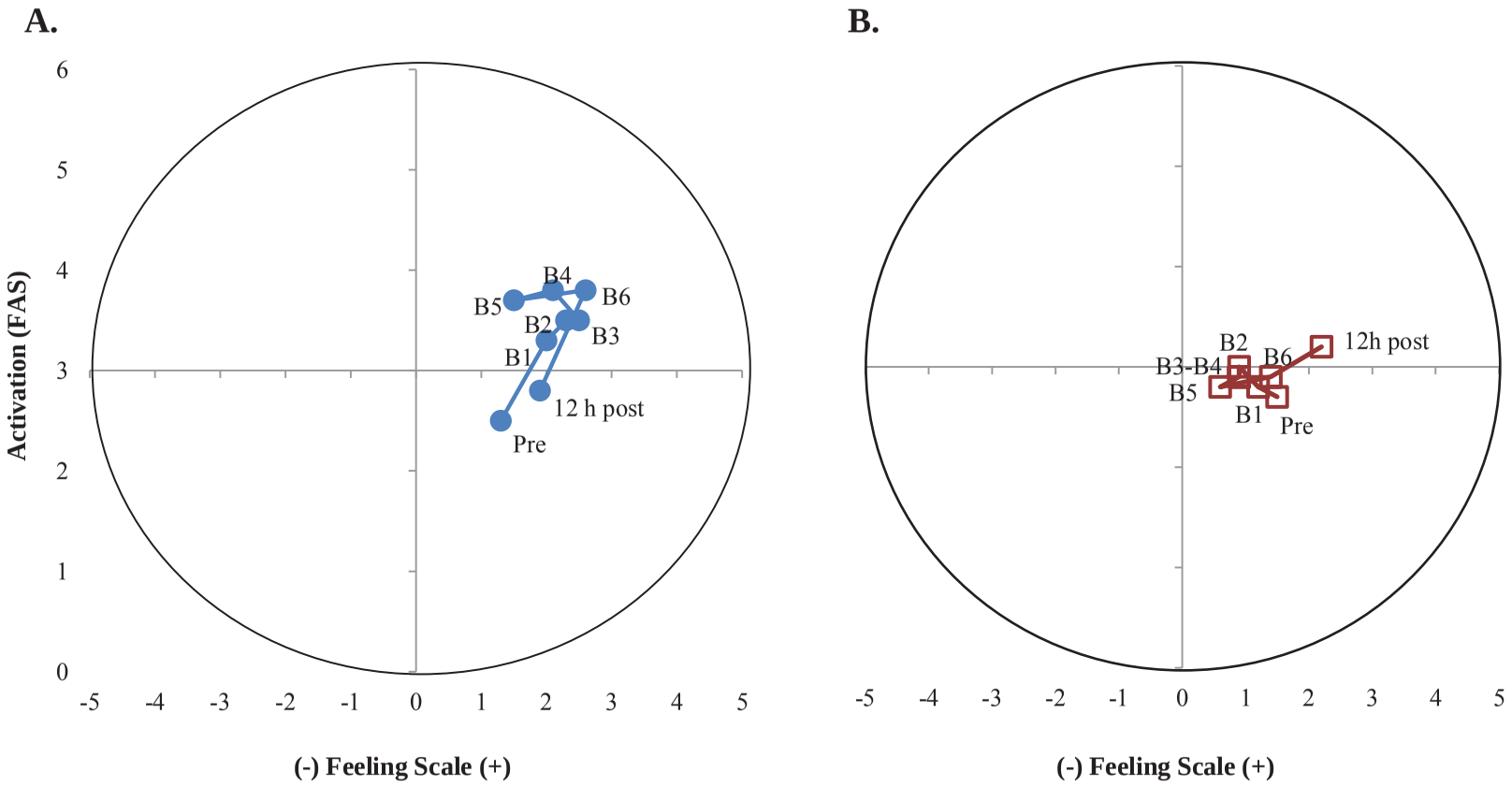

Figure 4.6 – Mean FS and FAS values plotted as Cartesian coordinates in a circumplex model. 4.6A represents caffeine values and 4.6B is placebo values.

4.4. Mood

Caffeine ingestion made no significant difference in overall POMS score (CAFF vs. PLA, 85.4 ± 27.9 vs. 101.3 ± 24.3; $t_{1,9} = -1.52$, $P = 0.163$). However the most relevant subsets to exercise, fatigue and vigour, showed varying effects. Figure 4.7A shows lower scores for fatigue on the caffeine compared to placebo trial (CAFF vs. PLA, 6.2 ± 4.8 vs. 8.3 ± 4.4; $F_{1,9} = 5.23$, $P = 0.048$). Although there was no main effect of caffeine ingestion on vigour (CAFF vs. PLA, 8.5 ± 4.2 vs. 7.5 ± 5.2; $F_{1,9} = 1.45$, $P = 0.259$), there was an interaction effect with vigour scores increasing in the caffeine trial but decreasing in the placebo trial (CAFF vs. PLA, 8.5 ± 4.2 vs. 7.5 ± 5.2; $F_{2,18} = 3.59$, $P = 0.049$; Figure 4.7B). Post-hoc analysis of vigour scores showed no specific differences at any of the time points.

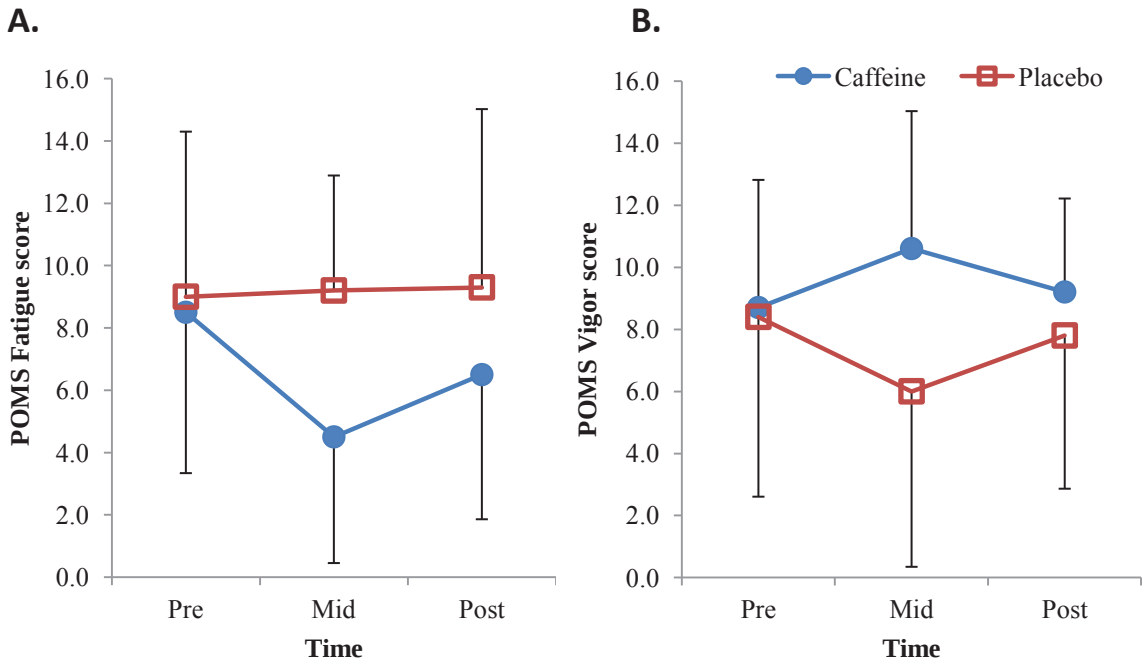

Figure 4.7 – Figure 4.7A depicts the fatigue scores in the POMS, and 4.7B depicts the vigour score. POMS – Profile of Mood States questionnaire.

4.5. Blood analysis

Minimal plasma caffeine levels were detected before participants commenced the trials (CAFF vs. PLA, 0.33 vs. 0.79 mmol·L^{-1}). Caffeine concentration increased over the duration of exercise (CAFF vs. PLA, 6.03 ± 3.61 vs. 0.70 ± 1.09 mmol·L^{-1}; $F_{4, 32} = 57.80$, $P \leq 0.01$). Plasma caffeine levels reached peak concentration approximately mid-way through the protocol, nearly 100 min after caffeine ingestion, after which there was a small decline ($F_{4, 32} = 53.82$, $P \leq 0.01$). The morning following the trial (~15 h post caffeine administration) plasma caffeine concentrations had still not returned to pre-exercise levels (Pre vs. 12 h post, 0.33 ± 0.31 vs. 5.44 ± 1.7 mmol·L^{-1}; $t_{1, 8} = -9.52$, $P \leq 0.01$; Figure 4.8). Individual responses to caffeine ingestion are shown in Figure 4.9.

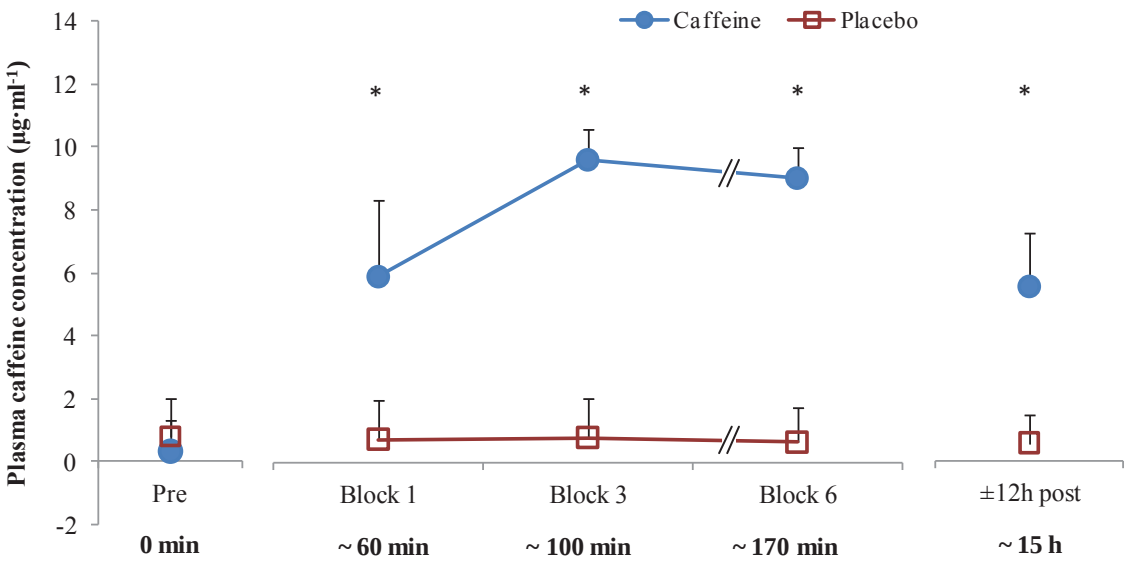

Figure 4.8 – Mean ± SD values of plasma caffeine concentration over duration of study. Time values on the horizontal axis indicate total time since 45 min prior to caffeine ingestion, which is represented as time 0 min. * significant difference between trials, P<0.05.

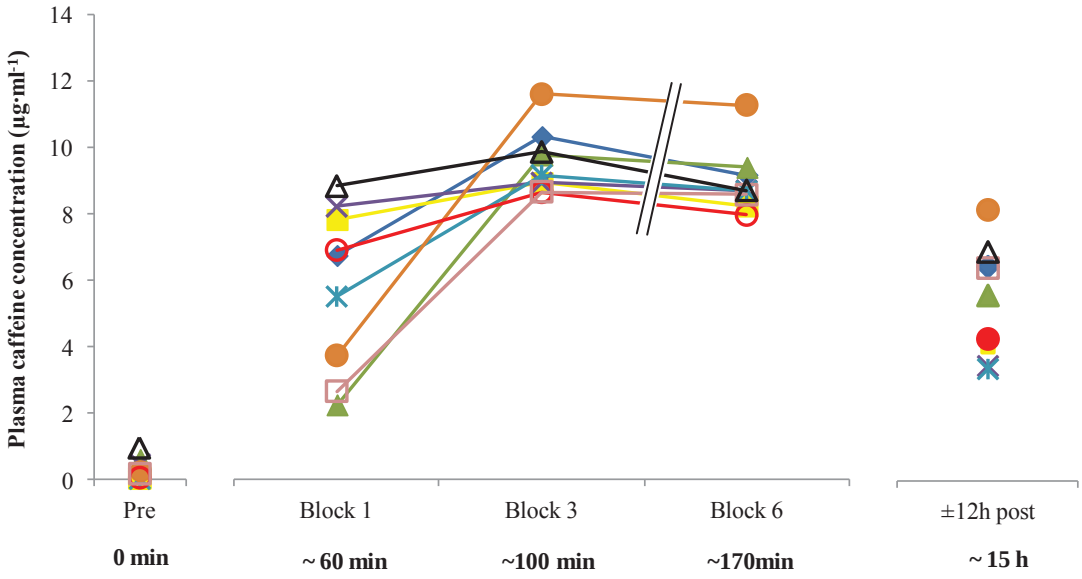

Figure 4.9 – Individual values for plasma caffeine concentration over duration of caffeine trial. Time values on the horizontal axis indicate total time since 45 min prior to caffeine ingestion, which is represented as time 0 min.

Plasma glucose concentrations were not affected by caffeine supplementation (CAFF vs. PLA, 4.89 ± 0.71 vs. 4.94 ± 0.59 mmol·L^{-1}; $F_{1, 8} = 0.059$. $P = 0.814$). There was a trend for plasma glucose concentrations to increase from the onset of exercise, reaching peak values during the first half of exercise before declining during the second half of the trial ($F_{3, 24} = 2.73$, $P = 0.066$).

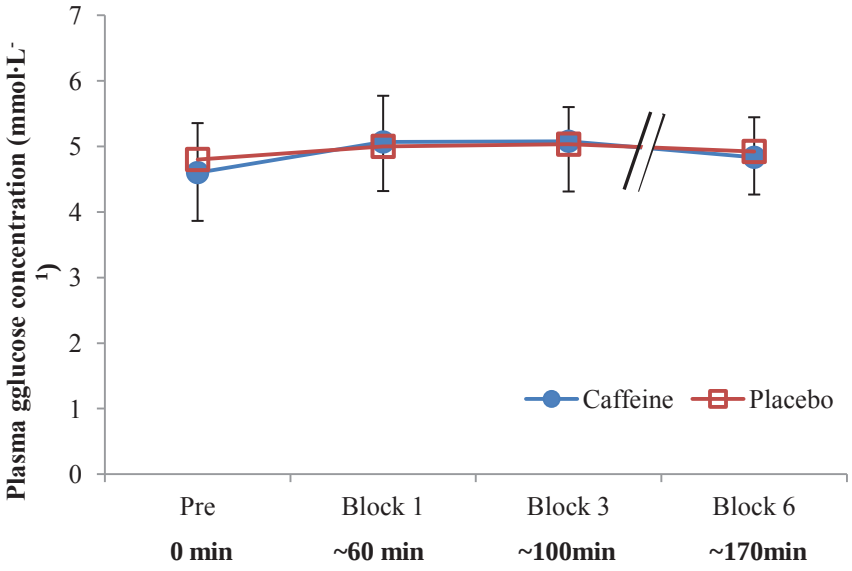

Figure 4.10 – Mean ± SD plasma glucose concentrations. Time values on the horizontal axis indicate total time since 45 min prior to caffeine ingestion, which is represented as time 0 min.

There was a trend for plasma insulin concentrations to decrease over the course of the trial ($F_{3, 21} = 3.12$, $P = 0.093$), but there was no effect of caffeine ingestion (CAFF vs. PLA, 7.92 ± 3.66 vs. 7.92 ± 4.75 mmol·L^{-1}; $F_{1, 7} = 0.00$, $P = 0.998$).

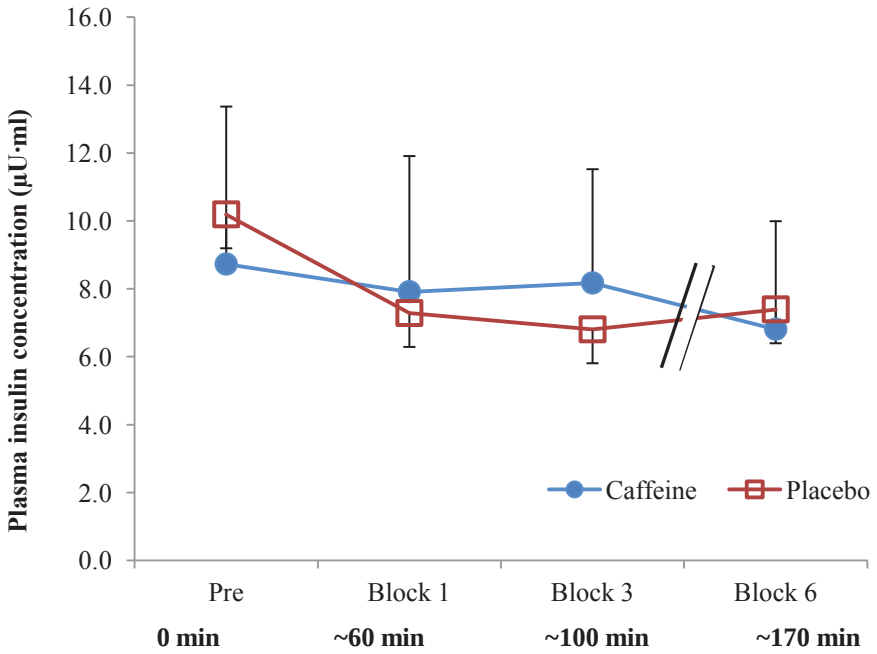

Figure 4.11 – Mean ± SD plasma insulin concentrations. Time values on the horizontal axis indicate total time since 45 min prior to caffeine ingestion, which is represented as time 0 min.

An increase in FFA concentration was seen with caffeine supplementation during intermittent exercise (CAFF vs. PLA, 0.95 ± 0.54 vs. 0.83 ± 0.48 mmol·L^{-1}; $F_{3, 24} = 29.2$, $P \leq 0.01$; Figure 4.12). FFA concentrations increased at a similar rate for the first hour following caffeine ingestion but then continued to rise at a far greater rate in the caffeine trial as noted by a significant interaction effect ($F_{3, 24} = 3.44$, $P = 0.033$). Post-hoc analysis revealed significant differences at block 6 ($P < 0.05$) only.

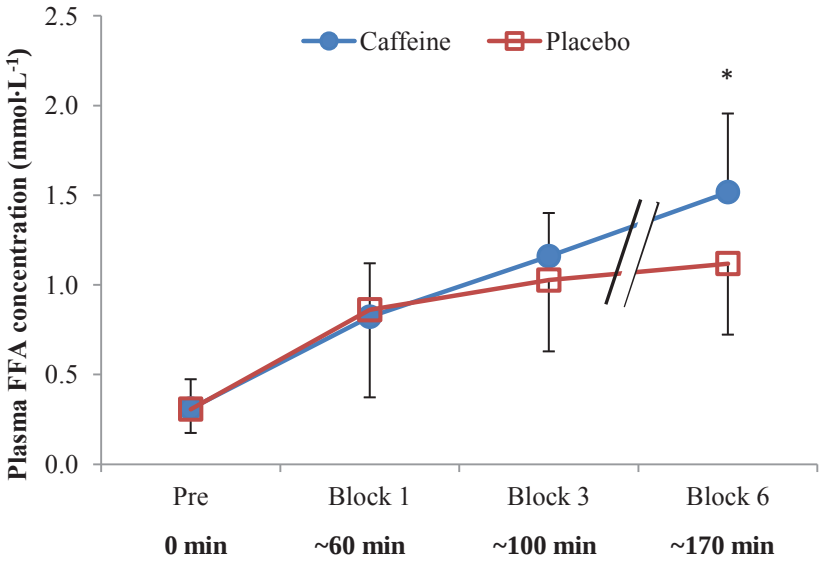

Figure 4.12 – Mean ± SD plasma FFA concentrations. Time values on the horizontal axis indicate total time since 45 min prior to caffeine ingestion, which is represented as time 0 min. * significant difference between trials, P<0.05.

4.6. Sleep quality

Certain aspects of sleep quality were negatively affected by caffeine supplementation (Table 4.2). Participants found it harder to get to sleep (CAFF vs. PLA, 5.9 ± 3.2 vs. 3.1 ± 1.7 cm; $t = 2.86$, $P = 0.019$), and also took longer to fall asleep (CAFF vs. PLA, 5.9 ± 3.2 vs. 2.8 ± 1.5 cm; $t = 3.45$, $P = 0.007$) following caffeine supplementation. Participants also felt less drowsy before they went to sleep in the caffeine trial (CAFF vs. PLA, 6.3 ± 2.7 vs. 3.3 ± 1.9 cm; $t = 3.01$, $P = 0.015$). In terms of quality of sleep, participants felt more restless after the

caffeine trial (CAFF vs. PLA, 7.1 ± 2.5 vs. 3.8 ± 2.3 cm; $t = 2.76$, $P = 0.022$), and there was a trend for participants to have more periods of wakefulness during the caffeine trial (CAFF vs. PLA, 7.0 ± 2.5 vs. 5.4 ± 2.6 cm; $t = 1.92$, $P = 0.087$). There were no significant effects of caffeine ingestion on awakening from sleep or on behaviour following awakening.

Table 4.1 - Data from the Leeds Sleep Evaluation Questionnaire

		Caffeine (cm)	Placebo (cm)	*P*-value
Getting to sleep	Easier or harder	5.9 ± 3.2	3.1 ± 1.7	0.019
	Quicker or slower	5.9 ± 3.2	2.8 ± 1.5	0.007
	Drowsiness level before sleep	6.3 ± 2.7	3.3 ± 1.9	0.015
Quality of sleep	Restful or restless	7.1 ± 2.5	3.8 ± 2.3	0.022
	Less or more periods of awakeness	7.0 ± 2.5	5.4 ± 2.6	0.087
Awakening	Easier or more difficult	4.3 ± 1.7	4.0 ± 1.9	0.553
	Shorter or longer time	4.1 ± 1.7	4.0 ± 2.0	0.823
Feeling on awakening	Alert or tired	5.6 ± 2.7	5.5 ± 2.6	0.814
Feeling now	Alert of tired	4.9 ± 2.3	4.0 ± 3.1	0.476
Balance/coordination on awakening	Less clumsy or more clumsy	4.4 ± 1.6	4.5 ± 1.9	0.649

Values are mean ± SD

4.7. Other data

There was no effect of caffeine ingestion on heart rate (CAFF vs. PLA, 142 ± 8.6 beats·min^{-1} vs. PLA, 144 ± 7.5 beats·min^{-1}; $F_{1, 3} = 3.51$; $P = 0.158$). USG was not significantly different pre-exercise between the caffeine and placebo trials (CAFF vs. PLA, 1.016 ± 0.01g·ml^{-1} vs. 1.016 ± 0.007 g·ml^{-1}; $t_{1, 9} = -0.34$, $P = 0.974$). The following morning after the trial, USG was not significantly different with a mean of 1.017 ± 0.007 g·ml^{-1} for both the caffeine and placebo trials. Table 3 shows that there were no significant differences between trials, in macronutrient composition of the participants' diets, and overall energy intake was not significantly different either.

Table 4.2 Nutritional composition of diet from 48 h food diaires

		Mean	SD	*P*-value
Energy (kJ)	CAFF	6088.8	1735.2	0.105
	PLA	7212.0	2170.6	
% Protein	CAFF	15.0	2.9	0.192
	PLA	17.0	4.1	
% Fat	CAFF	33.4	7.5	0.910
	PLA	33.2	8.6	
% CHO	CAFF	51.2	7.6	0.545
	PLA	49.8	12.2	

CHAPTER 5 – DISCUSSION

To the researcher's knowledge this is the first study to examine caffeine supplementation in an exercise setting using female intermittent games players taking a monophasic OCS. The main finding of this study is that caffeine improved perceptual responses, including higher pleasure and activation levels combined with a lowered perceived exertion during exercise. Although it had no effect on cognitive performance, caffeine improved aspects of mood including lower feelings of fatigue and higher levels of vigour. Caffeine also improved certain aspects of strength and power performance, but had a negative effect on certain areas of sleep.

Caffeine supplementation and the effects on strength and power performance is still a relatively recent area, and as such the research is still equivocal regarding an ergogenic effect of caffeine. This study found that caffeine enhanced average power and peak torque during eccentric contractions of the knee flexors and extensors, with small-moderate effect sizes (Figures 4.1 and 4.2). Astorino et al. (2010) also found that caffeine ingestion improved peak torque and average power during isokinetic contractions of the knee extensors and flexors; however they used recreationally active men, not trained individuals or females, as used in the current study. Woolf et al. (2008) performed a study examining strength and power during leg press, chest press, and Wingate test performance. The study found a higher peak power output in the Wingate test as a result of caffeine ingestion, but there was no effect of caffeine on the leg press exercise. This result was unusual as large muscle groups are contained within the leg and, if caffeine is expected to have an effect on skeletal muscle or fibre recruitment, then one would expect to see it in large muscle groups. Unfortunately the number of studies involving resistance exercise in females is scarce. Goldstein et al. (2010) found that caffeine significantly improved 1RM bench press exercise in resistance-trained

females, and Motl et al. (2006) found that caffeine significantly decreased ratings of leg muscle pain during cycling exercise at 60% O_2 max. However none of these studies examined measures of lower body strength or power, and as a result future research is needed with female participants to support the results of the present study.

An unexpected result of this study is that caffeine supplementation had no effect on concentric contractions, only on eccentric contractions. It is widely known that a single isokinetic concentric contraction is more reproducible than an isokinetic eccentric contraction and that knee extensor movements are more reproducible than knee flexor movements (Sauret, et al., 2009). As none of the participants were resistance trained athletes, it is probable that performing reproducible maximal contractions was challenging, and resulted in a large individual variation in the values. Therefore it is possible that there was greater individual variation in eccentric contraction performance combined with a reduced reproducibility. However, it is also likely that a learning effect was present as participants became more familiar with the eccentric exercise protocol and this would have minimised the extent of individual variation. In addition, Wretling and Henriksson-Larsen (1998) showed that adults do not perform a maximal contraction at the start of an endurance protocol. While the protocol used in the current study was not of an endurance nature, it is likely that participants did not perform maximal contractions at the start or in the middle of the intermittent treadmill running, in order to conserve energy or to delay fatigue. Due to the fact that the participants were not resistance trained in the current study, more habituation to the strength and power protocol would be prudent for future studies using non-resistance trained females.

Caffeine ingestion showed no effect on cognitive function as measured using the Stroop Test and Choice Reaction Time test (Section 4.2). These results are in disagreement with other

studies who found that caffeine ingestion improved cognitive performance using these tests (Hogervorst, et al., 2008; Hogervorst, et al., 1999; Kruk, et al., 2001; Lieberman, et al., 2002; Lieberman, et al., 1987). Indeed, there is little evidence to suggest that cognitive performance will be impaired following caffeine supplementation (Smith, 2002). The reasons for this lack of observed effect of caffeine supplementation are not clear. The caffeine dose administered in the current study is considered within the range likely to see ergogenic effects (Graham, 2001), so this is unlikely to be the cause. The age of the participants may be one factor that could account for the lack of effect. Younger participants are known to have faster reaction times, and generally score better on simple cognitive tasks that do not require complex functioning (Salthouse, 1996). Therefore greater effects of caffeine supplementation on cognition are likely to be more evident in older participants. The participants in the current study were 23.6 ± 4.1 years, and past studies have also used participants of a similar age and found a positive effect of caffeine (Brice & Smith, 2002; Childs & de Wit, 2006; Haskell, et al., 2005; Hindmarch, et al., 2000). Therefore, it is unlikely that the lack of a positive effect of caffeine was due to the dosage of caffeine or the age of the participants. It is possible that the sample size was not large enough in this study to detect a significant effect of caffeine on cognition as previous research has used sample sizes ranging from 19 (Hindmarch, et al., 1998) to 102 (Childs & de Wit, 2006).

Supplementation of 6 $mg \cdot kg^{-1}$ of caffeine was shown to improve ratings of perceived exertion, pleasure and activation during an intermittent treadmill-running protocol (Figures 4.3-4.6). Perceived exertion showed a trend towards lower values in the caffeine compared to placebo trial (P = 0.068), and this has been consistently shown in many exercise settings following caffeine ingestion (Cole, et al., 1996; Costill, 1978; Doherty, et al., 2004; MacIntosh & Wright, 1995). Mean perceived exertion values ranged from 12 – 13 on both caffeine and placebo trials which seems unusually low for 90-min of intermittent exercise,

with intensities ranging from 40 – 80% $_2$max. A free-running protocol such as the Loughborough Intermittent Shuttle Test (LIST) may have been more appropriate, and would have shown greater changes in values of perceived exertion, and future studies may use a protocol of this nature. A meta-analysis by Doherty and Smith (2005) confirmed this finding, however this meta-analysis examined studies that used a constant exercise load and intermittent exercise is of variable intensity and load. This meta-analysis was predominantly male based, with only 26% of the participants across all the selected studies being female. Sex-differences in perceived exertion are inconsistent across the literature, but a study by Robertson et al. (2000) found that it was unlikely that there would be differences in RPE between men and women performing exercise at an intensity of 70-90% O_2 max. More recent studies that have used team-sport athletes with intermittent exercise protocols, have failed to show a significant effect of caffeine on RPE (Glaister, et al., 2008; Schneiker, et al., 2006). The reasons for this are unclear but it may be that the repeated sprint protocols resulted in the participants working at a high-intensity where the RPE scale may not be sensitive enough to detect changes in perceived exertion. Also as nine out of ten participants correctly identified the caffeine and placebo treatments, there is a possibility that participants may have altered their responses according to which treatment they thought they had been given.

The circumplex model of analysis (Russell, 1980) was used to investigate how caffeine affected activation and pleasure-displeasure during intermittent exercise. To the researcher's knowledge this is the first time it has been used on female participants following caffeine administration. Caffeine ingestion was shown to result in a shift to an activated and pleasant state, from an unactivated pleasant state (Figure 4.6A). The participants stayed in this state for the remainder of the trial, and the 12 h post-exercise measure indicated a return to near pre-exercise values. This is in contrast to the placebo trial where the participants started in the

unactivated, pleasant state and remained there throughout the trial (Figure 4.6B). Only at the 12 h post measure did the participants move into the activated, pleasant quadrant.

The recently reported findings of Backhouse et al. (2011) support the results of this study with regards to RPE, and the positive effects of caffeine that were seen on pleasure and activation levels during exercise. Their study examined the effect of a 6 $mg \cdot kg^{-1}$ dose of caffeine in 12 endurance-trained men who cycled for 90 min at 70% O_2max. RPE was found to be significantly lower in the caffeine trial compared to placebo. This was combined with a significant interaction effect as participants found exercise less pleasurable on the placebo trial, but that feelings of pleasure were better maintained in the caffeine trial. Therefore it appears that caffeine supplementation is valuable in influencing feelings of pleasure, exertion, and activation during exercise.

The means by which caffeine affects pleasure and activation levels are not clear as caffeine has a multitude of effects throughout the body, and it is likely that the ergogenic response to caffeine is due to one, or more of these effects. Recently the main mechanism linking caffeine and improved performance has been said to be caffeine's role as an adenosine antagonist (Goldstein, Ziegenfuss, et al., 2010; Tarnopolsky, 2008). In this role caffeine competes with adenosine at the site of the receptor, where it binds to one of four receptors, which produces either an inhibitory or excitatory effect (Dunwiddie & Masino, 2001; Sawynok, 1998). Binding of caffeine to the A_1 receptors produces an inhibitory response, and binding to the A_2 receptors produces an excitatory response (Dunwiddie & Masino, 2001; Porkka-Heiskanen, et al., 2002). Consequently, it is likely that the binding of caffeine to the A_2 receptors produces an excitatory response that leads to an improvement in pleasure and activation levels, as was seen in the current study.

Further to this caffeine has been shown to reduce sensations of pain during exercise as it lowers the pain threshold by inhibiting the binding of adenosine to A_2 receptors. Interestingly a study by Motl et al. (2006) found that during cycling at 60% O_2 max for 30 min, leg pain perception was reduced in females but not in a dose-dependent fashion. This reduction in the pain response was far larger than the reduction experienced by male participants in an earlier study using the same protocol. The reasons for these sex differences may relate to caffeine pharmacokinetics and in particular to the fact that the menstrual cycle can potentially slow caffeine metabolism (Lane, et al., 1992). This would mean that women could experience higher plasma caffeine concentrations and a slower caffeine metabolism in comparison to men who received the same relative dose; the overall outcome being that any effect of caffeine is likely to be magnified in females, including the pain response to exercise. In terms of the current study, all the participants were taking a monophasic OCS which are known to slow caffeine metabolism (Abernathy & Todd, 1985; Patwardhan, et al., 1980). So while pain perception was not measured in this study, it is possible that the higher and prolonged levels of plasma caffeine in the female participants (Figure 4.8) may have enhanced any reduction in pain perception due to caffeine. The higher levels of plasma caffeine are also likely to have contributed to the improved mood and perception throughout the protocol, however activation level was the only measure to show a significant (but weak) correlation with plasma caffeine ($r = 0.285$, $P = 0.006$). Future studies should address the sex differences in the pain response to exercise with caffeine, and directly compare the perceptual and mood response to caffeine between men and women (specifically women taking an OCS).

Exercise has often been linked to enhancement of mood and it is often stated that this is a primary benefit of exercise (Berger & Motl, 2000). Further to this, feelings of fatigue and vigour are particularly relevant to exercise performance (Lieberman, 2001). In this study, caffeine supplementation resulted in participants feeling less fatigued but more vigorous

compared to the placebo trial (Figure 4.7). This is in agreement with other studies (Lieberman, 2001; Penetar, et al., 1993) who also reported a positive effect of caffeine on mood. Lieberman et al. (2002) found that a 300 mg dose of caffeine lowered fatigue at 1 h and 8 h post-ingestion, with no adverse effects on any other aspects of mood including anxiety.

However there are researchers who believe that the enhancements of cognition and mood are related to the caffeine withdrawal period imposed before testing a participant (Rogers, 2007). More specifically this theory suggests that caffeine withdrawal reduces cognitive performance and mood, and therefore supplementing with caffeine is only restoring cognitive performance and mood to baseline levels (Ruxton, 2008). However there are experimental examples of participants who, in the absence of caffeine withdrawal, still exhibit ergogenic effects of caffeine (Childs & de Wit, 2006; Smith, et al., 2005). Therefore it is unlikely that the positive effects of caffeine can be attributed to simply restoring baseline levels, but rather to caffeine's ergogenic nature. In this study the reduction of fatigue and the higher levels of vigour are likely to be due again to caffeine's central role as an adenosine antagonist, not due to an alleviation of withdrawal impairments on performance (Lieberman, et al., 2002; Ruxton, 2008).

Before both trials participants reported to the laboratory with minimal plasma caffeine levels illustrating a general compliance with caffeine restrictions before the study. Over the course of the trial, plasma caffeine concentrations increased after ingestion of the caffeine supplement, and no caffeine was present following placebo ingestion. Plasma caffeine levels peaked approximately 100 min after caffeine administration (Figure 4.8) and this has been seen in other studies (Benowitz, et al., 1995; Collomp, et al., 1991; Conway, et al., 2003). The 12 h post-exercise measure showed that plasma caffeine levels were still elevated

following a night's sleep even with no further caffeine ingestion. This is the first study to show the appearance and disappearance of plasma caffeine concentrations over a ~15 h period, with female intermittent games players who are taking a monophasic OCS. Regarding specific concentrations of caffeine's metabolites, paraxanthine, theophylline, and theobromine, this was beyond the scope of this thesis and will be measured at a later date. This will add further information to the literature regarding caffeine supplementation in female team sport athletes who are taking a monophasic OCS.

Caffeine supplementation did not affect plasma glucose concentrations (Figure 4.10) and this has also been shown in some (Graham, et al., 1998; Greer, et al., 1998) but not other (Doherty, 1998; Graham, et al., 2000; MacIntosh & Wright, 1995) studies . Hulston et al. (2008) also found that caffeine supplementation had no effect on glucose kinetics during exercise, and the authors noted that the large amount of variation in previous research is likely to be due to greater amounts of inter-individual variations in glucose kinetics. As there was no change in plasma glucose levels due to caffeine, insulin concentrations also remained unaffected by caffeine ingestion (Figure 4.11). This has been supported by some (Mohr, et al., 1998; Raguso, et al., 1996) but not all studies (Collomp, et al., 2002; Woolf, et al., 2008). However, this is to be expected given the methodological differences between those studies and the current study i.e. the studies by Woolf and Collomp involved participants eating a pre-exercise meal, while participants in the current study were required to report for testing after a 3 h fast.

While no change in plasma glucose or insulin concentrations was seen in the present study FFA concentration was higher following caffeine ingestion at the end of exercise ($P = 0.008$). This is an interesting result as many studies have reported no increase in FFA concentrations as a result of caffeine supplementation (Bell & McLellan, 2003; Greer, et al., 2000; Raguso,

et al., 1996), while others have (Cox, et al., 2002; Graham, et al., 2000; Mohr, et al., 1998). However RER values have not been consistently shown to be lowered by caffeine supplementation as would be expected if caffeine caused an increase in FFA (Graham, 2001). Many authors have speculated that the reason for this is that while caffeine may trigger an increase in lipolysis, the increased levels of FFA results in greater hepatic uptake of FFA (Graham, 2001). And, when taking the insulin and glucose data into consideration, it appears that caffeine does not affect carbohydrate or fat metabolism in active muscle, instead it is likely to have effects on the liver, adipose tissue and the central nervous system (Graham, et al., 2000).

However nearly all of the above mentioned studies have used male participants, and it is well known that differences in substrate metabolism occur between men and women, (see Tarnopolsky (2000) and Braun and Horton (2001) for reviews on the area). It is generally accepted that women will preferentially oxidise fat and have lower rates of carbohydrate oxidation during exercise (Braun & Horton, 2001), however the mechanisms for this are not clear. Further to these sex differences during exercise, caffeine is recognised as a lipolytic agent; however this does not always translate into observable effects on FFA levels or RER. This is in contrast to the current study where an increase in plasma FFA levels was seen; however, RER data is unavailable to support any assertions that fat oxidation was increased. Therefore further research is needed to determine the effects of caffeine on substrate metabolism, and the differences that may occur between male and female participants.

Caffeine is often anecdotally reported to have adverse effects on sleep quality (Brezinova, 1974; Hindmarch, et al., 2000; Nicholson & Stone, 1980; Youngstedt, et al., 2000). The current study found that a 6 $mg \cdot kg^{-1}$ dose of caffeine taken at approximately 6pm (± 1 h) made it harder and took longer to get to sleep, and reduced the quality of sleep, with more

periods of awakeness and feelings of restlessness (Table 4.2). Plasma caffeine levels at the end of block 6 were significantly correlated with all the getting to sleep variables, including finding it harder to get to sleep (r = 0.613, P = 0.007), taking longer to get to sleep (r = 0.650, P = 0.004), and feeling less drowsy before sleep (r = 0.618, P = 0.006). Plasma caffeine levels at the end of block 6 were also correlated with a more restless sleep (r = 0.550, P = 0.018).

Achieving a quality sleep is important to athletes as sleep is vital to the restoration of metabolic processes and in the regulation of hormone secretion (Driver & Taylor, 2000; Mougin, et al., 2001). Reduced sleep quality is known to adversely affect subsequent exercise performance (Mougin, et al., 1991; Oliver, et al., 2009; Skein, et al., 2011). Therefore if a 6 $mg \cdot kg^{-1}$ dose of caffeine in the early evening negatively affects sleep quality, it is likely that exercise performance the following day could be impaired. This is important for athletes to recognise who might be competing in a tournament situation. Caffeine's ergogenic benefits might improve performance during one match or training, but the reduced quality of sleep following this is likely to reduce performance in the next match or training (Oliver, et al., 2009; Skein, et al., 2011); an important consequence that must be taken into consideration.

An important factor overlooking this whole area of caffeine supplementation research is individual variation in the response to caffeine. Results from the current study showed that while all participants exhibited an increase in plasma caffeine concentrations followed by a decrease after ~100 min, there was considerable variation even though all had received the same relative dose (Figure 4.9). This is likely to be attributed to variation in the CYP1A2 gene which codes for the enzymes responsible for caffeine metabolism (Magkos & Kavouras, 2005). More than 150 single-nucleotide polymorphisms (SNPs) have been discovered for the CYP1A2 gene (Yang, et al., 2010), and ethnic differences have also been noted in CYP1A2

activity (Gunes & Dahl, 2008). More importantly individuals can be classified as having a "fast" or "slow" caffeine metabolism. An A to C substitution at position 734 on the CYP1A2 gene results in the CYP1A2*1F allele which is representative of "slow" metabolisers, while individuals homozygous for the CYP1A2*1A allele are "fast" metabolisers (Cornelis, et al., 2006). It was beyond the scope of this study to examine the genetic profile of the participants. However, future studies should try to include this as a screening measure where possible, as this is likely to play a significant role in explaining the observed effects of caffeine supplementation.

5.1 Conclusions

- For females taking monophasic OCS, a 6 $mg \cdot kg^{-1}$ dose of caffeine will improve perceptual and mood responses during intermittent exercise.
- Caffeine ingestion improved lower body eccentric strength and power.
- An ergolytic effect of caffeine on sleep quality was observed and should be taken into consideration before supplementing with caffeine.
- After caffeine supplementation, it appears that fat metabolism was increased while there was no effect on carbohydrate metabolism.

5.2 Limitations

There were a number of limitations with this study that should be addressed for future research in this area:

- The sample size was calculated based on power calculations using a strength and power study (Kalmar & Cafarelli, 1999a). More participants were likely to be needed to see statistically significant effects in the cognitive testing as other studies have used at least 19 participants (Hindmarch, et al., 1998).

- In an effort to minimise inter-individual variation, participants were all required to be on one of four brands of OCS (same hormonal composition), and also had to be a team sport athlete. These criteria made for a small selection pool, and recruitment was challenging due to the time constraints of this study.
- Problems were found with the expired air collection equipment, and this meant no RER or average $\dot{V}O_2$ data could be reported.
- In regards to the cognitive testing a larger cohort is needed and a more sensitive test, and where possible, one that is relevant to sportspeople.

5.3 Future directions

- The International Society of Sports Nutrition (Goldstein, Ziegenfuss, et al., 2010) highlighted the lack of research using female participants and the large variation in protocols used. It was stated that "*additional research is needed at all levels of sport to determine if caffeine is indeed effective for enhancing performance in women, either in a competitive or recreationally active setting*".
- More research is needed that uses well-designed protocols controlling for factors like menstrual cycle or OCS use, such as were administered in this study.
- Elucidate whether there is a difference in the ergogenic effect of caffeine in individuals who have variations in the CYP1A2 gene.
- Further investigate the mechanisms behind caffeine's ergogenic effects, particularly mechanisms of a central origin.
- While this study has been novel in its use of female intermittent games players, future research should seek to include a control group of men in order to better understand the consequence of sex differences on the effect of caffeine.

CHAPTER 7 – REFERENCES

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CHAPTER 6 – APPENDICES

Appendix 1 – Ethics approval from Massey University Human Ethics Committee

Appendix 2 – Participant information sheet

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APPENDIX 1

MASSEY UNIVERSITY

2 March 2011

Jemma O'Donnell
74A Woodcock Road
RD3
HAMILTON 3283

Dear Jemma

Re: HEC: Southern A Application – 10/86
Effect of caffeine supplementation on metabolism and physical and cognitive function in female intermittent games players taking a monophasic oral contraceptive

Thank you for your letter dated 1 March 2011.

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

JmBoddy

Prof Julie Boddy, Chair
Massey University Human Ethics Committee: Southern A

cc Dr Ajmol Ali
IFNHH
ALBANY

Dr Andrew Foskett
IFNHH
ALBANY

Prof Richard Archer, HoI
IFNHH
PN452

APPENDIX 2

Effect of caffeine supplementation on metabolism and physical and cognitive function in female intermittent games players taking a monophasic oral contraceptive

PARTICIPANT INFORMATION SHEET

Invitation to Participate in Research Study

I, Jemma O'Donnell, am a postgraduate student currently undertaking a thesis as a requirement of my Masters degree in Nutrition through Massey University under the supervision of Dr. Ajmol Ali, Dr. Andrew Foskett and Dr Pam von Hurst, and with the assistance of Mr. Simon Bennett, Mr David Wolf, Mr Naser Naser, and Miss Rebecca Watkin.

Caffeine is the most widely used supplement amongst athletes. The World Anti-Doping Agency removed caffeine from the list of banned substances in 2004 even though it has well demonstrated performance benefits during exercise, and in particular for endurance and time-trial exercise (Goldstein et al., 2010). There is little research regarding team sport athletes and caffeine supplementation, and even less for female team sport athletes. The research involving caffeine supplementation for women has often not taken into account influencing factors such as menstrual cycle or oral contraceptive use that can affect the actions of caffeine. Therefore the primary aim of this study is to investigate whether a caffeine supplement in female team sport athletes, who are currently taking oral contraceptives, will affect metabolism and/or physical and cognitive performance. A secondary aim will be to examine the effect of caffeine ingestion on immune function in female athletes.

As a participant in this study you will benefit in gaining a better knowledge of your fitness levels, and the procedures involved will be of benefit to your own training. You will also be able to examine if caffeine will improve your exercise performance, or see if it has any effects that that may discourage you from consuming it.

Participant Recruitment

Approximately 12 participants will be recruited to provide sufficient statistical power to the study. All participants will be reimbursed for travel expenses with $20 of MTA petrol vouchers per trial

To be included for this study you must be:

- A female team sports player
- 18 – 35 years old
- Using one of the following oral contraceptives for at least 3 months: Microgynon, Levlen ED, Nordette, or Monofeme
- A non-smoker
- Not taking any prescription or recreational drugs, including inhalers or corticosteroid creams
- Have a low to moderate caffeine intake (equivalent to no more than four cups of coffee a day). If you don't consume any caffeine then you cannot take part in this study.

Risks/Discomforts of the study could include:

- Feeling tired or fatigued after the exercise sessions.
- Mild soreness from blood sampling procedures.
- Possible impact on sleep quality after the main trials due to caffeine consumption.

Participating in this study will not interfere or interact with the oral contraceptive you are taking.

Project Procedures and Participant Involvement

Before taking part in this study you will be asked to complete a Caffeine Consumption Questionnaire, a Heath Screening Questionnaire, and a Menstruation Screening Questionnaire relating to your health and menstruation status, prior medical issues, and medications taken. These screening questionnaires are used to ascertain any information that may conflict with the study, (e.g. if you are taking any medications that may interfere with the outcome of the study), and may ultimately prohibit you from participating. If you have any medical condition listed in the Health Screening Questionnaire, or an irregular menstrual cycle, then we will have to exclude you from taking part. The information obtained on all study questionnaires is strictly confidential and will be used for the purposes of the present study only.

Preliminary trial

You will be asked to attend an initial session for a maximal oxygen uptake test to determine your fitness level. After a brief rest you will be asked to undergo a 45 min familiarization with the treadmill-running protocol (that will be used for the main trials). Following the exercise tests you will be shown how to complete the food record diary that you will keep for 48 h prior to each main trial, and you will be instructed to avoid foods/drinks that are high in caffeine, and any alcoholic drinks for the 48 h prior to the trials and up until the completion of the post 12 ± 1 h morning session. We will also give you further instructions regarding what you should do to prepare for the main trials.

Main trials

The main trials will consist of 6 x 15-min blocks of intermittent running exercise on a treadmill. You will be asked to come in for two trials: in one trial a capsule containing caffeine (equivalent to 4 cups of coffee) will be given to you 1 h before the trial, and in the other trial a capsule containing a placebo will be given to you 1 h before the trial. During the trials blood and saliva samples will be taken every 15 minutes and a countermovement jump performed. Blood samples will be analysed to determine levels of metabolites, such as glucose and free fatty acids, and levels of hormones, such as adrenaline, noradrenaline, and insulin. Saliva samples will be analysed for immune function measures (salivary IgA and alpha amylase). Expired air samples will be collected in the last two minutes of every 15-min block. Tests of perception (Profile of Mood States; POMS), cognition (trail-making test and Stroop colour and word test) and muscle power (using an isokinetic dynamometer) will be performed before, during and following exercise.

You will be invited to keep a food diary two days prior to the first trial and then asked to follow the same dietary intake on the days prior to the second trial. You will be asked to observe a 3-hour fast before each main trial, which will be conducted in the evening. The following morning you will be asked to come into the laboratory and complete a questionnaire about the previous night's sleep (Leeds Sleep Evaluation Questionnaire) as well as perform the muscle power testing.

Individuals trained in resuscitation (NZ Red Cross First Aid, Level 2) and 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 major issues.

Participant's Rights

You are under no obligation to accept this invitation. Should you choose to participate, you have the right to;

- decline to answer any particular question
- withdraw from the study at any time, even after signing a consent form (if you choose to withdraw you cannot withdraw your data from the analysis after the data collection has been completed)
- 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 (in the form of a summary sheet)

Confidentiality

All data collected will be used solely for research purposes and has the possibility of being presented in a professional journal. All personal information will be kept confidential by assigning numbers to each participant. No names will be visible on any papers on which you provide information. All data/information will be dealt with in confidentiality and will be stored in a secure location for five years on the Massey University Albany campus. After this time it will be disposed of by an appropriate staff member from the Sport and Exercise Science department.

Project Contacts

If you have any questions regarding this study, please do not hesitate to contact any of the following people for assistance:

Miss Jemma O'Donnell (Human Nutrition, IFNHH, Massey)
027 4698 499; jemmaod@gmail.com

Dr Ajmol Ali (School of Sport and Exercise, Massey)
(09) 414 0800 ext.41184; a.ali@massey.ac.nz

Dr Andrew Foskett (School of Sport and Exercise, Massey)
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Dr Pam von Hurst (Human Nutrition, IFNHH, Massey)
(09) 414 0800 ext 41205; p.r.vonhurst@massey.ac.nz

Mr Simon Bennett (Laboratory Manager – School of Sport and Exercise, Massey)
021 970 672; s.bennett1@massey.ac.nz

Committee Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 10/86. If you have any concerns about the conduct of this research, please contact Professor Julie Boddy, Chair, Massey University Human Ethics Committee: Southern A, telephone 06 350 5799 x 2541, 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 Injury Prevention, Rehabilitation and 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

Effect of caffeine supplementation on metabolism, and physical and cognitive function in female intermittent games players taking a monophasic oral contraceptive.

PARTICIPANT CONSENT FORM - INDIVIDUAL

This consent form will be held for a minimum period of five years.

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 agree to participate in this study under the conditions set out in the Information Sheet.

Signature: _____ **Date:** _____

Full name (printed): _____

Phone Number_____ **Age** _____ **Date of Birth** _____

Address_____

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 of the following questions by ticking only one box for each 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 upon 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 a pain in your chest when you do physical activity?

☐ No ☐

Qu 3. In the past month have you had 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 by vigorous exercise?

Yes ☐ No ☐

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

Yes ☐ No ☐

Qu 8. Have any immediate family had heart problems prior to the age of 60?

Yes ☐ No ☐

Qu 9. Have you been hospitalised 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 suffered from any diagnosed 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 creams?

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: _____

Signature of Parent: _____
or Guardian (for participants under the age of consent)

References

1. Thomas S, Reading J and Shephard 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 Sports Exerc* 28(4): 468-472

APPENDIX 5

Effect of caffeine supplementation on metabolism and physical and cognitive function in female intermittent games players taking a monophasic oral contraceptive

PARTICIPANT MENSTRUATION QUESTIONNAIRE

1. Do you use oral contraceptives? (If no, go to question 2) ☐ yes ☐ no

1a. Are you currently taking either:

Microgynon yes ☐ no ☐

Levlen ED yes ☐ no ☐

Nordette yes ☐ no ☐

Monofeme yes ☐ no ☐

1b. How often do you use it? _____

1c. How long have you been using this method of birth control? _____

2. Have you missed a period in the last 2 years? (If no, go to question 3) yes ☐ ☐ no

2a. Were the missed periods due to your method of birth control? yes ☐ ☐ no

2a. Approximately how many times in the past 2 years have you missed a period for reasons other than because of your birth control? _____

3. How many days does your period usually last for? (check one)

- 3 days or less ☐
- 4-6 days ☐
- 7 or more days ☐ If more than 7 days, please give an estimate: _____

4. How regular is your cycle? (check one)

- every 28 days ☐
- every 26-30 days ☐
- irregular ☐

5. When was the start of your last period? day_____ month_____ year_____

APPENDIX 6

Effect of caffeine supplementation on metabolism and physical and cognitive function in female intermittent games players taking a monophasic oral contraceptive.

CAFFEINE CONSUMPTION QUESTIONNAIRE

1) How often do you drink tea?

- ☐ Once a month or less
- ☐ 2-3 times a month
- ☐ 1-3 times per week
- ☐ 4-6 times per week
- ☐ Once a day
- ☐ 2-3 times per day
- ☐ 4-6 times per day
- ☐ More than 7 times per day

2) How often do you drink coffee?

- ☐ Once a month or less
- ☐ 2-3 times a month
- ☐ 1-3 times per week
- ☐ 4-6 times per week
- ☐ Once a day
- ☐ 2-3 times per day
- ☐ 4-6 times per day
- ☐ More than 7 times per day

3) What type of tea or coffee do you drink? *Espresso, decaf, instant, Earl Grey, herbal etc.*

.....

.....

4) How often do you consume energy drinks? *(Red Bull, V, Lift Plus, Monster, Demon, Ink, Mother)*

- ☐ Once a month or less
- ☐ 2-3 times a month
- ☐ 1-3 times per week
- ☐ 4-6 times per week
- ☐ Once a day
- ☐ 2-3 times per day
- ☐ 4-6 times per day
- ☐ More than 7 times per day

5) Describe how products containing caffeine normally affect you.

.....

.....

.....

.....

6) Do you normally drink coffee/tea/energy drinks after 8pm at night?

Yes ☐ No ☐

APPENDIX 7

Effect of caffeine supplementation on metabolism and physical and cognitive function in female intermittent games players taking a monophasic oral contraceptive

Daily Oral Contraceptive Diary

Please fill out this diary for the two days before your trial, the day of your trial, and the day following your trial.

Date	Time Taken	Comments

APPENDIX 8

Effect of caffeine supplementation on metabolism and physical and cognitive function in female intermittent games players taking a monophasic oral contraceptive.

PARTICIPANT NUTRITIONAL ANALYSIS FORM

Please write down all foods and drinks consumed for two days prior to participating in your first trial. This includes snacks, lollies, water, vitamins, supplements, etc. Be as specific as possible. You will be asked to eat the exact foods that you write down on this sheet on the two days prior to your second trial at approximately the same times.

• Please refrain from consuming foods/drinks high in caffeine (e.g. coffee, tea, soft-drinks, chocolate and energy drinks).
• Please refrain from consuming alcohol.
• Please do not consume any food 3 hours before your main trial. You will be allowed to drink as much water as you like but not sports drinks or any other food
• Please bring this form with you on the day of your trial.

Use this example as a guide:

Time	Description of Food/Drink Consumed	Amount
Breakfast		
7:30am	Eggs – scrambled w/ salt and pepper	2 eggs
	Toast – wheat with butter	2 slices
	Orange juice	1 glass
	Banana	1 large
	Multivitamin	1
Lunch		
12:30pm	Sushi take away from Tokyo – chicken teriyaki	6
	Water	2 glasses
Dinner		
6:00pm	Grilled chicken breast	1 breast
	Steamed vegetables (carrots, broccoli, capsicum)	1 cup
	Rice	1.5 cups (cooked)
	Milk	1.5 (tall) glasses
Snacks/Other		
11:00am	Yogurt – blueberry	1 cup
3:00pm	Pretzels	small handful
3:00pm	Orange juice	1 glass

DAY 1 (two days prior to your trial)

Time	Description of Food/Drink Consumed	Amount
Breakfast		
Lunch		
Dinner		
Snacks/Other		

DAY 2 (one day prior to your trial)

Time	Description of Food/Drink Consumed	Amount
Breakfast		
Lunch		
Dinner		
Snacks/Other		

APPENDIX 9

CAFFEINE IN FEMALES STUDY POMS

PSYCHOMETRIC SCALE ONE

SUBJECT ID _____ BLOCK/BEVERAGE _____

DATE _____ TIME _____ DAY _____

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 beside each item. Please check to make sure you have responded to all the 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

VIGOR	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

Grove, J.R., Prapavessis, H. Preliminary evidence for the reliability and validity of an abbreviated Profile of Mood States. *International Journal of Sport Psychology*. 1992 Apr-Jun Vol 23(2) 93-109.

SHONA L. HALSON,1,2 MATTHEW W. BRIDGE,1 ROMAIN MEEUSEN,3 BART BUSSCHAERT,3

MICHAEL GLEESON,1 DAVID A. JONES,1 AND ASKER E. JEUKENDRUP1 Time course of performance changes and fatigue markers during intensified training in trained cyclists. *J Appl Physiol 93: 947–956, 2002.*

Morgan WP, Brown DR, Raglin JS, O'Connor PJ, and Ellickson KA. Psychological monitoring of overtraining and staleness. Br J Sports Med 21: 107–114, 1987.

APPENDIX 10

THE LEEDS SLEEP EVALUATION QUESTIONNAIRE
A.C. PARROTT AND I. HINDMARCH (1978)

(to be filled-in by the patient)

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.

For example, this would indicate a moderate change for the worse:

"Good" ——————————|—————————— "Bad"

The purpose of this questionnaire is to evaluate the effect of the study medication on your sleep.

THE LEEDS SLEEP EVALUATION QUESTIONNAIRE

1. **How would you compare getting to sleep using the medication with getting to sleep normally, i.e. without medication?**

a) Easier than usual _____ Harder than usual |__|__|__|*

b) Quicker than usual _____ Slower than usual |__|__|__|*

c) Felt more drowsy than usual _____ Felt less drowsy than usual |__|__|__|*

2. **How would you compare the quality of sleep using the medication with non-medicated (your usual) sleep?**

a) More restful than usual _____ More restless than usual |__|__|__|*

b) Fewer periods of wakefulness than usual _____ More periods of wakefulness than usual |__|__|__|*

3. **How did your awakening after medication compare with your usual pattern of awakening?**

a) Easier than usual _____ More difficult than usual |__|__|__|*

b) Took shorter than usual _____ Took longer than usual |__|__|__|*

4. **How did you feel on waking?**

Alert _____ Tired |__|__|__|*

5. **How do you feel now ?**

Alert _____ Tired |__|__|__|*

6. **How was your sense of balance and coordination upon getting up?**

Less clumsy than usual _____ More clumsy than usual |__|__|__|*

** Do not write in the shaded areas.*

APPENDIX 11

"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 12

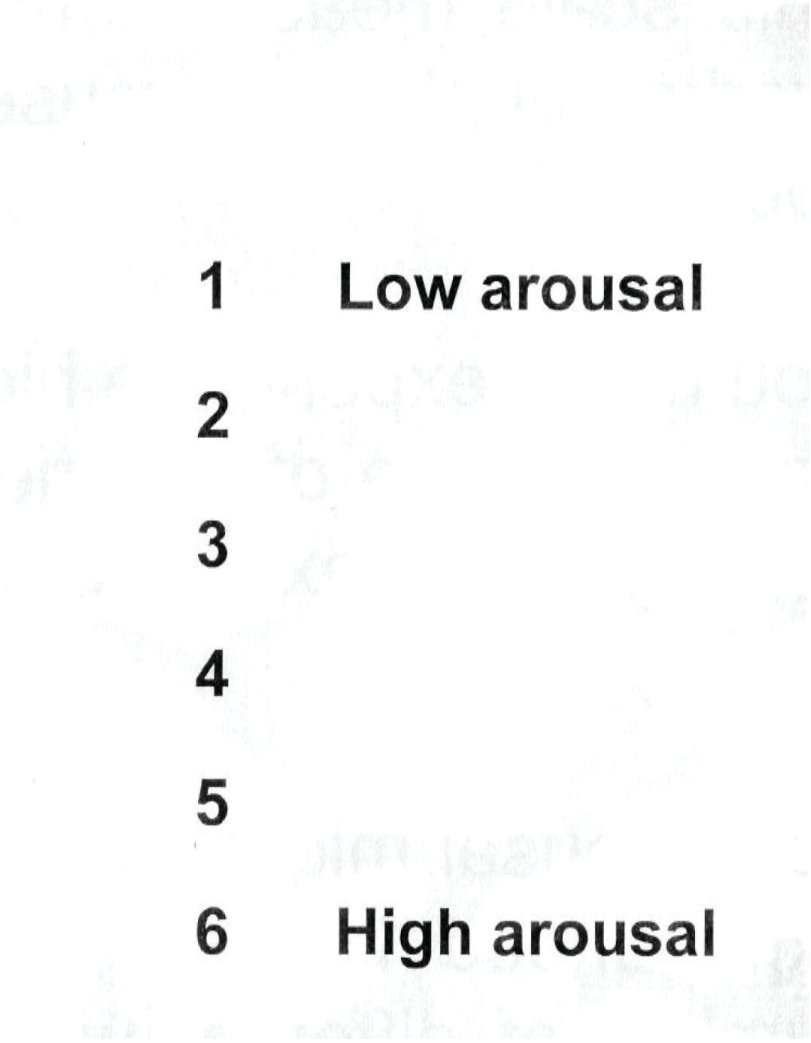
FEELING SCALE

+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 unpleasurable. 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 13

FELT AROUSAL SCALE



"This scale measures how 'worked up' or aroused you feel. You might experience high arousal in one of a variety of ways, for example as anxiety or anger. Low arousal might also be experienced by you in a number of different ways, for example relaxation or boredom or calmness."

Plasma Caffeine Assay

Preparation of plasma sample

It is important to suck up and dispense once before starting to aliquot into tubes for all repeated aliquots when using a single tip, i.e. the perchloric acid or NaOH.

Also the assay has been set up to use specific pipettes and tips.

1. Dispense 350 µL 0.8M Perchloric acid into labeled eppendorf tubes
2. Add 350 µL well mixed plasma
3. Vortex approximately 10s
4. Centrifuge 3-4 min at 11,500 g
5. Dispense 61.4 µL 0.4M NaOH into HPLC vials
6. Carefully remove 400 µLas 2 x 200 µL lots, and add to HPLC vial
7. Mix well

HPLC Analysis

Mobile phase: 25% methanol
Flow rate: 0.6 $ml \cdot min^{-1}$
Wavelength: 274 nm
Isocratic, 15 min run
20 µL injection volume
Standards 0.1-100 $\mu g \cdot ml^{-1}$ in methanol
Stock std 1 $mg \cdot ml^{-1}$

Glucose HK

Order Information

Cat. no.	Bottle	Contents
1447513	1	Buffer/ATP/NADP, 12 x 50 ml
	2	HK/G-6-PDH, 6 x 22 ml

Intended use

Enzymatic in vitro test for the quantitative determination of glucose in human serum, plasma and urine on COBAS® MIRA systems.

Summary[1-4]

Carbohydrates supply the body with glucose. Glucose is the most important monosaccharide in the blood; the postprandial concen-tration is 5 mmol of glucose per liter. Glucose substrate is an indispensable energy supplier which supports cellular function. Glucose degradation occurs in glycolysis. Glucose measurements are used in the diagnosis and monitoring of carbohydrate metabolism disorders including diabetes mellitus, neonatal hypoglycemia, idiopathic hypoglycemia, and pancreatic islet cell carcinoma.

The hexokinase method, based on the work of Schmidt and Peterson and Young, is a recognized reference method.

Test principle[3]

UV test

- Working reagent (buffer/ATP/NADP/HK/G-6-PDH), addition of sample and start of reaction:

  $$\text{glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

  Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP.

  $$\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^+$$

  Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phos-phate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and can be measured photometrically.

Reagent solution concentration

R1 Buffer/ATP/NADP
TRIS buffer*: 100 mmol/l, pH 7.8; Mg^{2+}: 4 mmol/l; ATP ≥1.7 mmol/l; NADP ≥1.0 mmol/l; preservative

R2 HK/G-6-PDH
HEPES buffer**: 30 mmol/l, pH 7.0; Mg^{2+}: 4 mmol/l; HK ≥8.3 U/ml (yeast); G-6-PDH ≥15 U/ml (E. coli); preservative

* TRIS = Tris(hydroxymethyl)-aminomethane
** HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethane sulfonic acid

Working reagent concentration

Buffer/ATP/NADP/HK/G-6-PDH (bottle 1 and 2)

TRIS buffer: 83 mmol/l; HEPES buffer: 5 mmol/l, pH 7.7; Mg^{2+}: 4 mmol/l; ATP ≥1.4 mmol/l; NADP ≥0.83 mmol/l; HK ≥1.4 U/ml (yeast); G-6-PDH ≥2.5 U/ml (E. coli); preservative

Reagent handling

R1: Ready for use

R2: Ready for use

Working reagent:
Combine 5 parts of bottle 1 with 1 part of bottle 2 (e.g. combine 5.0 ml of bottle 1 with 1.0 ml of bottle 2). Mix carefully.

Storage and stability

Unopened kit components: Up to the expiration date at 2–8°C.

Opened kit components: 3 months closed at 2–8°C.

Working reagent:
7 days (stored alternately in the refrigerator at 2–8°C and on the analyzer at 15–25°C; up to 8 hours opened in total on the analyzer).

Specimen collection and preparation

Collect serum using standard sampling tubes, Li-heparin, or K_3-EDTA plasma

Stability (no hemolysis)[5]: 8 hours at 15–25°C
72 hours at 2–8°C

Fluoride or iodoacetate plasma

Stability[5]: 24 hours at room temperature

Separate the sample from the cells (centrifuge) within 30 minutes of collection.[1]

Urine

Fresh random urine: Perform the assay immediately. If the assay cannot be performed immediately, store the samples in a refrigerator.[5]

24-hour urine: Collect the urine in a dark bottle and store on ice.[5]

Centrifuge samples containing precipitate before performing the assay.

Testing procedure

Materials provided

- Reagent solutions as described above

Additional materials required

- Calibrators and controls as indicated below

Calibration

Standardization: The glucose HK method was standardized against the ID-MS method.

Calibrator: C.f.a.s. (Calibrator for automated systems), Cat. No. 759350

Calibration frequency

Two-point calibration is recommended

- after reagent lot change
- as required following quality control procedures

Calibration verification: Not necessary.

Code No. 279-75401

Wako

NEFA C

(ACS-ACOD method)

この面は海外向けの添付文書です。
NEFA C は本品の輸出用名称です。
This page of the package insert is for use in countries outside of Japan.
NEFA C is the export name of this product.

Intended use

NEFA C is an in vitro assay for the quantitative determination of non-esterified fatty acids (NEFA) in serum.

Summary and explanation of the test

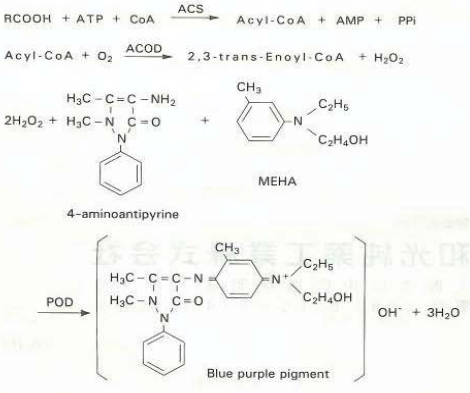
Non-esterified fatty acid (NEFA) in serum binding albumin, is used as an important energy source of peripheral tissues. The amount of NEFA in serum depends on a balance between intake in liver and peripheral tissues, and the release from adipose tissues. Amount of NEFA decreases by physical exercise, increases by starvation, cold, fear or smoking. And then increase or decrease of NEFA is observed in diabetes, hepatic diseases or endocrine diseases.
NEFA had been assayed by organic solvent extraction method, which was complicated to operate. Enzymatic method using Acyl-CoA oxidase (ACOD) has become widespread due to excellent specificity and concise procedure. NEFA C is the reagent kit for NEFA assay based on enzymatic method using 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) as a violet color agent.

Reagents

(1) Color A
(when reconstituted)
0.27 units/mL Acyl-CoA synthetase (ACS, *Pseudomonas sp.*)
0.73 mmol/L coenzyme A (CoA)
4.5 mmol/L adenosine 5'-triphosphate disodium salt (ATP, *Bacterium sp.*)
1.5 mmol/L 4-aminoantipyrine
2.7 units/mL ascorbate oxidase (AOD, *Cucurbita*)
Store at 2-10°C
(2) Solvent A
50 mmol/L Phosphate buffer, pH 7.0
Store at 2-10°C
(3) Color B
(when reconstituted)
5.5 units/mL Acyl-CoA oxidase (ACOD, *Arthrobacter sp.*)
6.8 units/mL peroxidase (POD, *Horseradish*)
Store at 2-10°C
(4) Solvent B
1.2 mmol/L 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA)
Store at 2-10°C
(5) NEFA Standard Solution
1 mEq/L oleic acid
Store at 2-10°C

Principle of the method

Non-esterified fatty acid (NEFA) in sample is converted to Acyl-CoA, AMP and pyrophosphoric acid (PPi) by the action of Acyl-CoA synthetase (ACS), under coexistence with coenzyme A (CoA) and adenosine 5'-triphosphate disodium salt (ATP). Obtained Acyl-CoA is oxidized and yields 2,3-trans-enoyl-CoA and hydrogen peroxide by the action of Acyl-CoA oxidase (ACOD). In the presence of peroxidase (POD), the hydrogen peroxide formed yields a blue purple pigment by quantitative oxidation condensation with 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) and 4-aminoantipyrine. Non-esterified fatty acids (NEFA) concentration is obtained by measuring absorbance of the blue purple color.



Reagent preparation and test procedure

1. Preparation of reagents

R1 : Prepare Reagent 1 by mixing one bottle of each Color A (for 10 mL) and Solvent A (10 mL).
After preparing the Reagent 1, store below 25°C and use within the day, or store at 2-10°C and use within 5 days.
R2 : Prepare Reagent 2 by mixing one bottle of each Color B (for 20 mL) and Solvent B (20 mL).
After preparing the Reagent 2, store below 25°C and use within the day, or store at 2-10°C and use within 5 days.

2. Materials required but not supplied

Test tube, pipette (for specimen, for reagent), water bath or heating block capable of maintaining 37°C, Spectrophotometer or Colorimeter that have 550 nm filter.

3. Standard procedure

	Sample (S)	Standard (Std)	Reagent Blank (Bl)	Sample blank※1 (SBl)
Specimen	Serum 0.05 mL	Standard 0.05 mL	Distilled or deionized water 0.05 mL	—
Reagent 1	1.0 mL	1.0 mL	1.0 mL	1.0 mL
Mix well and incubate for 10 minutes at 37°C.				
Reagent 2	2.0 mL	2.0 mL	2.0 mL	2.0 mL
Specimen	—	—	—	Serum 0.05 mL
Mix well and incubate for 10 minutes at 37°C.				
After cooling the solution to room temperature, measure the absorbance (E_S) of Sample (S) and the absorbance (E_{Std}) of Standard (Std) against Reagent Blank (Bl) within 30 minutes. Spectrophotometer 550 nm Colorimeter 550 nm filter				

※1 Sample Blank is not required for common samples, but required for lipemia or hemolysis sample.

4. Calibration

Prepare diluted standard solution as directed in Table below. Aliquot the diluted standard and assay the Standards as specimens according to the above standard procedure.
Plot the absorbance of each test tube along the ordinate against the concentration of NEFA along the abscissa.

Table

No.	Standard	Distilled or deionized water	Sample volume	Concentration of NEFA
1	1.0 mL	1.0 mL	0.05 mL	0.50 mEq/L
2	Undiluted	—	0.05 mL	1.00 mEq/L
3	Undiluted	—	0.10 mL	1.97 mEq/L※1

※1 The test sample volume is usually 0.05 mL, but 0.10 mL is taken in this case. The total volume is therefore increased slightly. The concentration of NEFA must be corrected accordingly as indicated in the table above.

5. Calculation

(1) Calculation method from calibration curve
Calculate NEFA concentration from the calibration curve which was made previously.
(2) Calculation method from expression

$$\text{NEFA (mEq/L)} = \frac{E_S}{E_{Std}} \times 1.0$$

Remarks
When sample blank is measured, calculate Es by subtracting sample blank from sample O. D. to get NEFA concentration.

1

Precautions for procedure

(1) Samples
  (a) Assay samples immediately after collection, because the enzymes such as lipoprotein lipase, phospholipase etc. hydrolyze lipids and form fatty acids.
  Freeze sample, when a serum is stored.
  (b) Hemolysis gives positive effect on the assay.
  (c) Bilirubin gives negative effect on the assay.
  (d) Ascorbic acid does not have significant effects on the assay.

Expected values[3]

131-445 μEq/L

Performance

(1) Sensitivity
  (a) When purified water is assayed, the absorbance is not more than 0.07.
  (b) When a standard of given concentration (non-esterified fatty acid 1 mEq/L) is assayed, the absorbance is 0.10-0.37
(2) Specificity
  When a control serum of known concentration is assayed, the assay value falls within the range of ± 15% of the known concentration.
(3) Precision
  When a sample is assayed not less than 5 times in a run, CV of absorbance is not more than 3%.
(4) Measurable range
  Up to 2 mEq/L NEFA. (In the case of using the standard procedure)

Correlation

Correlation coefficient $r = 0.995$ $(n = 30)$
Regression equation $y = 0.980\ x + 0.004$
y : NEFA C (mEq/L)
x : Wako NEFA
(Modified Duncombe method, mEq/L)

Precautions for assays

(1) Do not use the reagents described above in any procedures other than those described herein. Performance cannot be guaranteed if the reagents are used in other procedures or for other purposes.
(2) Operate the instruments according to operator's manuals under appropriate conditions. Consult the instrument manufacturer for details.
(3) Store the reagents under the specified conditions. Do not use reagents past the expiration date stated on each reagent container label.
(4) Do not use reagents which were frozen in error. Such reagents may give false results.
(5) After opening the reagents, it is recommended to use them immediately.
  When the opened reagents are stored, cap the bottles and keep them under the specified conditions.
(6) Do not use the containers and other materials in the package for any purposes other than those described herein.
(7) The vial is stoppered at reduced pressure. Slowly remove the stopper in order not to blow the powder in the vial.
(8) Do not use the reagents at other reaction temperatures and reaction times than described herein.
(9) The reaction induced by Reagent 1 will almost complete in 6 minutes at 37°C. Do not incubate more than 15 minutes.
(10) The reaction induced by Reagent 2 will almost complete in 5 minutes at 37°C. When the incubation at 37°C is continued, absorbance falls down time by time. Therefore, return it to room temperature immediately after the incubation for 10 minutes at 37°C.
(11) Avoid the direct sunlight during operation.

Precautions for protection from hazards

(1) If the reagents come in contact with mouth, eye or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
(2) Avoid pipetting by mouth. Use pipettors.
(3) Be careful not to cut yourself with the aluminum cap when remove it from the vial.

Precautions for disposal

(1) When discarding the reagents, dispose of them according to local or national regulations.
(2) All the devices including reagents and reagent bottles contacted with specimen should be considered potentially infectious.
(3) Color A and Solvent A contain sodium azide as a preservative (0.07% in Reagent 1 when reconstituted). NEFA Standard Solution contains 0.05% sodium azide.
  Sodium azide may react with lead or copper plumbing to form explosive compounds. Even though the reagent contains minute quantity of sodium azide, drains should be flushed well with a large amount of water, when discarding the reagents.

Precautions for results and diagnosis

This assay should not be used as the sole determinant for clinical diagnosis.

References

(1) Shimizu S., Yasui K., Tani Y., Yamada H., Biochem. Biophys. Res. Commun., **91**, 108-113 (1979).
(2) Shimizu S., Yasui K., Tani Y., Yamada H., Tabata K. and Murachi T., Japan Society of Bioscience, Biochemistry and Agrochemistry, Lecture content collection, **55**, 38 (1980). (in Japanese)
(3) Kushiro H., Takano K. and Fukui I., Journal of Medical Technology, **15**, 191-193 (1971). (in Japanese)

Ordering information

Code No.	Product	Package
279-75401	NEFA C	50 Tests
	Color A	6 × for 10 mL
	Solvent A	1 × 65 mL
	Color B	6 × for 20 mL
	Solvent B	1 × 130 mL
	NEFA Standard Solution	1 × 10 mL

Wako Pure Chemical Industries, Ltd.
1-2, Doshomachi 3-Chome, Chuo-Ku, Osaka 540-8605, Japan
Telephone : +81-6-6203-3749
Facsimile : +81-6-6203-1917
http://www.wako-chem.co.jp

06.10.23K04