THE PERFORMANCE AND PHYSIOLOGICAL EFFECTS OF CAFFEINE AND OCTOPAMINE SUPPLEMENTATION DURING ENDURANCE CYCLE EXERCISE

By

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A Doctoral Thesis

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Abstract

Caffeine consistently enhances endurance performance in temperate environmental conditions, while far less research has examined its ergogenic and physiological effects during prolonged exercise in the heat. Despite the performance benefit of an acute caffeine doses being less pronounced in regular caffeine users versus those not habituated to the drug, few studies have examined the influence of a prolonged period of controlled caffeine intake on endurance performance. The endogenous trace amine octopamine is purported to possess stimulant-like properties and influence fat metabolism, although no study has examined these effects in humans. The aim of this thesis was to further characterise the performance and physiological effects of caffeine during prolonged exercise, while elucidating a potential ergogenic role for octopamine.

The first two studies investigated the ergogenic and thermoregulatory effects of low to moderate caffeine doses during prolonged cycle exercise in the heat. Chapter 4 demonstrated that 3 $mg \cdot kg^{-1}$ caffeine, administered either as a single or split-dose (2 x 1.5 $mg \cdot kg^{-1}$) before exercise, improved endurance performance without influencing thermoregulation during prolonged exercise at a fixed work-rate. Dividing the caffeine bolus appeared to confer an additional performance benefit, suggesting repeated low dose may potentiate the efficiency of the same total caffeine dose under these conditions. Chapter 5 demonstrated that a 6 $mg \cdot kg^{-1}$ caffeine dose improved endurance cycle performance without differentially influencing thermoregulation than placebo.

The level of habituation to caffeine influences the ergogenic effect of an acute dose, yet previous studies have employed sub-chronic supplementation protocols. Chapter 6 investigated the effect of a twenty-eight day supplementation period on endurance cycle performance. Habituation to caffeine attenuated the ergogenic effect of an acute caffeine dose, without any change in circulating caffeine, substrate oxidation or hormonal concentrations. In chapter 7 the performance and metabolic effects of octopamine was investigated. Octopamine supplementation did not influence performance, hormonal concentrations or substrate oxidation, likely due to low serum concentrations of the drug.

Key words: Central fatigue, central nervous system, heat strain, habituation, stimulants

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Publications and Presentations

The findings of the studies reported in this thesis have been published and presented as follows:

Publications:

Chapter 5:

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Presentations:

Chapter 4:

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Chapter 6:

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List of Abbreviations

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AADC	Aromatic L-amino acid decarboxylase
ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
BCAA	Branched-chain amino acid
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CRF	Corticotropin-releasing factor
CV	Coefficient of variation
CYP	Cytochrome
ELISA	Enzyme-linked immunosorbent assay
FFA	Free fatty acid
GABA	Gamma-Aminobutyric acid
Hb	Haemoglobin
Hct	Haematocrit
HPLC	High performance liquid chromatography
L-DOPA	L-3,4-dihydroxyphenylalanine
LNAA	Large neutral amino acid
MRI	Magnetic resonance imaging

MCV	Maximal voluntary contraction
PET	Positron emission topography
PO/AH	Preoptic anterior hypothalamus
PVN	Paraventricular nucleus
RPE	Rating of perceived exertion
SNP	Single nucleotide polymorphism
SD	Standard deviation
STPD	Standard temperature and pressure for dry gas
TT	Time-trial
VA	Voluntary activation
VO_{2peak}	Peak oxygen uptake
VO_{2max}	Maximum oxygen uptake
W	Watts
WADA	World Anti-Doping Agency
W_{max}	Maximum workload

Chapter 1

General Introduction

The use of dietary supplements is widespread among athletes (Desbrow and Leveritt, 2006; Knapik et al. 2016) and university students (Lieberman et al. 2015). One of the primary reasons individuals consume supplements is to enhance performance (Lieberman et al. 2015), yet only a few appear to enhance performance. Stimulant-based compounds such as caffeine elicit consistent performance benefits in laboratory-based studies during prolonged exercise (>30 min) in temperate environmental conditions (20^{o}C; Burke, 2008). However, the influence of a high ambient temperature (~30^{o}C) on the ergogenic effect of caffeine has received little attention (Armstrong et al. 2007). Prolonged exercise in the heat places considerable strain on the thermoregulatory and cardiovascular systems (Cheuvront et al. 2010; Nybo, 2010). Several lines of evidence suggest that caffeine elevates the core temperature response to exercise in the heat (Cheuvront et al. 2009; Del-Coso et al. 2009; Ely et al. 2011; Roelands et al. 2011), which may precipitate the onset of fatigue (González-Alonso et al. 1999). However, a caffeine-induced increase in core temperature is not always observed, which may be due to the dosing strategy employed (Ganio et al. 2011; Pitchford et al. 2014; Roti et al. 2006). Indeed, larger intakes of caffeine (9 mg·kg^{-1}) elicit more consistent elevations in core and body temperature during exercise (Cheuvront et al. 2009; Ely et al. 2011), while the performance and thermoregulatory responses to lower doses (3-6 mg·kg^{-1}) are less clear (Del-Coso et al. 2009; Ganio et al. 2011; Pitchford et al. 2014; Roelands et al. 2011). Given the widespread intake of caffeine by athletes competing in warm environments (Desbrow and Leveritt, 2006), a deeper understanding of the performance and physiological effects following acute caffeine intake during exercise in the heat is warranted.

Despite such widespread intake of caffeine within the general population (Fitt et al. 2013), the influence of a prolonged period of controlled caffeine intake on endurance performance and exercise metabolism has not been investigated. The ergogenic effect of an acute 5 mg·kg^{-1} caffeine dose was less pronounced in regular caffeine users (>300 mg·day^{-1}) vs. their non-habituated counterparts (<50 mg·day^{-1}; Bell and McLellan, 2002). Similar

metabolic responses occurred during prolonged, fixed-intensity exercise when comparing low-and high-habitual caffeine users (Bangsbo et al. 1992). Furthermore, studies which employed sub-chronic supplementation protocols failed to report any change in metabolism or performance following the habituation period. Irwin et al (2011) reported that four-days of controlled caffeine intake (3 $mg \cdot kg^{-1}$) did not influence the magnitude of performance benefit from an acute 3 $mg \cdot kg^{-1}$ caffeine dose. Similarly, five-days of low (3 $mg \cdot kg^{-1}$) and moderate (6 $mg \cdot kg^{-1}$) caffeine intakes did not alter the physiological response to prolonged exercise in the heat (Roti et al. 2006). Based on these observations, it seems likely that a greater duration of controlled caffeine supplementation is required to influence the magnitude of performance benefit attained from acute supplementation.

The endogenous trace amine octopamine is suggested to possess stimulant-like properties and thus potentially enhance performance (Stohs, 2014). However, octopamine supplementation is currently prohibited by the World Anti-Doping agency (WADA), meaning its use is banned in competition by athletes (WADA, 2015). This is despite no evidence that octopamine influences performance in humans. Direct cerebral administration of octopamine increased locomotor activity in rats (Jagiełło-Wójtowicz, 1979), while data from mammalian cell-lines demonstrated the ability of octopamine to activate β_3 adrenoreceptors and stimulate lipolysis (Carpéné et al. 1999). Therefore, it would be of interest to examine the performance and physiological effects of acute octopamine supplementation in humans.

1.1 Thesis aims and outline

The aim of the work described in this thesis is to examine the performance and physiological effects of caffeine and octopamine during prolonged cycle exercise.

The main objectives are as follows:

- To investigate the performance and physiological responses following acute supplementation with low (Chapter 4) and moderate (Chapter 5) caffeine doses during prolonged exercise in the heat

- To examine the influence of a prolonged period of controlled caffeine intake on endurance cycle performance and exercise metabolism (Chapter 6)
- To determine the performance and metabolic effects of acute octopamine supplementation during prolonged exercise (Chapter 7)

A brief introduction to the subsequent chapters is provided below and a schematic of the experimental chapters is depicted in Figure 1.1.

Chapter 2 reviews the current evidence regarding the performance and physiological effects of caffeine supplementation during exercise in the heat, while also discussing the relatively few studies to investigate the influence of caffeine habituation on exercise performance. In addition, the animal and *in vitro* models relating to the effects of octopamine administration on physical activity and metabolism and how these responses may influence endurance cycle performance and *in vivo* human physiology are reviewed. Chapter 3 provides a detailed explanation of the methods employed during each of the investigations.

The study in Chapter 4 investigates the performance and physiological responses to prolonged exercise in the heat following two acute low-dose caffeine ingestion strategies (3 $mg \cdot kg^{-1}$). Chapter 5 examines the effects of an acute moderate caffeine dose (6 $mg \cdot kg^{-1}$) on cycle performance and thermoregulation during endurance exercise in the heat. It is hypothesised that acute caffeine supplementation in both studies will improve endurance cycle performance without adversely influencing thermoregulation versus placebo. Chapter 6 investigates whether a prolonged period (28 days) of controlled caffeine intake (1.5-3 $mg \cdot kg \cdot d^{-1}$) influences the performance or metabolic responses typical of acute supplementation. It is hypothesised that chronic caffeine intake will result in the development of tolerance to a subsequent acute caffeine dose. Chapter 7 examines the effects of an acute dose of octopamine (150 mg) on endurance cycle performance and exercise metabolism. It is hypothesised that octopamine supplementation will improve endurance cycle performance.

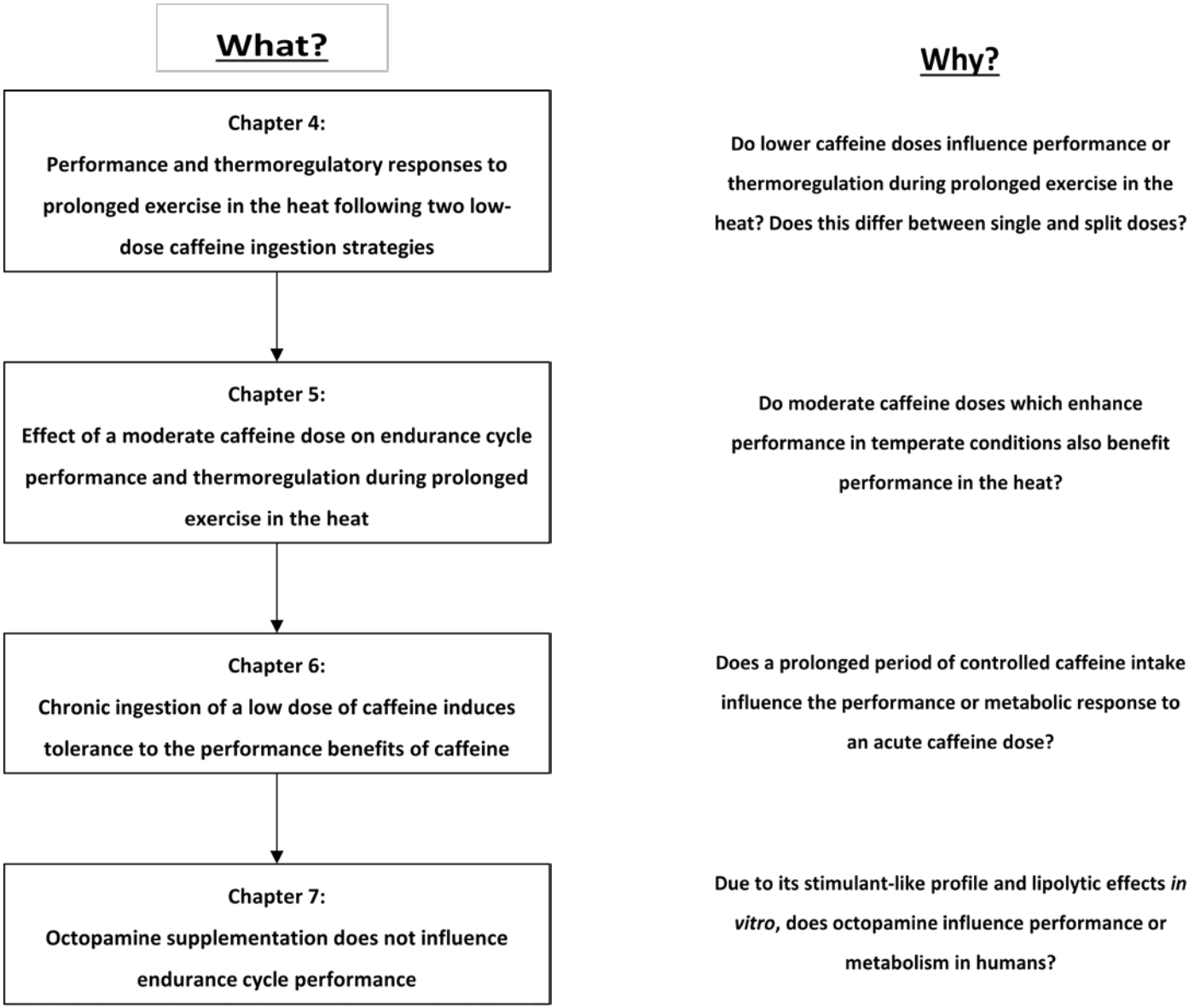

Figure 1.1 Schematic of experimental chapters

Chapter 2

Literature Review

2.1 Fatigue

Traditionally, fatigue is defined as the inability to maintain the required force or power output during exercise (Edwards, 1981) or any exercise-induced reduction in the ability to exert muscle force or power, regardless of whether the exercise task can be sustained (Bigland-Ritchie and Woods, 1984). The aetiology of fatigue has been divided into two camps: central and peripheral fatigue. In relation to cycle exercise, peripheral fatigue may manifest as biochemical changes within the exercising muscle leading to a reduced response to neural excitation (i.e. greater neural input to generate the same force; Abbiss and Laursen, 2005; Amann, 2011; Green, 1997). Conversley, central fatigue refers to events arising within the central nervous system (CNS) which reduces efferent stimulation of motor neurons via decending supraspinal pathways (i.e. reduced neural input to the exercising muscles; Enoka, 1992; Gandevia, 2001).

The concept of exercise-induced fatigue is not new. In 1891, August Waller suggested that the central component of fatigue served as a guard over peripheral fatigue. Subsequently, Alessandro Mosso crudely demonstrated a reduced capacity to perform physical work after a bout of mental exercise, resulting in the term ‘mental fatigue’ (Mosso, 1904). Francis Bainbridge later concluded that ‘muscular fatigue’ was superadded to ‘nervous fatigue’ (Bainbridge, 1919). Following the Nobel Prize winning work of Archibald Hill (Hill and Lupton, 1923; Hill et al. 1924), a great deal of emphasis was placed on the peripheral component of fatigue. During this time, the important link between blood glucose concentrations and marathon running performance was established (Gordon et al. 1925; Levine et al. 1924). The re-introduction of the muscle biopsy technique further emphasised the importance of substrate provision during prolonged exercise (Bergstrom et al. 1967). Despite the early proponents of central fatigue, a lack of available techniques to accurately assess CNS function likely precluded research into this area. However, with the introduction of twitch interpolation and transcranial magnetic stimulation (Barker, 1985; Grimby et al. 1981), a greater focus was placed on the central component of fatigue.

In recent years, various cause-and-effect models have been proposed to explain fatigue during prolonged exercise, with focus on the cardiovascular, biochemical, thermoregulatory, psychological/motivation and central govenour models (Abbiss and Laursen, 2005; Noakes et al. 2005). However, a complex intergrated model of fatigue which occurs during prolonged cycle exercise has been proposed (Amann, 2011; Figure 2.1).

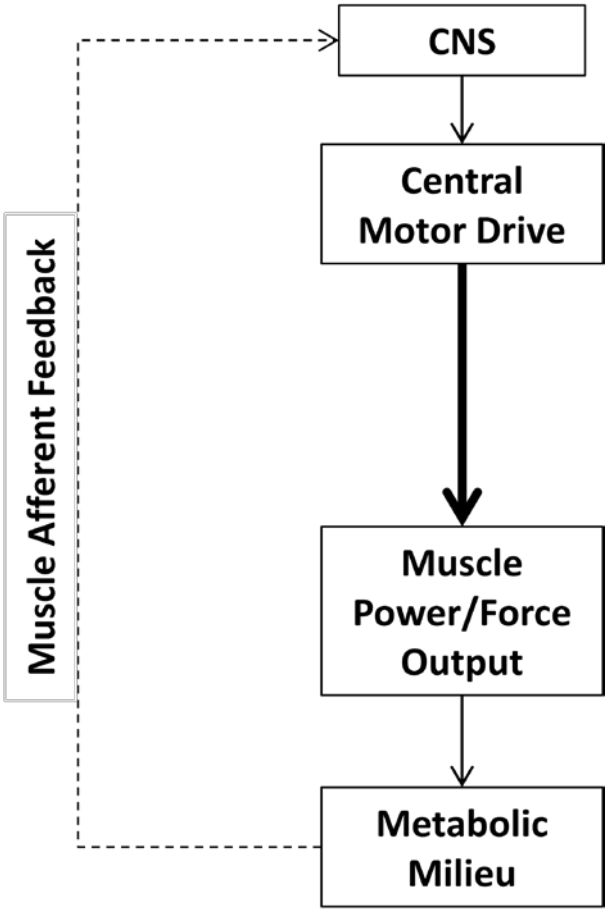

Figure 2.1 Schematic representation of the supraspinal reflex inhibition fatigue model of fatigue proposed by Amann (2011). The thick solid line represents neural input to the exercising muscles and the dashed line indicates afferent feedback to the CNS. This model suggests that muscle afferents exert inhibitory control on the magnitude of central motor drive during whole-body endurance exercise. The magnitude of central motor drive determines the power output of the locomotor muscles, which in turn determines the metabolic milieu within the exercising muscles. Finally, the metabolic milieu determines the magnitude of afferent (inhibitory) feedback, providing a feedback loop which restricts the state of locomotor muscle fatigue to an individual threshold.

Using this model, Amann (2011) argues that afferent feedback from peripheral locomotor muscles is an important determinant of endurance performance. However, rather than peripheral fatigue *per se*, the preceding biochemical changes within the exercising muscles provide feedback to the CNS during exercise (Amann et al. 2010). Specifically, metabosensitive group III/IV afferents relate exercise-induced metabolic changes in the muscle to the brain (Kaufman and Rybicki, 1987), with this inhibitory feedback determining the magnitude of central motor drive to the locomotor muscles (Amann, 2011). When severe peripheral fatigue is present (which appears to be highly individual; Amann et al. 2006), endurance exercise is voluntarily terminated (i.e. during a time to exhaustion task) or the exercise intensity is reduced though a decrease in central motor drive (i.e. during a TT task; Figure 2.1). The brain processes muscle afferent feedback to limit the development of peripheral fatigue to a critical threshold, beyond which the level of sensory input would not be tolerable. Therefore, the magnitude of biochemical changes within the exercising muscles exert an inhibitory effect on central motor drive and thus the development of central fatigue during whole-body endurance exercise (Amann, 2011).

A separate model to explain the fatigue which arises during prolonged cycle exercise suggests that afferent feedback from peripheral locomotor muscles is not particularly important in regulating self-paced endurance exercise (Marcora, 2010). Smilar to the integrated peripheral/central models of endurance performance (Amann, 2011; St Clair Gibson and Noakes, 2004; Noakes et al. 2005), Marcora (2010) does state that knowledge of the distance to cover during exercise, the distance already covered, and any previous experience of the exercise task are all important factors that will likely influence performance. However, a greater emphasis is placed on perceived exertion during exercise, which appears to be independent of afferent feedback from peripheral muscles, heart and lungs (Marcora, 2009). For example, when inducing peripheral muscle fatigue with epidural anesthesia, perceived exertion is either unchanged or increased during exercise (Kjaer et al. 1999; Smith et al. 2003). The latter effect is due to a compensatory increase in neural drive to the locomotor muscles in order to cycle at the same power output (Marcora et al. 2008). This increase in central motor command is detected, through collory discharges from motor to sensory areas of the cerebral cortex (Marcora, 2009), as an increase in perception of effort (Kjaer et al. 1999). Further evidence against the importance of afferent feedback from

skeletal muscles in determining endurance performance is the 'end spurt'. That is, a large increase in power output at the end of a TT despite a high prescence of fatigue-inducing metabolites (Amann et al. 2008). Therefore, according to this model, the conscious self-regualtion of power output during exercise, rather than inhibitory feedback from peripheral tissues, is more important in determining endurance performance. This is why it is called the 'psychobiological' model of endurance performance (Marcora et al. 2008; Marcora, 2010).

While the degree to which afferent feedback from peripheral tissues mediates the development of fatigue during prolonged exercise is still hotly debated (St Clair Gibson and Noakes, 2004; Marcora et al. 2008; Marcora, 2010; Noakes et al. 2005; Shephard, 2009), it is clear the brain plays a pivitol role (Meeusen and Watson, 2007). Nevertheless, the precise mechanisms responsible for the onset of fatigue will always depend on the duration, mode and intensity of exercise, as well as the environmental conditions. In recent decades, the central component has received increasing interest, especially in relation to changes in central neurotransmission (Meeusen and Watson, 2007; Newsholme et al. 1987), as discussed below.

2.2 The Central Fatigue Hypothesis

The most widely cited theory proposed to explain centrally-mediated fatigue was introduced by Newsholme and colleagues (1987). These authors built on the work of Chaouloff et al (1985, 1986), who reported increased brain serotonin concentrations in rats during prolonged exercise. Serotonin is a classical monoamine neurotransmitter typically associated with feelings of lethargy, sleepiness, and decreased arousal and mood (Jacobs and Azimita, 1992). Therefore, elevating cerebral serotonin concentrations would be expected to precipitate the onset of fatigue. The central fatigue hypothesis suggests that changes in peripheral substrate availability influence the rate of serotonin synthesis in the brain (Newsholme et al. 1987). The amino acid tryptophan (a precursor of serotonin) is loosely bound to albumin, with approximately 10% of the unbound amino acid present in the circulation (McMenamy and Oncley, 1958). During prolonged exercise, elevated free fatty acid (FFA) concentrations liberate tryptophan from albumin (Curzon et al. 1974), leading to a greater concentration of unbound tryptophan in the blood. This increases

cerebral uptake of free tryptophan by the large neutral amino acid (LNAA) transporter. Serotonin synthesis is dependent on the enzyme tryptophan hydroxylase, which is not saturated under normal physiological conditions (Jacobs and Azmitia, 1992). Hence, the increased delivery of tryptophan augments cerebral serotonin synthesis (Newsholme et al. 1987) and release (Schaechter and Wurtman, 1990).

The attractive concept of this hypothesis is undoubtedly the potential to influence brain serotonin through dietary manipulation. The branched chain amino acids (BCAA) compete with tryptophan for passage across the blood-brain barrier (BBB) by the LNAA transporter (Pardridge, 1983). During prolonged exercise, BCAA concentrations decrease due to enhanced uptake by skeletal muscle (Bloomstrand et al. 1988). This reduces the competition for tryptophan at the LNNA transporter, further increasing its entry into the brain. An early study reported enhanced marathon performance when BCAA's were consumed before exercise (Bloomstrand et al. 1991). Unfortunately, subsequent studies failed to confirm these initial findings (Blomstrand et al. 1997; Cheuvront et al. 2004; Watson et al. 2004). Additionally, the ingestion of tryptophan-free amino acid mixtures which deplete the pool of circulating tryptophan fails to influence endurance performance (Hobson et al. 2013). In rats, tryptophan administration augmented the exercise-induced increase in cerebral serotonin (>100%), but this did not induce early fatigue (Meeusen et al. 1996). Carbohydrate supplementation is another nutritional strategy purported to alter the balance of serotonin synthesis in the brain. Carbohydrate intake attenuates lipolysis, decreases the circulating concentration of FFA and ultimately limits the exercise-induced increase in unbound tryptophan. Supplementation with a 6% and 12% carbohydrate solution decreased FFA concentrations and the ratio of unbound tryptophan to BCAA's (Davis et al. 1992). While performance was enhanced compared with placebo, it is difficult to discern the precise contribution of the central versus peripheral effects of carbohydrate ingestion. More recently, Blomstrand et al (2005) demonstrated that supplementation with a 6% carbohydrate solution decreased cerebral tryptophan uptake during prolonged exercise compared with placebo. However, the sample size was small (n=5), and performance was not measured.

In addition to nutritional manipulation, pharmacological strategies have been employed to elucidate a potential role for serotonin in the development of fatigue during prolonged

exercise. The administration of a LNNA transporter blocker, which prevented cerebral tryptophan uptake and subsequent serotonin synthesis, prolonged exercise time in rats (Yamamoto and Newsholme, 2000). In a series of rat studies by Bailey et al (1992, 1993a), changes in exercise time were reported following the administration of specific serotonin agonists and antagonists. However, subsequent studies in humans failed to confirm these findings (Meeusen et al. 2006). Furthermore, augmenting synaptic serotonin concentrations with specific reuptake inhibitors can dose-dependently increase the activation of the entire motor pathway as assessed by magnetic resonance imaging (MRI; Loubinoux et al. 2002). In agreement, serotonin transmission is involved in stimulating locomotion in rats (Takahashi et al. 2000). While early evidence suggested that serotonin reuptake inhibition impairs endurance performance in humans (Wilson and Maughan, 1992), this might be due to disturbances in neuronal homeostasis via pre-and post-synaptic mechanisms, rather than by directly influencing the firing rate of serotonergic neurons (Struder and Weicker, 2001).

Bailey and colleagues (1993b) reported a decrease in dopamine tissue content at the point of fatigue, while serotonin concentrations remained elevated. These findings led to the suggestion that the ratio of serotonin to dopamine might be an important factor in the development of fatigue (Davis and Bailey, 1997). Hence, elevating dopamine concentrations during prolonged exercise would be expected to enhance performance. Tyrosine, a precursor for dopamine and noradrenaline synthesis, is subject to competitive transport across the LNAA-carrier system (Fernstrom et al. 1983). Synaptic dopamine is responsive to precursor supply during periods of acute stress (During et al. 1989), yet tyrosine supplementation does not influence endurance capacity (Watson et al. 2012) or performance (Tumilty et al. 2014) in the heat. Furthermore, augmenting cerebral dopamine synthesis and release through L-3,4-dihydroxyphenylalanine (L-DOPA) supplementation (Floel et al. 2008) does not prolong endurance capacity in a warm environment (Cordery et al. 2016). Conversely, dual dopamine-noradrenaline reuptake inhibition consistently enhances performance in warm, but not temperate conditions (Roelands and Meeusen, 2010). These contrasting findings suggest that simply influencing the ratio of serotonin to dopamine in the brain is not particularly important in the genesis of fatigue. Rather, the neurophysiological effects induced by different pharmacological interventions should be considered. For example, the dual dopamine-noradrenaline reuptake inhibitor Bupropion

changes the electrophysiology of the locus coeruleus (Nestler et al. 1999). While L-DOPA is a principle substrate for noradrenergic neurons within this brain region, it has no effect on locus coeruleus electrophysiology (Miguelez et al. 2011).

Despite promising results from early animal studies (Bailey et al. 1993b; Chaouloff et al. 1985, 1986), the role of serotonin in the development of fatigue is questionable. Several important factors should also be considered. First, the doses administered in previous animal models are typically much greater than the doses administered to humans. Second, the route of administration (i.e. direct injection in animals vs. oral ingestion in humans) will influence the pharmacokinetic profile of the drug. Third, the inter-species variation in the neurophysiological responses to nutritional and pharmacological interventions. Fourth, much of the early evidence was obtained from brain tissue homogenate, without the assessment of neurotransmitter concentrations over time (Meeusen et al. 2001; Meeusen et al. 2006). Even when microdialysis is employed, this method only enables the measurement of extracellular neurotransmitters in a single brain structure, which may not reflect changes in other cerebral regions. Despite the lack of consistent evidence substantiating a role for serotonin in the genesis of fatigue, events arising within the CNS appear to play a more important role in mediating endurance performance during exercise in the heat (Maughan et al. 2007; Nybo, 2010; Roelands et al. 2013).

2.3 Prolonged Exercise in the Heat

The capacity to perform prolonged exercise becomes impaired as the ambient temperature increases (Galloway and Maughan, 1997). Similarly, there is an inverse relationship between welt-bulb globe temperature and marathon race performance (Montain et al. 2007). The additional physiological challenge to thermoregulation is believed to play a key role in mediating this deterioration in performance (Nybo, 2010). Humans are approximately 20% efficient at converting chemical energy into mechanical work; the rest is lost as heat (Powers and Howley, 2009). During exercise, this excess heat will contribute to an elevation in core temperature, with the increase directly proportional to the power output (Neilsen, 1938). To maintain a stable internal environment, there are 4 avenues through which humans dissipate heat: conduction, convection, radiation, and evaporation. The first 3

mechanisms rely on a skin to environmental temperature gradient for heat loss to occur. Hence, when the environmental temperature increases, this temperature gradient narrows, and the evaporative route (i.e. sweating) becomes the primary method of heat loss (Neilsen, 1938). When sweat is deposited onto the surface of the skin, a large thermal input drives the conversion from water to vapour, resulting in heat loss to the environment. This process is dependent on a high skin temperature and the pressure gradient between the skin and the environment (Fortney and Vroman, 1985). Any increase in relative humidity will reduce this pressure gradient, thereby attenuating the capacity for heat loss. Recently, endurance capacity was reduced in a humidity-dependant manner during prolonged exercise at the same ambient temperature (30^{o}C; Maughan et al. 2012).

The mechanisms which closely describe the onset of fatigue during prolonged exercise in temperate conditions, such as the depletion of muscle glycogen and the accumulation of metabolites (Abbiss and Laursen, 2005; Green, 1997), are not particularly important in the genesis of fatigue when exercise is performed under conditions of heat stress (Neilsen et al. 1990; Neilsen et al. 1993; Parkin et al. 1999). The most widely-cited theory proposed to explain fatigue during prolonged exercise in the heat is the 'critical' core temperature hypothesis (Gonzalez-Alonzo et al. 1999; Neilsen et al. 1993). Despite commencing exercise with different core temperatures, participant's cycled to volitional exhaustion until remarkably similar core temperatures were achieved (~40^{o}C; Gonzalez-Alonzo et al. 1999). This response was also demonstrated in rats (Fuller et al. 1998), suggesting there may be a thermal limit beyond which exercise cannot continue. Additionally, impairments in voluntary muscle activation correlate more strongly with core temperature than local muscle temperature (Thomas et al. 2006). However, core temperature values at the point of exhaustion are greater in well-trained (Gonzalez-Alonzo et al. 1999) than untrained (Cheung and McLellan, 1998) individuals. Furthermore, highly-motivated athletes in the field can attain higher core temperatures than typically observed in a laboratory environment (Byrne et al. 2006). The administration of pharmacological agents also enable individuals to continue exercise with core temperatures >40^{o}C (Watson et al. 2005a). These observations suggest that fatigue is unlikely to occur only when core temperature reaches a specific value. Alternatively, there appears be a progressive inhibition of brain areas responsible for

motor activation as a consequence of the steadily rising hyperthermic state (Morrison et al. 2004).

The important link between brain temperature and exercise tolerance was demonstrated by Caputa and colleagues (1986). Thermo-elements were implanted into goats to independently manipulate core and hypothalamic temperature during exercise, with an increase in the latter precipitating the onset of fatigue. Neurons innervating the preoptic anterior hypothalamus (PO/AH) regulate changes in body temperature, based on afferent input from peripheral and spinal thermoreceptors, as well as the temperature of the blood flowing to the brain (Boulant, 2000). Brain temperature increases during prolonged exercise in the heat, remaining approximately 0.2^{o}C higher than the core, suggesting impaired heat removal via the blood (Nybo et al. 2002). Attenuated cerebral perfusion also accompanies hyperthermia (Nybo and Neilsen, 2001c), although restoring blood flow to non-hyperthermic levels does not prevent fatigue (Rasmussen et al. 2004). Prolonged exercise in the heat induces a gradual change in brain-wave activity, which is typically correlated with perceived exertion (Nybo and Nielsen, 2001b). Exercise-induced hyperthermia also attenuates the duration of sustained maximal voluntary contractions of the muscle (Nybo and Nielsen, 2001a) and augments cerebral ammonia uptake (Nybo et al. 2005). Ammonia is neurotoxic and easily penetrates the BBB (Suárez et al. 2002), which might have deleterious effects on glutamate and gamma-aminobutyric acid (GABA) neurotransmission (Nybo et al. 2005). Prolonged exercise in rats augments the permeability of the BBB (Sharma et al. 1991), which might modify the transport kinetics of substances important for CNS function. In humans, prolonged exercise in the heat increases circulating S-100ß (Watson et al. 2005b), a proposed peripheral marker of BBB permeability (Koh and Lee, 2014). However, sufficient fluid ingestion during exercise prevents the increase in S-100ß (Watson et al. 2006). Furthermore, exercise-induced hypohydration attenuates ventricular and cerebrospinal fluid volumes, while brain volume remains unchanged (Watson et al. 2010). Hence, despite the observed changes in S-100ß, there appears to be mechanisms in place to defend brain volume during exercise.

Cardiovascular function is also influenced during prolonged exercise in a warm environment (Cheuvront et al. 2010; Gonzalez-Alonso, 2007). The increased need to dissipate heat through the skin and the maintenance of muscle and cerebral blood flow requirements

result in an elevated cardiac output (Gonzalez-Alonso, 2007; Sawka et al. 2011). The accompanying hypohydration augments the reductions in blood volume and stroke volume, further increasing cardiovascular strain and reducing maximal oxygen uptake (Cheuvront et al. 2010; Gonzalez-Alonzo and Calbet, 2003). The increased cutaneous vasodilation in the presence of a narrowing core-to-skin temperature gradient is believed to play a more important role in the genesis of fatigue than an elevated core temperature (Sawka et al. 2012). An elevated core temperature during exercise would actually increase this temperature gradient, reduce skin blood flow requirements and thereby attenuate cardiovascular strain (Rowell, 1986; Sawka et al. 2012).

A complex feedforward-feedback model suggests that any change in pacing strategy during exercise is dictated by peripheral, central and emotional cues, ensuring the exercise task is completed long before catastrophic injury occurs (Noakes, 2011; Noakes et al. 2005; Tucker et al. 2004). The rate of heat storage during exercise was sufficient to modulate power output at a fixed level of perceived exertion (Tucker et al. 2006), suggesting a neural mechanism regulates self-paced exercise in the heat (Tucker, 2009). While the validity of this model has been questioned (Jay and Kenny, 2009), the aforementioned mechanisms proposed to regulate fatigue (cortical or subcortical) are all predicated on the avoidance of thermal injury.

While the precise cerebral mechanisms which mediate the onset of fatigue in the heat are not fully understood, influencing the CNS would seem a prudent strategy to enhance performance under these conditions. However, nutritional manipulation of CNS function does not appear to confer a performance benefit (Hobson et al. 2013; Tumilty et al. 2014; Watson et al. 2012). Conversely, pharmacological reuptake-inhibition of dopamine and noradrenaline consistently enhances performance in warm conditions (Roelands and Meeusen, 2010). However, these drugs enable participants to reach potentially dangerous core temperatures during exercise (Watson et al. 2005a), thus increasing the risk of heat illness. Furthermore, selective noradrenaline reuptake-inhibition with Reboxetine induced deleterious peripheral effects (Roelands et al. 2008). An alternative strategy is caffeine, a known CNS stimulant (Fredholm et al. 1999). Supplementation with caffeine consistently improves endurance performance in temperate conditions (Burke, 2008). Surprisingly, far less research has examined the ergogenic effects of caffeine during prolonged exercise in

the heat (Armstrong et al. 2007), despite a high prevalence of intake within the general population (Fitt et al. 2013) and by athletes competing in warm environments (Desbrow and Leveritt, 2006).

2.4 Caffeine

The methylxanthine caffeine (1,3,7-trimethylxanthine) is the most commonly consumed drug globally (Fredholm et al. 1999), with approximately 95% of UK adults consuming caffeine on a regular basis (Fitt et al. 2013). It was first isolated by Friedlieb Ferdinand Runge in 1819 (Runge, 1820) and later synthesised by Nobel Laureate Emil Fischer in 1895 (Fischer and Ach, 1895). Today, caffeine is present in a variety of foods, drinks, and over-the-counter medications. Early work by Rivers and Webber (1907) provided the first experimental evidence that caffeine ingestion increases muscular output. Subsequent research by Foltz and colleagues (1943) examined the influence of intravenous caffeine infusions to perform and recover from exhaustive exercise. During this time, American researchers investigated the effects of caffeine, cocaine, alcohol, strychnine and nitroglycerin on swimming performance (Haldi and Wynn, 1946). Interestingly, caffeine was the only intervention to enhance performance during prolonged exercise. Subsequent research further demonstrated caffeine's positive influence on exercise performance (Costill et al. 1978; Ivy et al. 1979; Weiss and Laties, 1962). In recent years, numerous studies have examined the potential for caffeine to improve physical performance (Burke, 2008; Graham, 2001), including both short-and-long duration exercise (Table 2.1).

Table 2.1 Effect of caffeine on exercise performance in temperate environmental conditions

	Intensity, mode, and duration of exercise	Performance effect
Short duration	• >90% VO_{2Peak} cycle and running-based exercise (<30 min)	Slight positive effect, varied results depending on protocol
	• >100% VO_{2Peak} cycle exercise (<5min)	Slight/no effect
	• >150% VO_{2Peak} running (<1 min)	No effect
	• Resistance training	Varied
Long duration	• 60-80% VO_{2Peak} cycle-and running-based exercise (>30 min); time trials (>30 min)	Generally positive, especially with longer duration exercise

References: Astorino and Roberson (2010); Burke (2008); Davis and Green (2009); Graham (2001); Spriet (1995); Spriet (2014).

As highlighted in table 2.1, caffeine's ergogenic effects are less consistent during high intensity, short duration exercise tasks, although a recent review indicated that caffeine provided a positive effect in approximately 50% of studies (Astorino and Roberson, 2010). Additionally, the lack of ergogenic effect in some investigations might be due to difficulties in quantifying and detecting small, worthwhile improvements in such types of exercise, where there is minimal potential for benefit. While there is some inter-individual variability in the response to caffeine supplementation (Jenkins et al. 2008), it is generally accepted that endurance performance (>30 min) is improved during a wide variety of exercise protocols (running, cycling, rowing etc.) when caffeine doses of 3-6 $mg \cdot kg^{-1}$ are consumed approximately 60 min before exercise (Burke, 2008; Graham, 2001). However, few studies have investigated the potential for caffeine to improve endurance performance when exercise is performed in the heat.

2.4.1 Endurance Performance, Thermoregulation, and Fluid-balance During Prolonged Exercise in the Heat

Supplementation with caffeine is typically discouraged during exercise in the heat due its purported ability to adversely influence heat-balance and hydration status. Caffeine ingestion reduces cutaneous blood flow (Daniels et al. 1998) and increases metabolic rate at rest (Belza et al. 2009; Poehlman et al. 1985), potentially elevating the rate of heat storage

during exercise. Furthermore, the caffeine-induced increase in sweat-rate (Kim et al. 2011) could augment the reductions in plasma volume and stroke volume, thereby increasing cardiovascular strain. These perturbations to physiological function are key factors involved in the genesis of fatigue during prolonged exercise in the heat (discussed in chapter 2.3).

Several early studies investigated the influence of varied caffeine doses on thermoregulation and fluid-balance during prolonged exercise in warm (25-29 ^{o}C) and hot (≥30^{o}C) environmental conditions (Bell et al. 1999; Falk et al. 1990; Gordon et al. 1982; Roti et al. 2006; Stebbins et al. 2001). As highlighted in table 2.2, these studies demonstrate that caffeine ingestion fails to adversely influence the rate of heat storage, fluid-balance, or circulatory strain during prolonged exercise. Despite a caffeine-induced elevation in heart rate, blood pressure and lactate in the study by Stebbins et al (2001), these responses did not influence the rate of heat storage or dissipation during exercise.

Table 2.2 Physiological effects of caffeine supplementation during exercise in the heat

Reference	Experimental design	Caffeine dose	Main outcomes
Roti et al (2006)	20 males; 37.7°C (56% RH); EHT (~90 min)	3 mg·kg^{-1}	Exercise time was greater compared with placebo. Sweat rate, T_{re}, T_{sk}, and VO2 were similar between trials
Gordon et al (1982)	2 groups of males (n=5); 24.5°C-28.9°C (41-54% RH); 100 min @ 89% VO_{2max}	5 mg·kg^{-1}	Sweat rate, sweat electrolytes, T_{re}, and plasma volume change were similar between groups
Cohen et al (1996)	5 males, 2 females; WBGT 24°C-28°C; 3 x 21-km road races	5 mg·kg^{-1}	Usg, plasma volume change, body mass loss, T_{ty}, lactate, glucose, and plasma sodium/potassium values were similar between trials
Bell et al (1999)	10 males; 40°C (30% RH); 50% VO_{2peak} until T_{re}=39.3°C	5 mg·kg^{-1} + 1 mg·kg^{-1} Eph	Exercise time was similar compared with placebo (120 min). Sweat rate, T_{re}, T_{sk}, and VO2 were similar between trials
Roti et al (2006)	19 males; 37.7°C (56% RH); EHT (90 min)	6 mg·kg^{-1}	Exercise time was similar compared with placebo (120 min). Sweat rate, T_{re}, T_{sk}, and VO2 were similar between trials
Stebbins et al (2001)	11 males; 38°C (40% RH); 45 min rest + 35 min @ 50% VO_{2max}	6 mg·kg^{-1}	Caffeine increased HR, BP, and lactate during exercise. T_{re}, T_{sk}, and FBF were similar across trials
Falk et al (1990)	7 trained males; 25°C (50% RH); TTE (70-75% VO_{2max})	7.5 mg·kg^{-1}	Heat storage, T_{re}, sweat rate and plasma volume change were similar between trials
Cohen et al (1996)	5 males, 2 females; WBGT 24°C-28°C; 3 x 21-km road races	9 mg·kg^{-1}	Usg, plasma volume change, body mass loss, T_{ty}, lactate, glucose, and plasma sodium/potassium values were similar between trials

Note: All studies provided a placebo condition for comparison. RH, relative humidity; TTE, time-to-exhaustion; Eph, Ephedrine; T_{re}, rectal temperature; T_{ty}, tympanic temperature; T_{sk}, skin temperature; VO2, oxygen uptake; VO_{2max}, maximum oxygen uptake; HR, heart rate; BP, blood pressure; FBF, forearm blood flow; EHT, exercise-heat tolerance test (treadmill walking); Usg, urine specific gravity; WBGT, wet-bulb globe temperature

Nonetheless, several important factors should be considered. The use of small sample sizes (n=5-7; Falk et al. 1990; Gordon et al. 1982), the between-group comparisons (Gordon et al. 1982; Roti et al. 2006), and the relatively short exercise protocols (35 min; Stebbins et al. 2001) make it difficult to substantiate caffeine's influence on thermoregulation, circulatory strain and fluid-balance under these conditions (Armstrong et al. 2007). Furthermore, the results of a previous field study (Cohen et al. 1996) were likely influenced by changes in environmental conditions within and between races. In addition, tympanic temperature was used as an index of core temperature, which is considered unsuitable to assess exercise-induced hyperthermia (Ganio et al. 2009a). In recent years, several well-controlled laboratory studies have provided further insight into caffeine's ergogenic and physiological effects during prolonged exercise in the heat.

Trained cyclists completed a 15 min performance test after 120 min of submaximal exercise in a warm (28.5^{o}C) environment (Cureton et al. 2007). Compared with a carbohydrate-electrolyte and placebo trial, performance was improved by 15-23% when participants received a drink containing both caffeine (5.3 mg·kg^{-1}) and carbohydrates. Furthermore, the reduction in maximal voluntary contraction (MVC) of the muscle after exercise was attenuated when caffeine and carbohydrates were co-ingested versus placebo and isolated carbohydrate intake (5% vs. 15%). However, voluntary activation (VA) was not improved, suggesting a direct effect of caffeine on the muscle (Cureton et al. 2007). Employing the same experimental protocol, these researchers failed to report any influence of caffeine on cardiovascular strain, thermoregulation, or fluid-balance during prolonged steady-state exercise (Millard-Stafford et al. 2007). However, in both of these studies the caffeine and carbohydrate drink contained additional compounds (B vitamins, taurine, and carnitine) that were not present in the placebo and carbohydrate treatments, which might have influenced the results.

Subsequently, Del-Coso et al (2008) assessed MVC and VA before and after 120 min of submaximal cycle exercise in a hot (36^{o}C) environment. Using a repeated-measures design, endurance-trained cyclists received either water to replace 97% of fluid loss during exercise, the same volume of a 6% carbohydrate-electrolyte solution, no fluid, or each of these treatments with 6 mg·kg^{-1} caffeine. These authors demonstrated that caffeine, when combined with a carbohydrate-electrolyte solution, increased maximal leg force after

exercise (2%) by augmenting VA (i.e. reducing central fatigue). These results contrast with that of Cureton et al (2007), which could be due to the timing of the testing procedures. Del-Coso et al (2008) assessed maximal leg force immediately after exercise while participants remained in the environmental chamber, while Cureton et al (2007) removed cyclists from the chamber and assessed MVC after 8, 18, and 28 min of rest in temperate conditions (21^{o}C). Removing participants from the chamber will have lowered their core temperature, attenuated the influence of hyperthermia on central fatigue and thereby reduced the capacity for the caffeine and carbohydrate-electrolyte solution to influence VA.

Utilising the same experimental protocol, these researchers examined the influence of caffeine on thermoregulation and fluid-electrolyte balance during prolonged exercise in the heat (Del-Coso et al. 2009). Using trained, heat-acclimated cyclists, the addition of caffeine to a carbohydrate-electrolyte drink induced a higher core temperature at the end of exercise than isolated carbohydrate intake (0.2-0.3^{o}C; *P*=0.07). This difference was not due to enhanced carbohydrate oxidation, which has been suggested (Yeo et al. 2005), and measures of heat dissipation were not different between the two trials (i.e. skin temperature and skin blood flow; Del-Coso et al. 2009). Furthermore, when caffeine was ingested alone or in combination with water and the carbohydrate-electrolyte solution, urine excretion was greater than the trials without caffeine (21%, 43%, and 15%, respectively). Due to the high variation across participants, these individual differences were not statistically significant. However, when collapsing the data (i.e. caffeine trials vs. non-caffeine trials), urine excretion was significantly greater after caffeine ingestion. It should be noted that the majority of fluid loss during exercise was due to sweating, and caffeine failed to influence whole-body sweat rate when ingested in isolation or with the rehydration drinks (Del-Coso et al. 2009).

Cheuvront et al (2009) reported that 9 mg·kg^{-1} caffeine elicited a higher core temperature than placebo (0.2-0.3^{o}C) during 30 min of steady-state exercise (50% VO_{2peak}) in 40^{o}C. Additionally, total work produced (kJ) during a subsequent 15 min performance task was similar between trials. Given the caffeine-induced increase in core temperature was small, remained constant throughout exercise, and was undetected by participants (i.e. no difference in perceived thermal stress across trials), these authors suggested that a high ambient temperature might negate the efficacy of otherwise well-established ergogenic aids

(Cheuvront et al. 2009). In agreement, carbohydrate ingestion did not improve endurance capacity in a warm (28^{o}C) environment (Nassif et al. 2008), but supplementation prolonged time to exhaustion in temperate conditions (Bergstrom et al. 1967). Similar to the findings of Cheuvront et al (2009), the provision of 9 mg·kg^{-1} caffeine marginally increased mean body temperature and the rate of heat storage during 30 min of steady-state exercise (50% VO_{2peak}) in 40^{o}C (Ely et al. 2011). Unfortunately, endurance performance was not measured.

More recently, Roelands et al (2011) supplemented well-trained cyclists with 6 mg·kg^{-1} caffeine before prolonged exercise in the heat (30^{o}C). During the initial hour of fixed-intensity exercise (55% W_{max}), there was a greater increase in core temperature during the caffeine trial than placebo, with values reaching approximately 0.5^{o}C higher at the end of the preload (Roelands et al. 2011). The authors attributed this caffeine-induced increase in core temperature to the lack of ergogenic effect during a subsequent 30 min TT (P=0.462). However, it seems likely that such an elevation in core temperature would actually precipitate the onset of fatigue, rather than enable similar performance times to those attained in the placebo trial. Perhaps this provides further evidence that an elevated core temperature is not as important in the genesis of fatigue during prolonged exercise in the heat as suggested (Gonzalez-Alonso et al. 1999).

Employing a repeated-measures design, Ganio et al (2011) examined the ergogenic and physiological effects of 6 mg·kg^{-1} caffeine during prolonged exercise in cool (12^{o}C) and hot (33^{o}C) environmental conditions, respectively. Trained male participants cycled for 90 min at 60% and 70% maximum oxygen uptake (VO_{2max}; alternating every 15 min), immediately followed by a 15 min performance task (total kJ produced). During the steady-state exercise in both environmental conditions, caffeine failed to differentially influence the thermoregulatory, cardiovascular, and metabolic variables than placebo (Ganio et al. 2011). However, caffeine enhanced performance to a similar magnitude in both ambient conditions, with a 9 kJ (Placebo: 233 ± 37 kJ vs. Caffeine: 242 ± 38 kJ) and 12 kJ (Placebo: 185 ± 33 vs. Caffeine: 197 ± 38) increase in the cool and hot environment, respectively. Unfortunately, these authors did not provide a direct comparison between the caffeine and placebo trial in the hot condition only. When applying magnitude-based inferences to the data from this manuscript (Hopkins, 2000), assuming a smallest worthwhile change in performance of 3% (Ganio et al. 2009a), the 12kJ increase represents an 82.2% chance of

benefit (*P*=0.082). The findings of this investigation clearly contrast with that of Roelands et al (2011), despite both studies supplementing well-trained participants with the same total caffeine dose. However, one important difference is the dosing strategy employed. Roelands et al (2011) supplemented individuals with a single 6 mg·kg^{-1} caffeine dose 60 min before exercise, whereas Ganio et al (2011) provided participants with a 3 mg·kg^{-1} caffeine dose 60 min before and 45 min during exercise. Hence, the splitting of the caffeine bolus by Ganio and colleagues (2011) might have distributed the thermogenic effects of caffeine, thereby enabling participants to perform better during the subsequent performance task. While dividing the caffeine bolus confers a similar performance benefit compared with single-dose administration in temperate conditions (Conway et al. 2003), no study has investigated the performance and thermoregulatory responses to single-and split-caffeine doses during prolonged exercise in the heat.

Pitchford et al (2014) recently investigated whether a low dose of caffeine could improve endurance performance in the heat (35^{o}C), without negatively influencing thermoregulation during exercise. Well-trained participants completed an energy-based cycle TT (~60 min) after ingesting a single 3 mg·kg^{-1} caffeine dose or placebo. Performance was improved after caffeine intake (*P*=0.06), with these authors calculating an 85.8% chance of benefit (Pitchford et al. 2014). Furthermore, core temperature and estimated sweat rates were similar between trials. Despite participants completing the set amount of work quicker after caffeine ingestion, rating of perceived exertion (RPE) was similar between trials, suggesting a reduced relative perception of effort during exercise (Pitchford et al. 2014). However, these authors did not employ a period of fixed-intensity exercise prior to the performance task, which makes comparing the physiological data between trials difficult to interpret due to fluctuations in power output.

2.4.2 Influence of Habituation

Given the widespread intake of caffeine (Desbrow and Leveritt, 2006; Fitt et al. 2013; Fredholm et al. 1999), it is important to consider whether the level of habitual intake of the drug influences the physiological or ergogenic effects attained from acute supplementation. Robertson et al (1981) reported that 4 days of regular caffeine intake (750 mg) developed

tolerance to the humoral and hemodynamic responses to acute caffeine intake (250 mg) at rest, including blood pressure, heart rate, plasma renin activity, and urinary and plasma catecholamine concentrations. Subsequently, Bangsbo and colleagues (1992) assessed the metabolic response to steady-state exercise following acute and chronic caffeine supplementation. Trained participants were supplemented with daily doses of placebo (n=6) or 500 mg caffeine (n=6) for 6 weeks. The control group completed a placebo trial before and after the chronic supplementation period, while the caffeine group undertook these placebo trials and one acute 500 mg caffeine trial before and after the supplementation period (Bangsbo et al. 1992). After 6 weeks of caffeine intake, the adrenaline response to acute caffeine ingestion was attenuated, while there was no change in the activity of several key metabolic enzymes associated with fat metabolism. However, it is important to note that participants in both groups were permitted to consume their normal intake of caffeine throughout the 6 week period. Before testing, both groups reported habitual intakes of approximately 5 cups of coffee per day (range: 1-15; Bangsbo et al. 1992). If an average cup of coffee contains approximately 100 mg caffeine (Desbrow et al. 2007; Fitt et al. 2013), and the caffeine group ingested an additional 500 mg·day^{-1}, the daily intake of these individuals would have been approximately 14 mg·kg^{-1} (mean body mass: 71.1 kg); this level of intake is far greater than habitually consumed by the general population (Fitt et al. 2013). Unfortunately, these authors did not determine whether participants in the caffeine group actually continued with their habitual intake of caffeine throughout the study period.

Van Soeren and colleagues (1993) further examined the influence of chronic caffeine supplementation on the metabolic response to steady-state exercise (60 min at 50% VO_{2max}). Caffeine naive individuals (n=7) completed one placebo trial and one acute 5 mg·kg^{-1} caffeine trial. Habitual caffeine users (n=7) completed one placebo trial and two acute 5 mg·kg^{-1} caffeine trials; the two caffeine trials were separated by 6 days of randomised daily caffeine (2 x 2.5 mg·kg^{-1}) or placebo intake. No additional dietary caffeine was permitted during the study period. In the caffeine naive group, the exercise-induced increase in plasma adrenaline was augmented by caffeine, while plasma adrenaline responses were similar across trials in the caffeine users group. Furthermore, noradrenaline, glycerol, FFA, and plasma and urinary caffeine concentrations were similar between groups (Van Soeren et al. 1993).

To determine whether the ergogenic effect of caffeine is influenced by the level of habitual intake of the drug, Dodd et al (1991) examined caffeine naive (<20 mg·day^{-1}; n=8) and regular caffeine users (>300 mg·day^{-1}; n=9) during three incremental exercise tests to exhaustion. One hour before exercise, participants ingested either placebo or caffeine at 3 mg·kg^{-1} or 5 mg·kg^{-1}. Despite caffeine ingestion augmenting resting heart rate and circulating FFA's in the control group, time to exhaustion was similar between and within groups. Subsequently, Bell and McLellan (2002) compared caffeine users (>300 mg·day^{-1}; n=13) and non-users (<50 mg·day^{-1}; n=8) during a cycle time to exhaustion (80% VO_{2max}) after ingesting 5 mg·kg^{-1} caffeine. These authors also assessed the duration of caffeine's ergogenic effect, with the exercise protocol commencing 1, 3, or 6 hours post-caffeine ingestion, respectively (Bell and McLellan, 2002). Interestingly, both the magnitude and the duration of caffeine's ergogenic effects were influenced by the level of habitual intake. In the caffeine users group, performance was enhanced at 1 and 3 hour post-caffeine ingestion, but was similar to placebo at 6 hours. Conversely, performance was enhanced at 1, 3, and 6 hours post-caffeine ingestion in the caffeine naive group. Furthermore, time to exhaustion was longer during all three caffeine trials in the non-users group versus those habituated to caffeine (Bell and McLellan, 2002). These data strongly suggest that the level of habitual intake of caffeine can influence the performance benefits attained from acute supplementation. However, these models do not provide a timeframe by which regular caffeine intake induces the development of tolerance. Furthermore, in the Bell and McLellan (2002) study, no information was provided regarding the length of time participants in the caffeine group were habitually consuming >300 mg·day^{-1} prior to testing. It seems logical to suggest that the length of time participants were regularly consuming caffeine (i.e. weeks, months, years etc.) would influence the response to acute supplementation.

Several animal studies have investigated the influence of chronic caffeine intake on physical activity. In rats, repeated exposure to caffeine in the drinking water (14 days) induced the development of tolerance to the performance benefit of a subsequent acute caffeine dose (Karcz-Kubicha et al. 2003). This response was confirmed in other animal models (Ciruela et al. 2006; Quarta et al. 2004), although large doses were used (i.e. 130 mg·kg·day$^{-1;}$ Quarta et al. 2004). Roti and colleagues (2006) stratified 59 participants by age, body composition and body mass into 3 groups (n=19-20). From days 1-6, all participants ingested 3 mg·kg^{-1}

caffeine. From days 7-12, each group consumed either placebo or caffeine at 3 mg·kg^{-1} or 6 mg·kg^{-1}. Caffeine was ingested in two equal doses; the first in the morning, followed by the second in the afternoon. On the afternoon of day 12, one hour post-capsule ingestion, all participants undertook an exercise-heat tolerance test (graded treadmill walking) in 37^{o}C. Exercise time was greater in the 3 mg·kg^{-1} caffeine group, while thermoregulation, cardiovascular responses and various markers of hydration status were similar between groups (Roti et al. 2006).

Recently, Irwin et al (2011) examined the influence of a short withdrawal and habituation period on the acute ergogenic effects of caffeine. Using a repeated-measures design, trained participants were supplemented with placebo or 3 mg·kg^{-1} caffeine for 4 days, followed by an acute placebo or 3 mg·kg^{-1} caffeine trial on day 5 (Irwin et al. 2011). Acute caffeine ingestion enhanced performance compared with placebo, regardless of the intervention during the preceding 4 days, while there was no difference between the two caffeine trials. These data suggest that a short habituation period fails to influence the ergogenic effects of an acute 3 mg·kg^{-1} caffeine dose. Hence, a longer period of controlled caffeine intake might be required to elicit changes in endurance performance typical of acute caffeine ingestion. Given the performance differences between low-and high-habitual caffeine users in response to acute supplementation (Bell and McLellan, 2002), and the high prevalence of habitual caffeine intake within the general population (Fitt et al. 2013), this hypothesis warrants investigation.

2.4.3 Pharmacokinetics

After oral ingestion, caffeine is almost completely absorbed from the gastrointestinal tract (>99%), and peak blood concentrations are achieved within 30-60 min (Blanchard and Sawers, 1983; Graham and Spriet, 1995; Mumford et al. 1996). However, the rate of absorption can be influenced by the pH and composition of the drug formulation (Bonati et al. 1982). Caffeine is absorbed quicker from gum than capsules (Kamimori et al. 2002), from capsules than cola and chocolate (Mumford et al. 1996), and from coffee than cola (Marks and Kelly, 1973). Nonetheless, it appears that when the caffeine dose and administration volume are the same, these differences are not observed (Liguori et al. 1997). Recently,

Hodgson et al (2013) supplemented participants with pure (anhydrous) caffeine or coffee, with both trials providing 5 $mg \cdot kg^{-1}$ caffeine dissolved in 600 mL of water. These authors reported similar circulating caffeine concentrations and performance benefits (versus placebo) between the two caffeine trials (Hodgson et al. 2013).

The hydrophobic nature of caffeine enables its passage across all biological membranes and thus it is readily distributed throughout all tissues of the body (Fredholm et al. 1999). After oral ingestion, caffeine dilutes in total body water and reaches a volume of distribution between 0.5-1.3 $L \cdot kg^{-1}$ (Kamimori et al. 2002; Lelo et al. 1986). In rats, caffeine concentrations in the extracellular fluid compartments of several tissues (liver, adipose tissue, muscle, brain) follow a similar time course to that observed in plasma (Stahle et al. 1991a), while in man the distribution of caffeine in the extracellular fluid of subcutaneous adipose tissue is not closely correlated with plasma concentrations (Stahle et al. 1992b). This might be due to differences in albumin content between the two fluid compartments. In mice, intracellular caffeine concentrations in several organs (brain, heart, liver) are similar to the levels reported in plasma (Burg and Werner, 1972), while data from humans is lacking. The pharmacokinetic distribution of caffeine in plasma can be explained by first-order, linear kinetics (Kamimori et al. 2002; Newton et al. 1981).

Caffeine is metabolised by the cytochrome P450 (CYP450) system in the liver (Fredholm et al. 1999; Gu et al. 1992). Like most xenobiotics, liver microsomes metabolise caffeine to produce over 25 derivative compounds (Arnaud, 1987; Somani and Gupta, 1988). The most important of these biotransformations are the N3-demethylation to paraxanthine, N1-demthylation to theobromine, and N7-demethylation to theophylline; these account for approximately 80%, 11%, and 5%, respectively, of caffeine metabolism *in vivo* (Lelo et al. 1986). Once formed, these compounds undergo extensive metabolism to form a range of other metabolites (Arnaud, 1987). The remaining ~4% is attributed to the biotransformations leading to trimethyluric acid and trimethyluracil, as well as renal excretion of unchanged caffeine (Lelo et al. 1986). A simplified schematic of caffeine metabolism is shown in Figure 2.2.

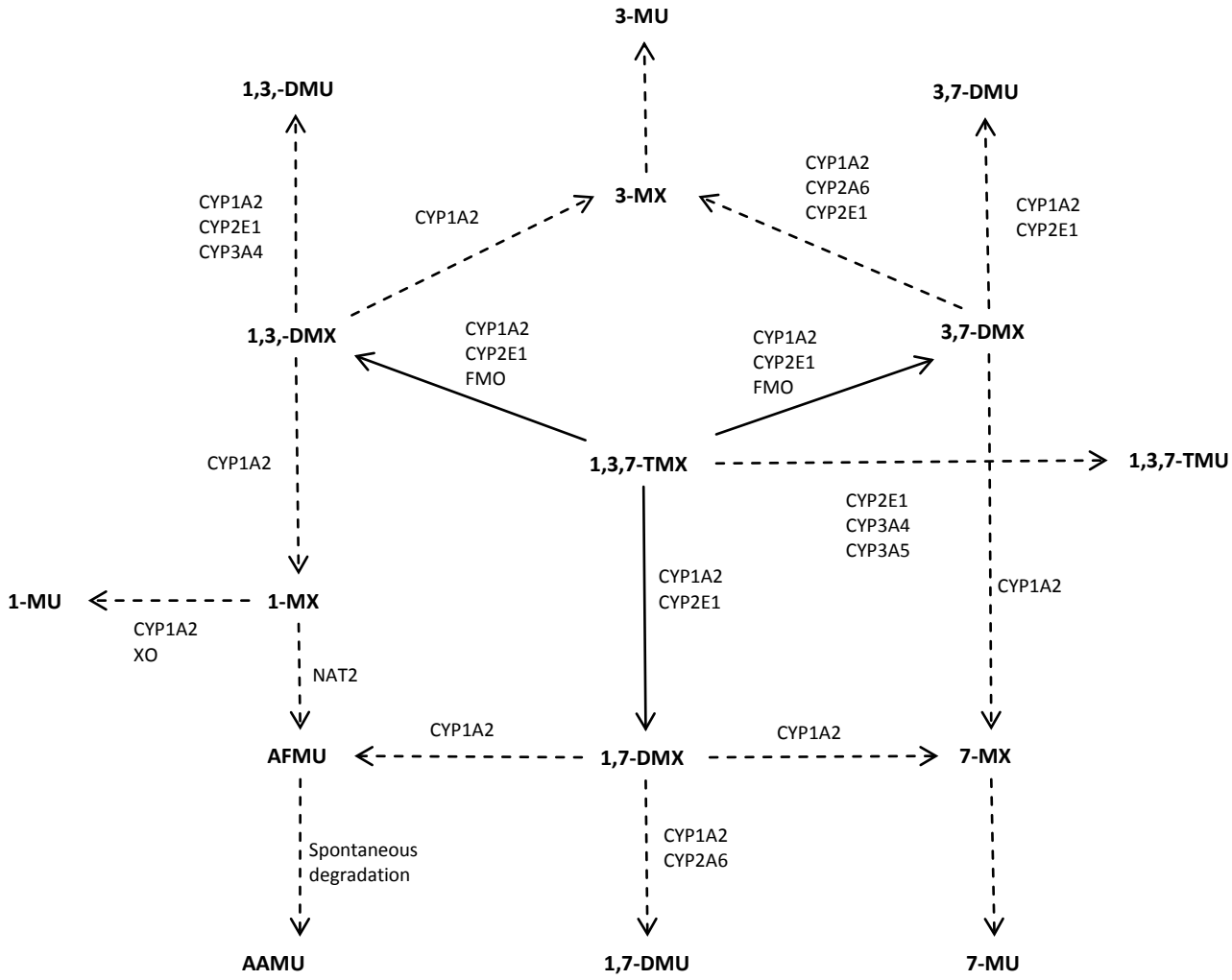

Figure 2.2 Simplified schematic of caffeine metabolism *in vivo*. Full and broken arrows represent major and minor pathways, respectively. 1,3,7-TMX, 1,3,7-trimethylxanthine (caffeine); 1,7-DMX, 1,7-dimethylxathine (paraxanthine); 3,7-DMX, 3,7-dimethylxanthine (theobromine); 1,3-DMX, 1,3-dimethylxanthine (theophylline); 1-MX, 1-methylxanthine; 3-MX, 3-methylxanthine; 7-MX , 7-methylxanthine; 1,3,7-TMU, 1,3,7-trimethyluric acid; 1,3-DMU, 1,3-dimethyluric acid; 1,7-DMU, 1,7-dimethyluric acid; 3,7-DMU, 3,7-dimethyluric acid; 1-MU, 1-methyluric acid; AFMU, 5-acetylamino-6-formylamino-3-methyluracil; AAMU, 5-acetyl-6-amino-3-methyluracil; CYP, cytochrome P450 (various subtypes); NAT2, N-acetyltransferase; XO, xanthine oxidase; FMO, flavin-containing monooxygenase. References: Arnaud (1987); Caubet et al (2002); Gu et al (1992); Magkos and Kavouras (2005); Lelo et al (1986); Somani et al (1988).

The biological half-life of plasma caffeine ranges from 3.5-5.5 hours (Arnaud, 1987), with a clearance rate of approximately 1-3 $mL \cdot kg \cdot min^{-1}$ (Kamimori et al. 2002). Inter-individual differences in caffeine pharmacokinetics is largely due to hepatic CYP1A2 activity (Rasmussen et al. 2002), which accounts for approximately 95% of caffeine metabolism (Gu et al. 1992; Figure 2.2). While genetic differences can influence the activity of this enzyme (Chen et al. 2005; Rasmussen et al. 2002), a variety of lifestyle factors are also important. For example, smoking increases CYP1A2 activity, resulting in lower plasma caffeine concentrations (Vitisen et al. 1991). Conversely, the use of oral contraceptives decreases CYP1A2 activity, resulting in elevated levels of circulating caffeine (Rietveld et al. 1984). Urinary excretion of unchanged caffeine accounts for approximately 3% of the ingested dose (Tag-Liu et al. 1983). Until 2004, caffeine was placed on the WADA prohibited list, where it now remains on the monitoring programme (WADA, 2015). A urinary threshold of 12 $\mu g \cdot mL^{-1}$ was enforced, with greater concentrations prompting a doping violation. However, caffeine intakes of 13 $mg \cdot kg^{-1}$ are necessary to increase urinary concentrations beyond this cut-off point (Pasman et al. 1995). Supplementation with 3-6 $mg \cdot kg^{-1}$ is deemed sufficient to provide the maximum performance benefit (Burke, 2008; Desbrow et al. 2012), and these doses elicit urinary caffeine concentrations well below the previous threshold (Pasman et al. 1995). Furthermore, the dosing strategy employed can influence urinary caffeine concentrations (i.e. lower with split-versus single-doses; Conway et al. 2003). These data likely contributed to the removal of caffeine from the WADA list of prohibited substances

2.4.4 Mechanism of Action

Several mechanisms have been proposed to explain caffeine's ergogenic effects. The first of these is caffeine's influence on skeletal muscle, which was demonstrated over a century ago (Veley and Waller, 1910). Specifically, caffeine was shown to influence calcium release from the sarcoplasmic reticulum (Rousseau et al. 1988), probably through its interaction with the ryanodine receptor (McPherson et al. 1991). However, mM concentrations are required to influence intracellular calcium *in vitro*, which would prove toxic in humans (Fredholm and Hedqvist, 1980; Fredholm et al. 1999). After oral intakes of 3-9 $mg \cdot kg^{-1}$, plasma caffeine

reaches 10-70 uM (Graham and Spriet, 1995), levels which are too low to increase the development of muscle tension when applied directly to isolated, fatigued muscles (James et al. 2004). Furthermore, supplementation with 6 $mg \cdot kg^{-1}$ caffeine enhanced isometric leg strength, time to fatigue, maximal voluntary torque, and maximal voluntary activation (Kalmar and Cafarelli, 1999; Plaskett and Cafarelli, 2001). However, in both of these studies caffeine failed to influence the contractile properties of the muscle, suggesting an alternative mechanism.

Caffeine and theophylline were shown to be non-selective phosphodiesterase inhibitors (Butcher and Sutherland, 1962). Phosphodiesterase enzymes participate in various signal transduction pathways and hydrolyse the phosphodiester bond in second messenger molecules such as cyclic adenosine monophosphate (cAMP; Soderling and Beavo, 2000). This inhibits cAMP breakdown and stimulates lipolysis through the activation of hormone-sensitive lipase (Fain et al. 1972). However, similar to caffeine's influence on intracellular calcium, supraphysiological doses are required to inhibit phosphodiesterase enzymes *in vitro* (Fredholm et al. 1999).

Probably the most well-known theory proposed to explain caffeine's ergogenic effect during prolonged exercise is its purported ability to enhance fat oxidation (Costill et al. 1978; Ivy et al. 1979). Supplementation with 330 mg caffeine 60 min before exercise increased time to exhaustion and enhanced estimated fat oxidation rates and plasma concentrations of FFA's and glycerol (Costill et al. 1978). Subsequently, Ivy et al (1979) demonstrated enhanced work production and estimated fat oxidation rates when caffeine was ingested before and during prolonged cycle exercise. To directly assess substrate utilisation during exercise, Essig et al (1980) took muscle biopsies before and after 30 min of submaximal exercise and reported a significant increase in muscle triglyceride oxidation during the caffeine trial. Furthermore, plasma FFA's increased, while the respiratory exchange ratio (RER) decreased (Essig et al. 1980). This model is predicated on the assumption that enhancing fat oxidation during prolonged exercise (via adrenaline-mediated FFA release from adipose tissue) will spare limited muscle glycogen, thereby prolonging exercise capacity (Essig et al. 1980).

However, subsequent investigations failed to confirm these initial findings (Graham et al. 2000; Graham, 2001). Using stable-isotopic tracers to estimate substrate oxidation rates

during prolonged exercise, Hulston and Jeukendrup (2008) failed to report any change in glucose kinetics after participants ingested a drink containing carbohydrates and caffeine (5.3 $mg \cdot kg^{-1}$), despite a 4.1% increase in performance versus the carbohydrate only trial. Furthermore, glucose appearance/disappearance in the blood and estimated fat oxidation rates during 60 min of submaximal exercise were not influenced by 6 $mg \cdot kg^{-1}$ caffeine (Roy et al. 2001). Similarly, supplementation with theophylline did not influence fat metabolism during prolonged exercise (Raguso et al. 2006). Furthermore, a recent review concluded that caffeine ingestion exerts little to no effect on skeletal muscle metabolism during prolonged exercise (Graham et al. 2008). Finally, the strongest evidence against this model comes from observations that individuals with tetraplegia, in which there is no sympathetic activation, demonstrate improved performance after caffeine ingestion (6 $mg \cdot kg^{-1}$; Mohr et al. 1998). These authors also reported a caffeine-induced elevation in plasma FFA's, without any change in circulating catecholamines. Hence, blood-borne mechanisms alone appear untenable to explain caffeine's ergogenic effects.

While additional mechanisms may partly account for caffeine's positive influence on performance, such as the inhibition of glycogen phosphorylase and the stimulation of the sodium/potassium pump (Magkos and Kavouras, 2005), there is compelling evidence that caffeine enhances performance through direct actions within the CNS (Fredholm et al. 1999). Specifically, the ergogenic effects of caffeine depend on its ability to antagonise adenosine receptors in the brain (Ferré, 2008; Ferré, 2010; Fredholm et al. 1999). In order to identify the specific neurophysiological effects elicited by adenosine receptor blockade, the role of adenosine should be addressed. Adenosine is a purine nucleoside which modulates neural activity (Dunwiddie and Masino, 2001), although it is not classed as a neurotransmitter (Fredholm et al. 2001). Neurotransmitters such as dopamine, noradrenaline, and serotonin are stored within presynaptic vesicles, released in a calcium-dependant manner, and bind to post-synaptic receptors. Adenosine is not accumulated into vesicles and its presence within the synapse is not entirely dependent on calcium (Dunwiddie and Masino, 2001). There are two main sources of synaptic adenosine. First, high rates of cellular activity increase the dephosphorylation of adenosine triphosphate (ATP) within neurons, thus elevating intracellular adenosine. Virtually all cells are equipped with nucleoside transporters (Cass et al. 1998), ensuring a constant flux of intracellular

adenosine into the extracellular space. Second, ATP is co-released with glutamate from neurons and glia (Chuna, 2001), which is rapidly metabolised by ecto-nucleotidases to generate adenosine (Zimmerman and Braun, 1999). In both scenarios, the concentration of synaptic adenosine is dependent on the workload of the cell (Schiffman et al. 2007).

The physiological effects exerted by adenosine are determined by the presence of specific receptors. To date, four G-protein-coupled receptors have been cloned: A_1, A_{2A}, A_{2B}, and A_3 (Fredholm et al. 2001; Fredholm et al. 2011). Basal concentrations of adenosine preferentially act on the A_1 and A_{2A} subtypes, whereas supraphysiological doses are required to tonically activate the A_{2B} and A_3 isoforms (Feoktistov and Biaggioni, 1997; Fredholm et al. 1999; Jacobson, 1998). This is partly due to a low affinity of adenosine for these receptors (i.e. A_{2B}; Fredholm et al. 2001b), but is also attributed to the sparse expression of the A_{2B} and A_3 subtypes throughout the brain (Table 2.3). These receptors likely become important during periods of illness, when the concentration of adenosine in the interstitial fluid increases 100-1000-fold (i.e. ischemia; Hadberg et al. 1987). Differences in tissue distribution, intracellular signalling, and binding potencies of each receptor subtype is shown in table 2.3.

Table 2.3 Adenosine receptor distribution, signalling and binding potency

	A_1	A_{2A}	A_{2B}	A_3
High expression	Cerebral cortex; cerebellum; septal nuclei; hippocampus; dorsal horn of spinal cord; eye; adrenal gland; atria	Caudate-putamen; nucleus accumbens; olfactory tubercle; olfactory bulb; spleen; thymus; leucocytes; platelets	Caecum; colon; bladder	Testis; mast cells
Intermediate expression	Other brain regions; skeletal muscle; adipose tissue; liver; kidney; salivary glands; oesophagus; colon; antrum; testis	Heart; lung; blood vessels	Hypothalamus; lung; blood vessels; eye; mast cells	Cerebellum; hippocampus; lung; spleen; liver
Low expression	Lung; pancreas	Other brain regions	Other brain regions; adipose tissue; adrenal gland; kidney; pituitary gland	Testes; heart; other brain regions; intestine
G-protein	$G_{i1/2/3}$; G_o	G_s; G_{olf}	G_s	$G_{i2/3}$
Cellular effect	↓cAMP ↑IP_3/DAG ↑ Arachidonate (PLA_2) ↑ Choline (PLD)	↑ cAMP ↑IP_3	↑ cAMP ↑IP_3/DAG (PLC)	↓cAMP ↑IP_3/DAG (PLC)
Adenosine (EC_{50}, uM)	0.31	0.73	23.5	0.29
Caffeine (K_D, uM)	33.8	12.3	15.5	>100

cAMP, cyclic-adenosine monophosphate; IP_3, Inositol triphosphate; DAG, diacylglycerol, PLA_2, phospholipase A_2; PLD, phospholipase D; PLC, phospholipase C. References: Distribution = Fredholm et al (1999); Fredholm et al (2001a); Mishina et al (2007); Salvatore et al (1993); Svenningsson et al (1997); Cellular effects = Fisone et al (2004); Fredholm et al (1999); Fredholm et al (2000); Binding potencies (mean values) = Fredholm et al (2001b).

The neurophysiological effects exerted by habitual, low-doses of caffeine are primarily mediated through the central blockade of A_1 and A_{2A} receptors (Ferré, 2008; Fredholm et al. 1999). This was demonstrated by Davis et al (2003) when caffeine and a selective A_1/A_{2A} agonist were directly administered into the brain of rats. Running time was enhanced after caffeine, decreased with the A_1/A_{2A} agonist, while exercise time was similar to placebo when both drugs were co-administered (i.e. they cancelled each other out; Davis et al. 2003). However, this study could not discern the precise contribution of the A_1 and A_{2A}

receptor. While the A_1 isoform is located throughout the brain, the A_{2A} receptor is densely enriched within the dopamine-innervated areas of the basal ganglia, especially the striatum (El Yacoubi et al. 2001; Table 2.3). This brain region is the main input structure of the basal ganglia and plays key role in the control of voluntary movements, reinforcement, reward, motivation, and addictive behaviours (Gerfen, 1992). Global-knockout of the A_{2A} receptor attenuates the locomotor-stimulating effects of caffeine (Lindskog et al. 2002; El Yacoubi et al. 2000), while more selective gene-deletion strategies highlight a specific role for the striatum in meditating the performance benefits of caffeine (Lazarus et al. 2011). Hence, the ergogenic effects of caffeine, at least in part, can be explained by striatal A_{2A} receptor blockade. Another facet to this mechanism is the influence of caffeine on the intra-molecular cross-talk in the postsynaptic striatal A_2/D_2 heteromer. Adenosine-and caffeine-mediated occupancy of the A_{2A} receptor in this heteromer attenuate and augment, respectively, dopamine signalling via the D_2 receptor (Ferré et al. 1991; Ferré et al. 1993; Ferré et al. 1997a). The caffeine-induced potentiation of D_2 signalling results in the disinhibition of upper motor neurons, thereby enhancing performance through an increase in central motor drive (Fisone et al. 2004; Figure 2.3).

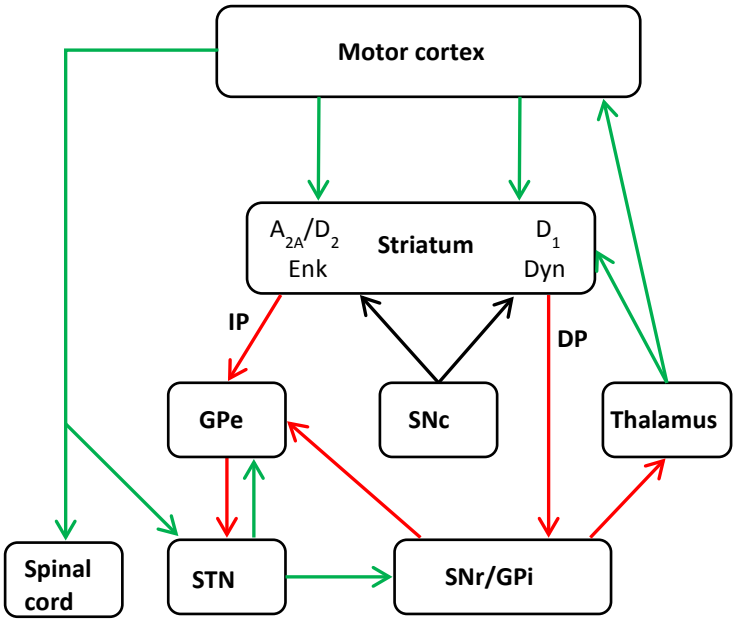

Figure 2.3 Simplified schematic of the basal ganglia circuitry including the direct and indirect output pathways of the striatum. Green, red and black arrows indicate stimulatory (glutamate), inhibitory (GABA) and modulatory (dopamine) input, respectively. SNc, substantia nigra pars compacta; GPe, external globus pallidus; STN, subthalamic nucleus; SNr, substantia nigra pars-reticulata (rodents); GPi, internal globus pallidus (primates); Enk, enkephalin; Dyn, dynorphin; DP, direct pathway; IP, indirect pathway. References: Fisone et al (2004); Morelli et al (2007); Schiffmann et al (2007).

The striatum is primarily comprised of medium-spiny projection neurons (>95%) which use GABA as their principle neurotransmitter (Gerfen, 2004; Smith and Bolum, 1990). These output neurons influence the activity of several basal ganglia nuclei via two main efferents: the direct (dynorphin) and indirect (enkephalin) pathway (Gerfen, 1992). The striatum receives stimulatory input (glutamate) from the motor cortex, thalamus and limbic areas, while receiving modulatory input (dopamine) from the substantia nigra pars compacta. From here, the direct and indirect pathways have opposing actions on movement. The direct pathway inhibits the activity of the internal globus pallidus, disinhibits thalamic neurons and thus augments stimulatory input to the motor cortex. Conversely, the indirect pathway, via the external globus pallidus and subthalamic nucleus, inhibits thalamic neurons and thus attenuates glutamate projections to the motor cortex (Gerfen, 1992; Figure 2.3). Dopamine activates, via the D_1 receptor, the direct pathway and inhibits, via the D_2 receptor, the indirect pathway (Gerfen, 2004). Adenosine, via the A_{2A} receptor, attenuates dopamine-mediated inhibition of the indirect pathway. Caffeine, by potentiating D_2

signalling in the A_{2A}/D_2 heteromer (Ferré et al. 1997), augments dopamine-mediated inhibition of the indirect pathway (Fisone et al. 2004). Of course, this is a gross simplification of how caffeine can influence the basal ganglia circuitry, especially since the precise functions of these striatal output neurons are only just being elucidated (Wall et al. 2013).

There is also evidence to suggest that caffeine exerts it stimulatory actions by increasing striatal dopamine release through the blockade of presynaptic A_1 receptors (Borycz et al. 2007; Solanis et al. 2002; Quarta et al. 2004). However, these results were not confirmed in other animal models (Acquas et al. 2002; De Luca et al. 2007). Interestingly, the findings of De Luca et al (2007) suggest that previous reports of enhanced striatal dopamine release after caffeine administration (Solanis et al. 2002) might be due to inadequate placement of the microdialysis probe. Furthermore, the caffeine-mediated increase in striatal dopamine release may be sub-regional, with more consistent elevations in the medial compartments (Borycz et al. 2007). Nevertheless, supplementation with a low dose of caffeine (300 mg) failed to influence *in vivo* dopamine release in human striatum (n=20) as assessed by positron emission topography (PET) and [^{11}C] raclopride (Volkow et al. 2015). These data support those of a previous PET study after participants (n=8) ingested 200 mg caffeine (Kaasinen et al. 2004). Researchers from both investigations suggested that physiologically relevant doses of caffeine elicit changes in dopamine signalling via striatal A_{2A} receptor blockade, without influencing dopamine release. Unfortunately, no human PET study has investigated whether caffeine can influence neurotransmitter release in other cerebral compartments.

The ability of caffeine to increase arousal is well-established (Rainnie et al. 1994), which is likely due to enhanced cholinergic activity in the basal forebrain and prefrontal cortex (Acquas et al. 2002; Benington et al. 1995). This response was shown to be dependent on A_1 receptor blockade (Van Dort et al. 2009). Additionally, caffeine prolonged exercise time in rats in association with increased synaptic dopamine concentrations in the PO/AH (Zheng et al. 2014). A follow-up study by the same research group showed that caffeine inhibited the adenosine receptor agonist-induced decrease in running time and dopamine release in the same brain region (Zheng and Hasegawa, 2016). This suggests the ergogenic effect of caffeine is dependent on adenosine receptor blockade-induced dopamine release, at least in the PO/AH. Although large doses were administered in both of these studies (≥ 10 mg·kg^{-1}),

the influence of caffeine on neurotransmitter release in non-striatal regions should not be discounted.

While the above studies provide strong evidence that caffeine enhances performance through a central mechanism, these data were obtained from animal models, cell cultures or resting human participants. However, a number of exercise studies in humans have provided further insight into the role caffeine may play in attenuating central fatigue. Recently, De Morree et al (2014) reported that caffeine supplementation reduced the motor-related cortical potential amplitude during isometric muscle contractions, despite participants being able to produce the same amount of force compared with placebo. The authors attributed this response to enhanced CNS excitability following caffeine ingestion. Indeed, caffeine may increase CNS excitability at a spinal or supraspinal level, including excitation of the alpha motor neuron pool and increases in motor evoked potentials and self-sustained firing of human motor units (Kalmar and Cafarelli, 2004a). An increase in self-sustained firing is suggested to enhance motor neuron excitability and thus reduce the level of descending neural drive necessary to maintain a required power output during exercise (Walton et al. 2002). Similarly, the positive effect of caffeine of motor cortex excitability (Cerqueira et al. 2006) would reduce the magnitude of excitatory inputs from pre motor areas necessary to produce the same degree of primary motor cortex output. Furthermore, when single-pulse TMS was used to estimate central excitability during exercise, caffeine increased the postactivation potentiation of cortical evoked potentials (Kalmar and Cafarelli, 2004b), suggesting enhanced central motor drive to the locomotor muscles. Therefore, based on these observations, it seems caffeine can enhance power output during exercise by augmenting descending neural drive to the locomotor muscles and/or reducing the neural cost necessary to generate a specific power output (i.e. lowering the threshold for neuronal activation).

2.5 Octopamine

While the vast majority of athletes consume supplements to enhance performance (Lieberman et al. 2015), few actually hold up to the scrutiny of scientific research. As mentioned above, caffeine consistently enhances endurance performance due to its ability

to influence the CNS (Fredholm et al. 1999). Therefore, it would be of interest to determine whether other stimulant-based compounds can also influence endurance performance. Octopamine is a trace amine structurally similar to the neurotransmitter noradrenaline which functions as a principal stimulatory neurotransmitter in the invertebrate CNS (Farooqui, 2012); this is likely the reason it is currently on the WADA list of prohibited substances (WADA, 2015). In recent years, there have been reports of positive doping samples among athletes (WADA, 2011), despite no evidence from humans that octopamine actually improves exercise performance.

2.5.1 Pharmacokinetics

Octopamine exists either in the ortho (*o*-), meta (*m*-), or para (*p*-) form, depending on the location of the hydroxyl group on the benzene ring (Brown et al. 1998). In nature, *p*-octopamine in synthesised from the precursor amino acid L-phenylalanine, which is converted to L-tyrosine via tyrosine hydroxylase. L-tyrosine is converted to the trace amine *p*-tyramine via aromatic L-amino acid decarboxylase (AADC) and then to *p*-octopamine via dopamine β-hydroxylase (Brandau and Axelrod, 1972; Figure 2.4). There are reports of *m*-octopamine in mammalian nerves tissue and brain (Ibrahim et al. 1985), while *o*-octopamine does not occur in nature and is only derived through chemical synthesis. Given the role of *p*-octopamine has been extensively studied, the specific involvement of the *o*-and *m*-isomers will no longer be considered in great detail.

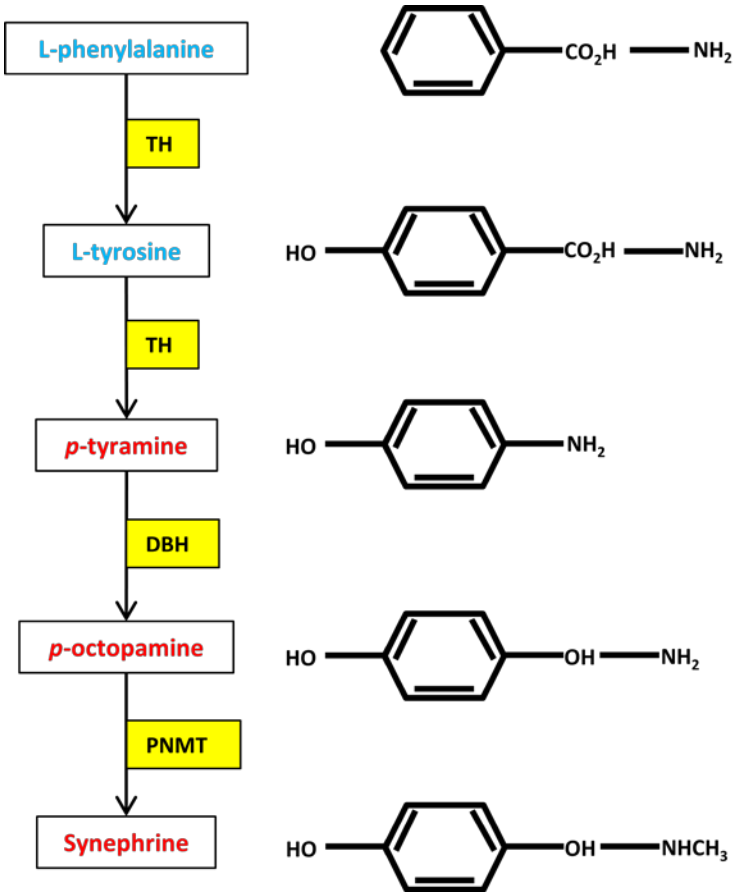

Figure 2.4 Biosynthesis of octopamine. Blue and red font indicate precursor amino acids and primary trace amines, respectively. Enzymes (yellow background) TH, tyrosine hydroxylase; AADC, aromatic L-amino acid decarboxylase; DBH, Dopamine beta-hydroxylase; PNMT, Phenylethanolamine N-methyltransferase. References: Berry (2004); Broadley (2010); Lindemann and Hoener (2005).

The physiological disposition of [^{3}H] octopamine was determined by Hengstmann et al (1974). After oral ingestion, 93% of the dose was excreted in the urine compared with 82% via the intravenous route, suggesting the drug is well absorbed. Furthermore, peak serum concentrations were achieved 30-60 min post-ingestion (Hengstmann et al. 1974). Deamination and conjugation are the two major metabolic pathways responsible for octopamine clearance, with deamination by monoamine oxidase to *p*-hydroxmandelic acid constituting the major route of elimination (~66%; Hengstmann et al. 1974). These authors also showed that over eleven times more conjugated octopamine appears in the urine after

oral ingestion compared with intravenous infusion, suggesting the drug is rapidly metabolised via the oral route.

2.5.2 Mechanism of Action

Following the identification of octopamine in the salivary glands of the octopus (Erspamer, 1948), a broad range of functions within the insect CNS were established, specifically signal-transduction mechanisms mediated through the activation of octopamine receptors (Farooqui, 2012). These receptors are believed to represent the counterparts to adrenoreceptors in humans (Farooqui, 2007), yet octopamine receptors in insects and adrenoreceptors in mammals differ in their ligand binding characteristics. Hence, it is not possible to extrapolate the results obtained from one system to another (Stohs, 2014). For example, in invertebrates, octopamine binds to its α-adrenoreceptors with a greater affinity than adrenaline, noradrenaline, and dopamine (Evans et al. 1988; Klaassen and Kammer, 1985; Park and Keeley, 1998). Conversely, in humans, octopamine exhibits a 30-fold (brown et al. 1988) and 2000-fold (Fregly et al. 1979) lower α-adrenergic activity than noradrenaline. However, an interesting observation is that octopamine can selectively and potently bind to mammalian β_3-adrenoreceptors (Carpéné et al. 1999). These receptors are located in white and brown adipose tissue and their activation enhances lipolysis (Arch, 2002). When comparing several biogenic amines, octopamine was shown to be the most potent and selective for β_3-adrenoreceptors, stimulating lipolysis in rat, hamster and dog adipose tissue. Furthermore, octopamine expressed only a two-fold lower binding affinity for β_3-adrenoreceptors than noradrenaline in a Chinese hamster ovary cell line (Carpéné et al. 1999).

While specific octopamine receptors are not present in humans, the identification of the G-protein-coupled trace amine-associated receptors (TAARs) has elucidated a potential central role for octopamine (Borowsky et al. 2001; Bunzow et al. 2001). Of the various TAAR subtypes identified (>20), octopamine binds to TAAR1 with highest affinity (Borowsky et al. 2001). This receptor regulates cerebral neurotransmitter release (Liberles, 2015; Lindemann et al. 2008) and is expressed in the monoaminergic-enriched areas of the brain, including the striatum, substantia nigra, ventral tegmental area, locus coeruleus, amygdala, and raphe

nuclei (Berry, 2004; Borowsky et al. 2001; Linderman et al. 2008; Xie et al. 2007). Furthermore, TAAR1 is implicated in the functional regulation of the dopaminergic system (Miller, 2011), potentially influencing dopamine release and reuptake inhibition (Pei et al. 2016; Xie et al. 2007; Xie and Miller, 2009). However, cerebral concentrations of octopamine are several hundred-fold lower than dopamine, noradrenaline, and serotonin (Berry, 2004), suggesting endogenous levels may act in a neuromodulatory capacity (Burchett and Hicks, 2006). Basal concentrations are detectable in plasma (~1 $ng \cdot mL^{-1}$; D'Andrea et al. 2010) and can fluctuate during various disease states. Lower concentrations are present in the early stages of Parkinson's disease (D'Andrea et al. 2010) and Bulimia (D'Andrea et al. 2009), while plasma levels are elevated in patients with migraine (D'Andrea et al. 2012) and liver disease (Mousseau and Butterworth, 1995). These changes likely reflect a dysregulation in the metabolism of the precursor amino acids and/or their respective enzymes. For example, irregular activity of the enzyme tyrosine decarboxylase is a key feature of Parkinson's disease (Haavik and Toska, 1998).

Under the trade name Norphen, octopamine was administered to patients for the treatment of hypotensive and circulatory disorders. Doses of 450-600 $mg \cdot day^{-1}$ resulted in mild increases in systolic blood pressure (6-14 mm Hg), without the presence of adverse effects (Braasch et al. 1971; Mestrovic, 1972; Kuske, 1969; Stucke, 1972; Ziegelmeyer, 1972). A variety of dosing protocols were employed, including repeated administration of drops and chewable capsules (Kuske, 1969), sustained release tablets or capsules (Braasch et al. 1971; Stucke, 1972; Ziegelmeyer, 1972), and intravenous infusions (Mestrovic, 1972). However, these studies did not investigate the physiological effects of octopamine in normotensive individuals, and no study has examined its performance effects in humans. Intracerebroventricular administration of octopamine increased locomotor activity in rats (Jagiełło-Wójtowicz, 1979; Jagiełło-Wójtowicz and Chodkowska, 1984), but this response was not confirmed elsewhere (Delacour and Guenaire, 1983). Furthermore, octopamine administration decreased GABAergic transmission in rats (Jagiełło-Wójtowicz and Chodkowska, 1984), while endogenous levels are purported to play a role in the central stress response (Ennaceur et al. 1986). When rats were subjected to bouts of uncontrolled stress, a decrease in octopamine in the hypothalamus and brain stem was observed (Ennaceur et al. 1986). Although the functional significance of this response is not clear,

neurons within the hypothalamus and brain stem mediate the top-down stress response (Joëls and Baram, 2009).

2.6 Pituitary Hormones as Indirect Measures of Central Neurotransmission

While the advent of brain imaging techniques such as PET and MRI has provided new avenues to monitor the central effects of various nutritional and pharmacological interventions, these methods are typically limited to clinical and diagnostic research. Furthermore, factors such as expense, the need for trained operators, and logistical problems with performing exercise in or close to the equipment precludes their application in exercise physiology-based research. As such, the measurement of peripheral hormones is typically used an as index of central neurotransmission (Checkley, 1980). This is due to the role of central monoamines in the control of hormone release from the anterior and posterior pituitary gland (Smith and Vale, 2006).

Neurons within the paraventricular nucleus (PVN) of the hypothalamus synthesise and release corticotropin-releaing factor (CRF), a major regulator of the central stress response (Rivier and Vale, 1983). Noradrenergic projections from the brain stem induce CRF release from the PVN, which acts on the pituitary gland to secrete adrenocorticotropic hormone (ACTH) into the blood. Circulating ACTH then stimulates cortisol release from the adrenal gland (Tsigos and Chrousos, 2002). The influence of central serotoninergic and dopaminergic activity on pituitary hormonal release is also fairly well-established. Serotonin was initially believed to stimulate prolactin release (Van de Kar, 1997), with little influence from noradrenaline (Freeman et al. 2000). However, prolactin secretion is under tonic inhibition from tuberoinfundibular dopamine neurons, with a decrease in prolactin suggesting an increase in dopamine (Ben-Jonathan and Hnasko, 2001). Serotonin augments circulating ACTH and thus cortisol (Dinan, 1996), while noradrenaline has opposing actions at α-and ß-adrenoreceptors on growth hormone release (Checkley, 1980; Van der Kar, 1997).

Monitoring the hormonal response to a given nutritional or pharmacological intervention is a common tool in exercise physiology and provides useful information regarding alterations in central neurotransmission. The central effects of drugs which act via noradrenergic

(Cooper et al. 1994) and dopaminergic (Floel et al. 2008) mechanisms are in accordance with the hormonal responses reported in previous exercise studies (Watson et al. 2005a; Cordery et al. 2016). However, due to the complex functional integration of the monoamine neurotransmitter systems, it is difficult to determine the precise contribution of a single neurotransmitter to a given hormonal response. Furthermore, the involvement of other neurotransmitters systems (i.e. glutamate, GABA, orexin), neuropeptides, and endogenous opioids should be considered (Meeusen et al. 2006). There also remains a degree of uncertainty regarding the endocrine response to physiological and psychological stress (Dinan, 1996; Smith and Vale, 2006), which may conflate the significance of a given hormonal response.

2.7 Summary

Few studies have investigated the performance and thermoregulatory effects of caffeine during prolonged exercise in the heat (Armstrong et al. 2007). Similarly, the influence of habituation to caffeine has received little attention (Bell and McLellan, 2002; Irwin et al. 2011). This is despite a high prevalence of intake by athletes during competition (Desbrow and Leveritt, 2006) and across the general population (Fitt et al. 2013). While octopamine is currently placed on the WADA prohibited list (WADA, 2015), no study has examined its performance or metabolic effects during prolonged exercise in humans.

The studies described in chapters 4 and 5 are designed to further explore the performance and physiological effects of acute caffeine supplementation during prolonged exercise in the heat. Specifically, chapter 4 was the first study to examine the thermoregulatory and ergogenic effects of a low dose of caffeine, administered either as a single-or split-dose prior to prolonged exercise. Due to the lack of consistent findings within the literature (Ganio et al. 2011; Roelands et al. 2011), the aim of chapter 5 was to determine whether a moderate caffeine dose influences endurance performance or thermoregulation during prolonged cycle exercise. Chapter 6 was designed to assess the influence of a prolonged period of controlled chronic caffeine intake on endurance performance. As previous studies utilised sub-chronic supplementation protocols (i.e. 4 days; Irwin et al. 2011), the habituation protocol employed in chapter 6 was more representative to that observed within the

general population (Fit et al. 2013). The precise role of octopamine in humans is not clear, although it may possess stimulant-like properties (Stohs, 2014; WADA, 2015) and influence fat metabolism (Carpéné et al. 1999). As such, the study described in chapter 7 investigated whether an acute dose of octopamine could influence endurance performance or metabolism.

Chapter 3

General Methods

3.1 Ethical Approval

All experimental protocols were approved by the Ethics Approvals (Human Participants) Sub-Committee at Loughborough University, UK. The reference numbers for the procedures described in chapters 3, 4, 5, and 6 are R12-P115, R15-P104, R14-P76, and R15-P072, respectively. All prospective participants were first approached either in person, or via email and poster advertisements. The full nature and purpose of the investigation was explained verbally and documented in the respective participant information sheet; this included information regarding any potential risks or discomfort that might arise during the study. For example, in chapters 3, 4, and 5, participants were informed about the mild side effects associated with caffeine ingestion. Additionally, participants in chapter 6 were informed that octopamine is currently on the WADA list of prohibited substances. Following an opportunity to ask questions, those interested in participating completed a health screen questionnaire to ensure eligibility for the study. After this, a written statement of consent was signed. Each participant was fully aware they could withdraw from the study at any point, without having to provide their reasons for doing so.

3.2 Participants

All participants were male, aged 18-40 years, and were recruited from the staff and student population at Loughborough University, as well as local sports clubs. Given the physically demanding nature of the investigations, all participants were familiar with the sensation of strenuous and prolonged exercise. Individuals with a history of metabolic disease or those which presented illness at the time of the study were excluded. In chapters 4 and 5, participants were unaccustomed to exercise in a warm environment at the time of the study. In chapter 6, only participants with a habitual caffeine intake of <75 $mg \cdot kg^{-1}$ were allowed to take part in the study. Those who failed to meet the inclusion criteria were thanked for their interest and informed their help would not be required.

3.3 Experimental Design

All investigations employed a placebo-controlled, repeated-measures design. In chapters 4, 5, and 7, all trials were randomised using a Latin-square design and were conducted in a double-blind manner. In chapter 6, the initial 2 trials were randomised and double-blind while the final trial was single-blind. Furthermore, a control group was used to enable a double-blind chronic supplementation period (discussed in chapter 6.3). Hence, chapter 6 employed a mixed-measures design protocol (i.e. between and within factors), whereas chapters 4, 5, and 7 utilised a within-measures design.

3.4 Measurement of Peak Oxygen Uptake

During each investigation, participants had their peak oxygen uptake (VO_{2peak}) established at least 72 hours before the familiarisation trial. This determined the workload used during the subsequent familiarisation and experimental trials. This test consisted of continuous incremental exercise on an electronically braked cycle ergometer (Lode Corival, Groningen, The Netherlands) set in hyperbolic mode (i.e. cadence independent).

Participants began exercise at a workload of 95 Watts (W), with the intensity increasing by 35W every 3 min until volitional exhaustion. Participants received verbal encouragement from the investigators to help ensure a maximum effort. At the end of exercise, maximum workload (W_{max}) was calculated according to Jeukendrup et al (1996). In chapter 6, participants completed a second VO_{2peak} test to account for any potential change in fitness over the study period; this followed the same procedure described above.

In chapters 4 and 6, expired gas samples were collected during the final min of each stage using the Douglas bag method. If participants indicated they could not finish their current stage, a final sample was collected during the last min of exercise. In chapters 5 and 7, expired gas was collected during the final min of exercise only. In both tests, VO_{2peak} was defined as the highest VO_2 measured. Heart rate (Polar Beat, Kempele, Finland) and RPE (Borg, 1982) were recorded at the end of each stage and at exhaustion.

Expired gas was analysed for oxygen and carbon dioxide concentrations with a paramagnetic analyser (Servomex 1400, Sussex, UK) calibrated against gases of known concentration (British Oxygen Company, London, UK). Total volume was quantified (Harvard Dr Gas Meter, Harvard Apparatus, USA) and gas temperature was determined with an electronic sensor (Edale Instruments Ltd, Cambridge, UK). All gas volumes were expressed as standard temperature and pressure for dry gas (STPD) and barometric pressure was recorded with a standard mercury barometer. Oxygen uptake and carbon dioxide production were determined using published equations (Frayn, 1983). In chapters 4 and 6, the oxygen uptake to work-rate relationship was used to calculate the intensity of exercise during each trial. In chapters 5 and 7, the workload was calculated relative to W_{max}.

3.5 Standardisation of Conditions

Before the experimental trials, all participants undertook one familiarisation trial. This visit ensured that participants were accustomed to the procedures employed during the investigation, to minimise learning or anxiety effects, and to ensure attainment of a maximum effort during the performance task. This visit was identical to the experimental trials in all respects, although no treatment was administered.

To ensure metabolic conditions were similar across trials conditions, participants were provided with a dietary and physical activity diary to complete in the 24 hours before the familiarisation trial and to replicate this during the 24 hours preceding each subsequent experimental trial. Participants were also instructed to refrain from strenuous physical activity and alcohol ingestion during this period. The caffeine-specific standardisation protocols for each experimental chapter are fully described in their respective methods section. During each study, participants were provided with a list of commonly consumed caffeinated foods and drinks to help achieve these requirements. Compliance to these measures was verified at the start of each trial, before any data collection.

All trials were performed in the morning (start: 7-9am) after an overnight fast (8-12 hours), although participants ingested 500 mL of plain water 60 min before arriving at the laboratory. The environmental conditions in chapters 6 and 7 were approximately 20^{o}C and

50-60% RH. Similar environmental conditions were maintained during the pre-exercise rest period in chapters 4 and 5. However, during exercise the environmental conditions were 30^{o}C and 50% RH; these conditions were maintained using a climatic chamber (Weiss Technik, Loughborough, UK). Furthermore, the familiarisation and experimental trials were separated by 7-10 days to limit the development of heat acclimation (Barnett and Maughan, 1993). All trials were performed at the same time of day to minimise circadian-type variations in performance; this also controlled for diurnal changes in thermoregulation in chapters 4 and 5 (Reilly and Brooks, 1986). Participants used the same ergometer during each visit and were supervised by the same investigator. Additionally, saddle height was recorded during the VO_{2peak} test, which was used during each subsequent trial.

3.6 Experimental Trials

The exercise protocol employed during each investigation was a preloaded performance task. After a prolonged period of exercise at a fixed work-rate (30-60 min), the ergometer was programmed for the performance task. Participants were instructed to produce as much work (kJ) as possible within 30 min; this method is consistent with previous studies (Cheuvront et al. 2009; Ganio et al. 2011). Before starting, participants were encouraged to produce a maximal effort. The initial workload was set at 75% VO_{2peak} or W_{max}, but participants were free to adjust the intensity of exercise as desired from the outset. During exercise, participants received information regarding time elapsed and cadence, but no other information or verbal encouragement was provided. Each participant was provided with two chances to practice pacing and control of the ergometer; once shortly after completing the initial VO_{2peak} test, and again during the familiarisation trial.

In each study, post-void nude body mass was recorded upon arrival to the laboratory (Adam AFW-120 K, Milton Keynes, UK) and again immediately after exercise, after participants towelled dry (chapters 4 and 5). The change in body mass, corrected for urine output and fluid intake, was used to estimate sweat rate. These values were not corrected for respiratory water losses or changes in metabolic water production due to substrate oxidation. In chapters 4 and 5, participants ingested a radio-telemetry pill (CorTemp, HG Inc, Palmetto, Florida, USA) the evening before each experimental trial; this was used as an

index of core temperature. This method significantly correlates with rectal temperature during prolonged exercise in high ambient temperature (Ganio et al. 2009a). Furthermore, skin surface temperature thermistors (Grant Squirrel SQ800, Cambridgeshire, UK) were attached to four sites (chest, upper arm, thigh, and calf) for the calculation of weighted mean skin temperature (Ramanathan, 1964); these were held in place with Transpore medical tape (3M, Loughboorugh, UK). During all trials, heart rate was recorded using short-range telemetry (Polar Beat, Kempele, Finland). Perceived exertion was recorded throughout exercise using the 15-point Borg scale (Borg, 1982). In chapters 4 and 5, perceived thermal stress (using a 21 point scale ranging from -10, unbearable cold, to +10, unbearable heat) was recorded at rest and during exercise. In chapters 4, 6, and 7, expired gas samples were collected every 15 min during the fixed-intensity exercise. In chapter 5, expired gas samples were collected every 30 min. These values were used to estimate the rates of fat and carbohydrate oxidation during exercise (Peronnet and Massicotte 1991). During the fixed-intensity exercise, plain water (temperature: ~20^{o}C) was provided every 15 min; the amount administered was 150 mL in chapters 4 and 5 and 100 mL in chapters 6 and 7, respectively.

3.7 Blood Collection and Analysis

To prevent postural changes in blood volume, participants rested in a supine position for 15 min before the first venous sample was collected. During all trials, a 21 g butterfly cannula was inserted into an antecubital forearm vein; this was attached to a three-way tap (BD Connecta, Helsingborg, Sweden) to enable repeated blood sampling throughout each trial. After each sample was collected, the cannula was flushed with 2.5 mL of saline to ensure patency. The volume of blood collected during each sample was 7 mL in chapters 4, 5 and 6 and 12 mL in chapter 7, respectively.

All venous samples were drawn directly into dry syringes. During all trials, 2 mL was dispensed into tubes containing K_2EDTA. Duplicate 100 µL sub-samples were rapidly deproteinised in 1 mL of ice-cold 0.3 N perchloric acid. These were centrifuged, with the resulting supernatant used for spectrophotometric determination of plasma glucose in duplicate using a commercially available assay (GOD-PAP, Randox Ltd, UK). Haemoglobin

(Hb; cyanmethemoglobin method) was measured in duplicate with spectrophotometric detection while haematocrit (Hct) was measured in triplicate by microcentrifugation (Hawksley, Sussex, UK). Both Hb and Hct were determined on the same day the samples were collected; these data were used to estimate percentage changes to blood and plasma volumes relative to the resting sample (Dill and Costill, 1974). During all trials, 5 mL of whole blood was dispensed into tubes containing clotting activator (Sarstedt, Germany). In chapter 7, a further 5 mL was dispensed into tubes containing K_2EDTA (Sarstedt, Germany). These were left on ice for at least 60 min prior to centrifugation at 1750 g for 10 min at 4^{o}C to yield serum and plasma, respectively. The supernatants were transferred into eppendorf tubes and stored at -21^{o}C until analysis. In each study, serum cortisol and prolactin were measured via Enzyme-linked immunosorbent assay (ELISA; DRG diagnostic, Germany). In chapters 4, 5, and 6, serum caffeine was determined with reverse-phase high performance liquid chromatography (HPLC) with ultraviolet detection (Holland et al. 1998). In chapter 7, serum octopamine was measured with reverse-phase HPLC with fluorescence detection (Wood and Hall, 2000) and plasma FFA (Randox laboratories Ltd, Crumlin, UK) by colorimetric methods (ABX Pentra 400, Horiba Medical, UK). All standard curves were measured in duplicate, while participant samples were measured in singlicate. Section 3.8 provides the intra-assay coefficient of variation (CV) for plasma glucose, haemoglobin, and haematocrit. The CV'S for caffeine, cortisol, prolactin, octopamine, and FFA's are provided in the relevant experimental chapters.

3.8 Coefficient of Variation of Methods (n=20)

Measure	Method	Mean	SD	CV
Plasma glucose (mmol·L^{-1})	GOD-PAP (Randox)	4.57	0.08	1.86
Haemoglobin (g·dL^{-1})	Cyanmethaemoglobin	14.79	0.11	0.74
Haematocrit (%)	Microcentrifugation	44.10	0.35	0.79

Note: CV=(SD/Mean)*100

Chapter 4

Performance and Thermoregulatory Responses to Prolonged Exercise in the Heat Following Two Low-Dose Caffeine Ingestion Strategies

4.1 Abstract

The aim of this study was to examine the performance and thermoregulatory effects of two low-dose caffeine ingestion strategies in high ambient temperature. Ten recreationally active males completed an incremental exercise test, one familiarisation trial and three experimental trials. Participants ingested a single 3 $mg \cdot kg^{-1}$ caffeine dose 60 min before exercise (single-caffeine), a 1.5 $mg \cdot kg^{-1}$ dose 60 min and immediately before exercise (split-caffeine) or a placebo. Trials consisted of 60 min of cycle exercise at 60% peak oxygen uptake followed by a 30 min performance task (total kJ produced) in 30˚C and 50% RH. Performance was improved in the single-caffeine (341.9 ± 45.9 kJ; Cohen's *d* effect size [*d*]=0.42; *P*=0.029; 95% CI: 2.4-43.1 kJ; 90.2% chance of benefit) and split-caffeine trial (357.6 ± 53.3 kJ; *d*=0.67; *P*<0.05; 95% CI: 23.7-53.1 kJ; 99.6% chance of benefit) compared with placebo (319.2 ± 62.0 kJ). The split-dose strategy further enhanced performance than single-dose administration (*d*=0.32; *P*=0.059; 95% CI: 0.6-31.9 kJ; 78.5% chance of benefit). Caffeine did not influence thermoregulation, hormonal concentrations or substrate oxidation (all *P*>0.05). Perceived exertion was lower throughout the initial 60 min of exercise during both caffeine trials than placebo (*P*<0.048), but no difference was observed between the two caffeine trials (*P*=0.506). While performance was enhanced after single-dose administration, dividing the caffeine bolus conferred additional benefit. Consequently, repeated low-doses could potentiate the efficacy of the same total caffeine dose when exercise is performed in the heat.

4.2 Introduction

Caffeine (1,3,7-trimethylxanthine) is a popular nutritional supplement consumed by athletes (Desbrow and Leveritt, 2006). When exercise is performed in temperate environmental conditions (~20 ^{o}C), caffeine has well-established positive effects on endurance performance (Burke, 2008). However, the influence of a high ambient temperature on caffeine's ergogenic potential has received less attention. Supplementation with 6 mg·kg^{-1} enhanced maximal voluntary contraction of the quadriceps (Del Coso et al. 2008) and enabled participants to produce more work (kJ) during a 15 min performance task compared with placebo (Ganio et al. 2011). Conversely, TT performance was not influenced by caffeine doses of 6 mg·kg^{-1} (Roelands et al. 2011) or 9 mg·kg^{-1} (Cheuvront et al. 2009). Furthermore, 21 km race performance was not enhanced with caffeine intakes of 5 or 9 mg·kg^{-1} in hot, humid conditions (Cohen et al. 1996). Hence, caffeine's ergogenic effects are less consistent when exercise is undertaken in a warm environment, at least when moderate to large doses (i.e. 5-9 mg·kg^{-1}) are consumed.

It is well-documented that the capacity to perform prolonged exercise becomes impaired as the ambient temperature increases (Galloway and Maughan, 1997). The increased physiological burden to dissipate heat through the skin and the concomitant elevation in core temperature are key factors mediating this deterioration in performance (Cheuvront et al. 2010; Nybo, 2010). The resulting hyperthermia influences several aspects of central nervous system function, including altered brain activity (Nielsen et al. 2001c), reduced maximal voluntary contractions (Nybo and Nielsen, 2001a) and increased perception of effort (Nybo and Nielsen, 2001b), leading to a decreased drive to continue exercise (Nybo, 2010). Previous studies have demonstrated that caffeine can influence thermoregulation during exercise in high ambient conditions (Cheuvront et al. 2009; Ely et al. 2011; Roelands et al. 2011). Caffeine has elicited large (Roelands et al. 2011) and small (Cheuvront et al. 2009; Ely et al. 2011) increases in core temperature compared with placebo. Additionally, sweat-electrolyte losses were greater after caffeine intake in the heat (Del Coso et al. 2009). Hence, these challenges to thermoregulation and fluid-balance could preclude the ergogenic effects of caffeine when exercise is performed in the heat. Interestingly, these responses are typically observed when moderate to large caffeine doses (i.e. 6-9 mg·kg^{-1}) are employed (Cheuvront et al. 2009; Del Coso et al. 2009; Ely et al. 2011; Roelands et al.

2011). Therefore, the provision of smaller doses (~3 mg·kg^{-1}) could prove a more prudent strategy when aiming to enhance performance in the heat, thus avoiding the deleterious physiological effects induced by larger caffeine intakes. To date, only one study has employed this approach (Pitchford et al. 2014). These authors reported improved cycle TT performance when 3 mg·kg^{-1} of caffeine was administered prior to prolonged exercise in the heat (35^{o}C and 25% RH). While core temperature was similar between trials, the self-paced TT employed makes comparing the physiological data between trials difficult to interpret due to fluctuations in power output. Additionally, these authors did not measure skin temperature. It therefore remains to be determined if a low-dose of caffeine can influence thermoregulation during prolonged fixed-intensity exercise in the heat.

Differences in caffeine-dosing strategies can influence the core temperature response to prolonged exercise in the heat (Ganio et al. 2011; Roelands et al. 2011). Both studies provided 6 mg·kg^{-1} and examined the influence of caffeine at a fixed work-rate. Roelands et al (2011) reported an elevation in core temperature from 25 min until the end of exercise after the provision of a single 6 mg·kg^{-1} dose. Conversely, Ganio et al (2011) did not observed a caffeine-induced increase in core temperature after dividing the caffeine bolus into two 3 mg·kg^{-1} doses. This discrepancy led to the suggestion that splitting the caffeine bolus could distribute its thermogenic effects during prolonged exercise in high ambient temperature (Pitchford et al. 2014). Dividing the caffeine bolus in a temperate environment confers a similar performance benefit compared with single-dose administration (Conway et al. 2003; Cox et al. 2002). Moreover, the optimum caffeine dose required to enhance performance in temperate conditions plateaus at 3 mg·kg^{-1} (Desbrow et al. 2012). However, no study has investigated the effects of a single and divided low-dose of caffeine on endurance performance and thermoregulation in the same participant group during prolonged exercise in the heat.

Hence, the aim of this study was to examine the influence of a low-dose of caffeine, administered either as a single-or split-dose, on endurance performance and thermoregulation during prolonged exercise in the heat.

4.3 Methods

Ten healthy, recreationally active males (Age: 22 ± 4 y; body mass: 82.0 ± 9.8 kg; height: 1.80 ± 0.08 m; VO_{2peak}: 49.0 ± 3.4 $mL \cdot kg^{-1} \cdot min^{-1}$) took part in this double-blind, placebo controlled, randomised, cross-over study. All participants completed one initial incremental exercise test to exhaustion, one familiarisation trial, and three experimental trials. The preliminary trial consisted of an incremental exercise test to volitional exhaustion conducted on an electronically braked cycle ergometer (Lode Corival, Groningen, Holland) to determine VO_{2peak} and the power output required to elicit 60% and 75% VO_{2peak}. This test was conducted in temperate conditions (~20˚C). After 5-7 days, the familiarisation trial was undertaken to ensure participants were fully accustomed to the procedures employed during the investigation and to minimise any learning or anxiety effects. This visit was performed in environmental conditions maintained at 30°C and 50% RH and was identical to the experimental trials in all respects, although no treatment was administered.

The trial overview is depicted in figure 4.1. The pre-trial dietary and exercise standardisation procedures are outlined in chapter 3.5. No caffeine was permitted during the 24 hours before the familiarisation and experimental trials. Participants were given a telemetry pill to ingest the evening before each experimental trial to enable the measurement of core temperature. On the day of testing, participants arrived at the laboratory (7-9am) and post-void nude body mass was recorded. After 15 min of seated rest, a 21 g cannula was inserted into an antecubital vein to enable repeated blood sampling. An initial 7 mL resting blood sample was collected after which participants received a capsule containing 3 $mg \cdot kg^{-1}$ (single-caffeine) or 1.5 $mg \cdot kg^{-1}$ (split-caffeine) of anhydrous caffeine (BDH Ltd, Poole, UK) or 250 mg of starch (BDH Ltd, Poole, UK; Placebo) with 50 mL of plain water. A telemetry heart-rate band was then positioned (Polar Beat, Kempele, Finland) and skin surface temperature thermistors (Grant Squirrel SQ800, Cambridgeshire, UK) were attached to four sites (chest, upper arm, thigh, and calf) to determine weighted mean skin temperature (Ramanathan, 1964). After 45 min of seated rest in 20 °C, core and skin temperature and heart rate were recorded at 5 min intervals. A second venous sample (7 mL) was collected 60 min post-capsule ingestion. Next, participants consumed a second capsule containing 250 mg of starch (single-caffeine and placebo) or 1.5 $mg \cdot kg^{-1}$ of caffeine (split-caffeine). All capsules were indistinguishable with regards to dimension, weight and colour

Participants then entered a climatic chamber (Weiss-Gallenkamp, UK) maintained at 30˚C and 50%. Trials consisted of 60 min of cycle exercise at a workload corresponding to 60% VO_{2peak}. During this period, core and skin temperature and heart rate were recorded every 5 min. Perceived exertion (Borg 1982) and perceived thermal stress (using a 21 point scale ranging from -10, unbearable cold, to +10, unbearable heat) were measured every 10 min. Expired gas samples (1 min) were collected into Douglas bags every 15 min to determine the rates of fat and carbohydrate oxidation (Peronnet and Massicotte 1991). After each sample, participants were provided with 150 mL of plain water and venous samples (7 mL) were collected every 20 min.

Following the fixed intensity exercise, there was a 2-3 min delay whilst the ergometer was set up for the performance task. Participants were instructed to complete as much work (kJ) as possible within 30 min. Exercise began at a workload corresponding to 75% VO_{2peak}, but from the outset participants were free to increase or decrease their power output as desired. Core and skin temperature and heart rate were measured every 5 min while RPE and thermal stress were recorded at 10 and 20 min, respectively. Immediately after the performance task, a final venous sample (7 mL) was collected while participants remained seated on the ergometer. Participants then returned to a comfortable environment (~20˚C) where the cannula, telemetry band and skin thermistors were removed. Thereafter, nude body mass, corrected for fluid intake, was recorded to enable the estimation of sweat rate.

Whole blood (2 mL) was used to measure Hb, Hct, and glucose. The remaining blood (5 mL) was used to yield serum for the subsequent analysis of serum prolactin and cortisol with ELISA (DRG diagnostics, Germany) and caffeine with reverse-phase HPLC (Holland et al. 1998) as described in chapter 3.7. The intra-assay CV for serum prolactin, cortisol, and caffeine was 5.9%, 3.1%, and 3.3%, respectively.

All data were analysed using IBM SPSS statistics version 21.0. Normality was assessed with the Shapiro Wilk test. To evaluate differences in exercise performance, fasting plasma glucose, pre exercise nude body mass, initial core temperature and sweat rate across trials conditions, a one-way repeated-measures analysis of variance (ANOVA) was employed. Cohen's *d* effect size for differences in total work produced during the experimental trials was calculated ([mean 1 - mean 2]/pooled SD) and interpreted as trivial (0-0.19), small (0.2-

0.49), medium (0.5-0.79) or large (>0.8) as previously described (Cohen 1992). Variables measured throughout each trial were analysed using a two-way (trial x time) repeated-measures ANOVA. Where the assumption of sphericity had been violated, the degrees of freedom were corrected with a Greenhouse-Geisser as appropriate. Main effects and interactions were followed up with Bonferroni-adjusted paired *t*-tests for normally distributed data or Bonferroni-adjusted Wilcoxon Signed Rank tests for non-normally distributed data. In addition to null-hypothesis testing, magnitude-based inferences examined whether the observed changes in performance were meaningful (Hopkins 2000). Based on previous findings (Ganio et al. 2009b), the smallest worthwhile change in performance was set at 3% (10 kJ). Data are presented as means ± standard deviation (SD) throughout, unless otherwise stated. Statistical significance was accepted at $P<0.05$.

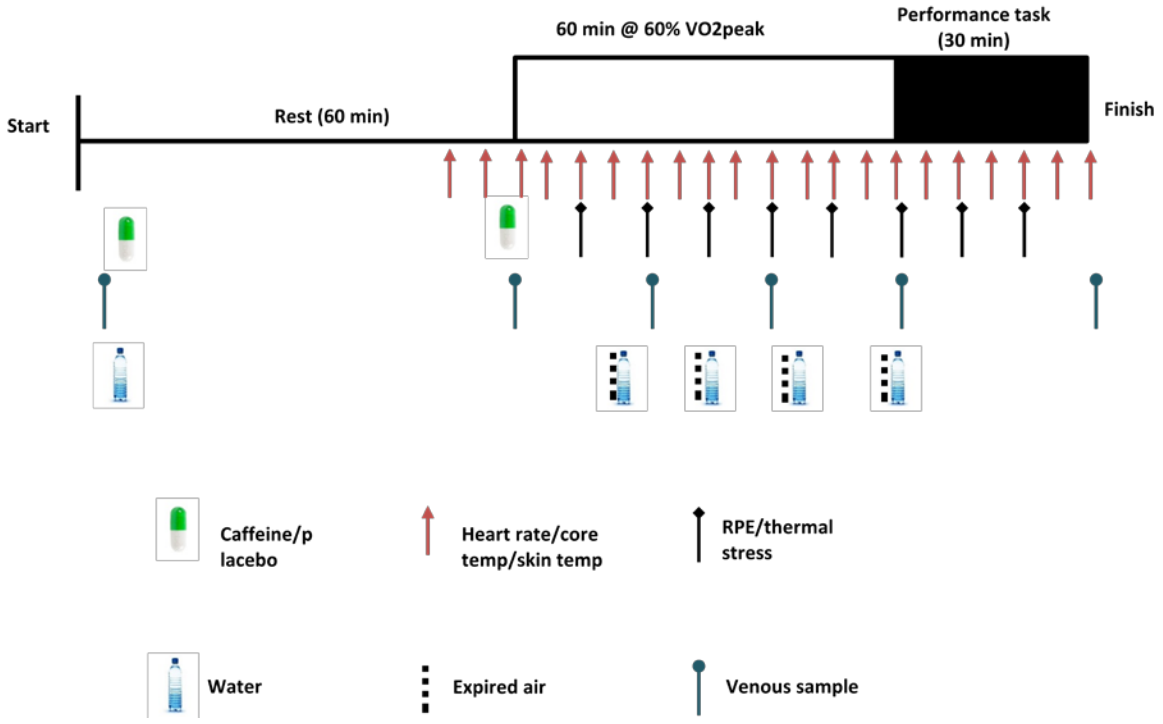

Figure 4.1 Trial overview

4.4 Results

Self-reported habitual caffeine intake revealed that participants were low caffeine consumers (mean: 75 ± 77 mg·day^{-1}). No side effects related to the treatment were reported during any of the trials. There were no main effects of trial for pre-exercise nude body mass (P=0.528), initial core temperature (P=0.369) or fasting plasma glucose (P=0.625), suggesting participants began each trial in a similar physiological state.

There was a main effect of trial for exercise performance (P<0.05). Total work produced was greater during the single-caffeine (341.9 ± 45.9 kJ; d=0.42; P=0.029) and split-caffeine trial (357.6 ± 53.3 kJ; d=0.67; P<0.05) compared with placebo (319.2 ± 62.0 kJ; Figure 4.2). Based on the smallest worthwhile change of 10 kJ, the chance these increases represent a beneficial, trivial or harmful influence on performance is 90.2%, 9.7% and 0.2% (95% CI: 2.4 to 43.1 kJ) for the single-caffeine trial and 99.6%, 0.4% and 0.0% (95% CI: 23.7 to 53.1 kJ) for the split-caffeine trial, respectively. There was a small increase in work produced during the split-caffeine trial compared with single-caffeine (d=0.32; P=0.059); the chance this difference represents a beneficial, trivial or harmful influence on performance is 78.5%, 21.2% and 0.3% (95% CI: -0.6 to 31.9 kJ), respectively.

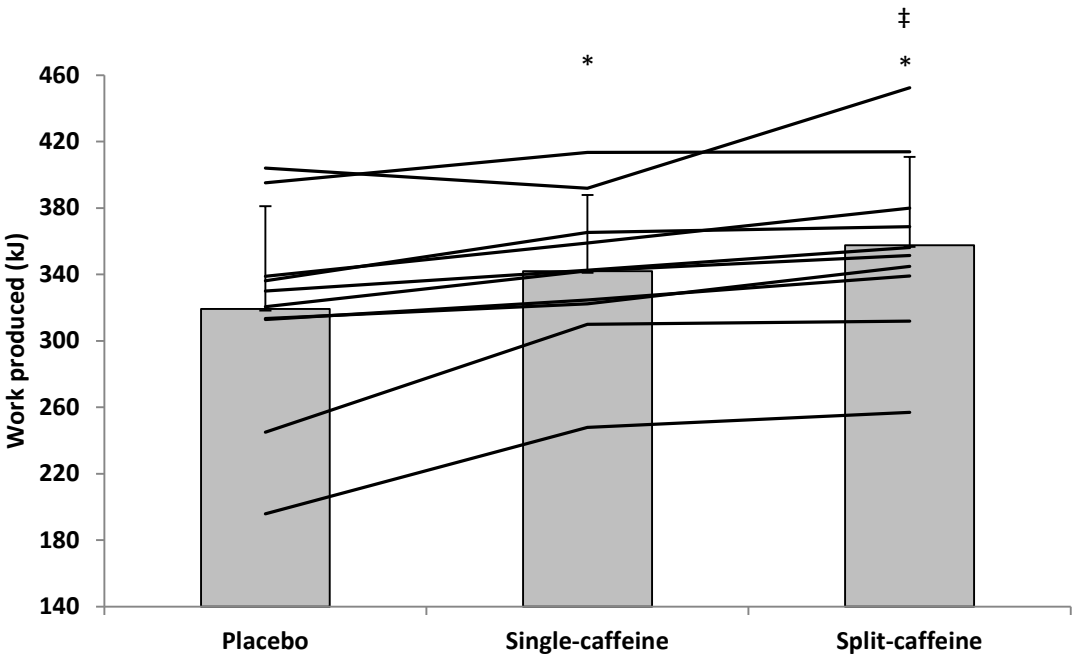

Figure 4.2 Mean (bars) and individual (lines) work produced during the performance task. *denotes single-caffeine and split-caffeine greater than placebo (P<0.029). ‡denotes P=0.059 compared with single-caffeine. Values are mean ± SD.

All 10 participants performed best during the split-caffeine trial, 1 performed worst during the single-caffeine trial and 9 performed worst during the placebo trial (Figure 4.2). Only 1 participant correctly guessed the order of the trials, suggesting that the blinding treatment was successful (i.e. less than chance).

There were no differences across trials for pre-exercise core temperature (P=0.255; Figure 4.3A). There was a main effect of time for core temperature during the fixed-intensity exercise (P<0.05), but no main effect of trial (P=0.123) or trial x time interaction (P=0.808). During the performance task there was a main effect of time (P=0.022), trial (P<0.05), and a trial x time interaction (P<0.05). Higher values were recorded from 20 to 30 min during the split-caffeine trial compared with single-caffeine and placebo (P<0.05). Furthermore, higher values were recorded at 25 and 30 min during the single-caffeine trial compared with placebo (P<0.05; Figure 4.3A). There were no differences across trials for pre-exercise skin temperature (P=0.459; 4.3B). There was a main effect of time for skin temperature during the fixed-intensity exercise (P<0.05), with values steadily increasing and reaching a plateau after 25 min. No main effect of trial (P=0.610) or trial x time interaction (P=0.864) was apparent. Similarly, during the performance task there was a main effect of time (P=0.019), but no main effect of trial (P=0.250) or interaction effect (P=0.862; Figure 4.3B).

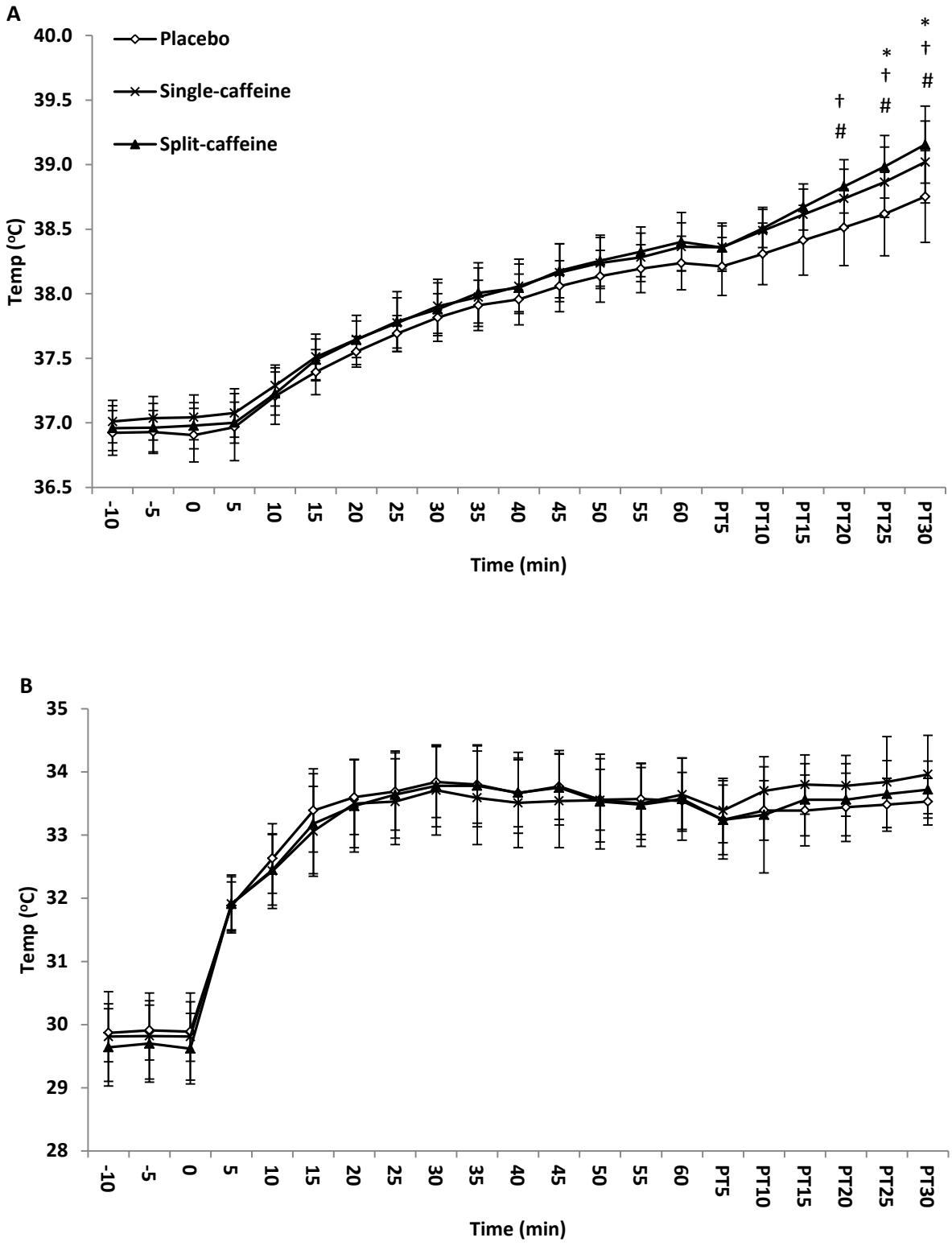

Figure 4.3 Core (A) and skin (B) temperature during the experimental trials. Single-caffeine and split-caffeine greater than placebo are denoted as * and #, respectively. Split-caffeine greater than single-caffeine is denoted as †. All notations are $P<0.05$. PT, performance test. Values are mean ± SD.

There was a main effect of time (P<0.05), trial (P<0.05) and a trial x time interaction (P<0.05) for serum caffeine. Baseline concentrations were similar across trials (P>0.05), remaining below the limit of detection for most participants. Circulating concentrations were 2.24 ± 0.73 $\mu g \cdot mL^{-1}$ and 1.16 ± 0.28 $\mu g \cdot mL^{-1}$ 60 min post-ingestion during the single-caffeine and split-caffeine trials, respectively (P<0.05; Figure 4.4). Caffeine concentrations remained higher throughout the initial 40 min of exercise during the single-caffeine trial compared with split-caffeine (P<0.05). After the performance task, higher values were recorded during the split-caffeine trial than single-caffeine (P<0.05; Figure 4.4).

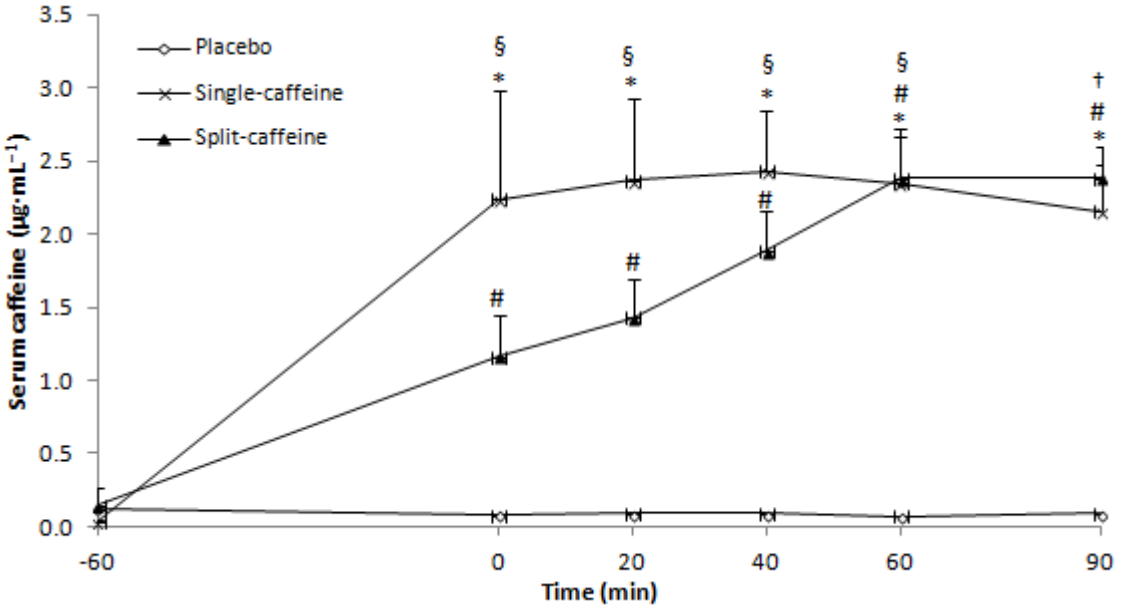

Figure 4.4 Circulating caffeine during the experimental trials. Single-caffeine and split-caffeine greater than placebo are denoted as * and #, respectively. Single-caffeine greater than split-caffeine is denoted as §. Split-caffeine greater than single-caffeine is denoted as †. All notations are P<0.05. Values are mean ± SD.

Circulating cortisol showed a main effect of time (P=0.001), but no main effect of trial (P=0.404) or trial x time interaction (P=0.545; Figure 4.5A). There was a main effect of time for circulating prolactin (P<0.05), with higher values recorded from 20 min until the end of exercise compared with baseline (P<0.05; Figure 4.5B). No main effect of trial (P=0.978) or trial x time interaction (P=0.832) was apparent.

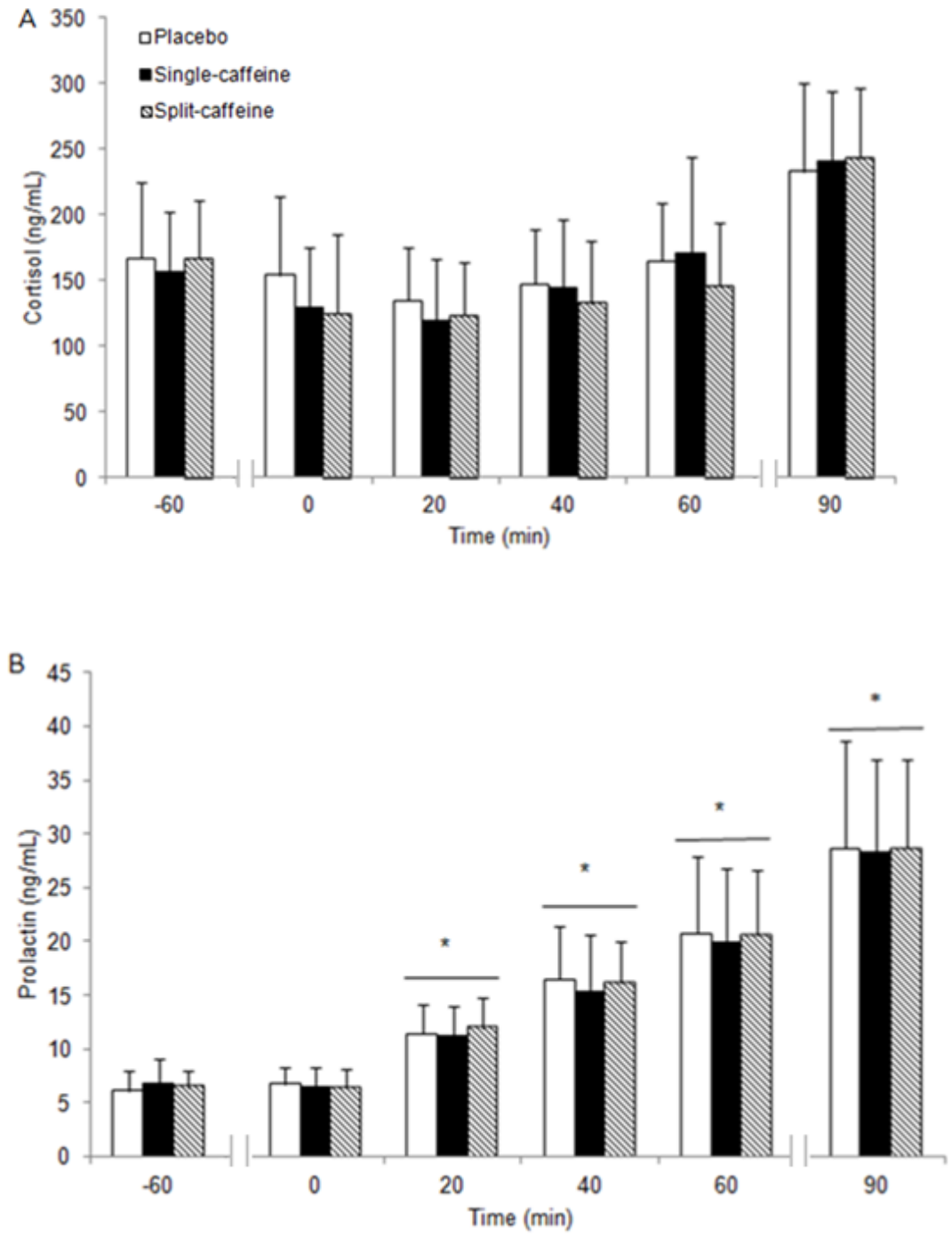

Figure 4.5 Circulating cortisol (A) and prolactin (B) during the experimental trials. *denotes a significant difference (P<0.05) from -60. Values are mean ± SD.

Plasma glucose showed a main effect of time (P<0.05), but no main effect of trial (P=0.415) or interaction effect (P=0.693; Figure 4.6).

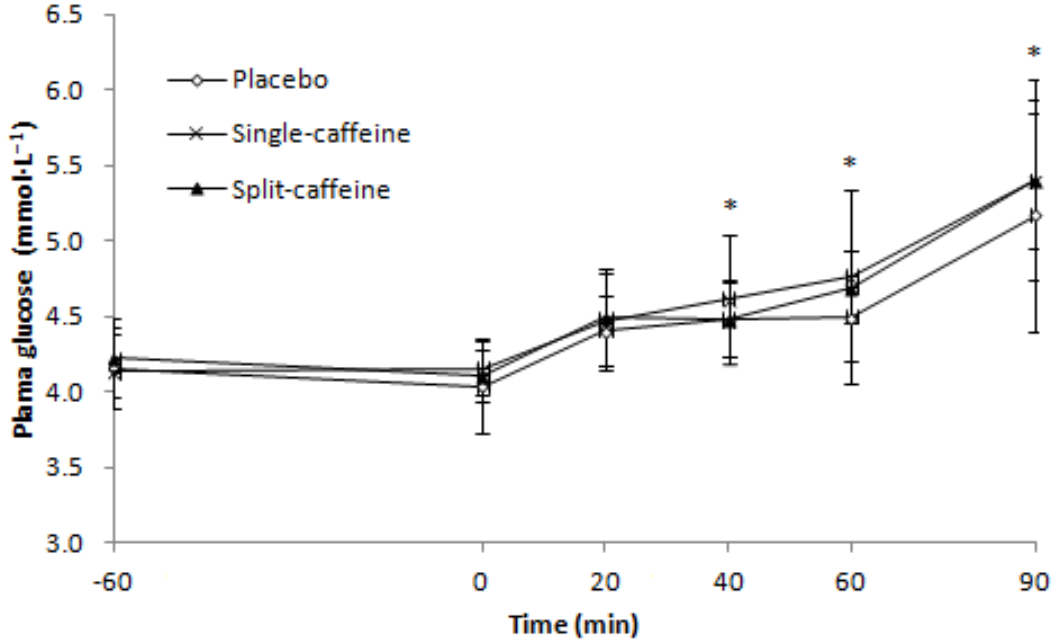

Figure 4.6 Plasma glucose during the experimental trials. *denotes a significant difference (P<0.05) from -60. Values are mean ± SD.

Changes to blood and plasma volume showed main effects of time (P<0.05), but no main effects of trial (P>0.345) or trial x time interactions (P>0.387) were apparent. There was no main effect of time (P=0.248), trial (P=0.638) or a trial x time interaction (P=0.699) for changes to cell volume.

During the fixed-intensity exercise RPE showed main effects of time (P<0.05) and trial (P<0.05), but no interaction effect (P=0.155). Lower values were recorded during the single-caffeine (P=0.048) and split-caffeine trial (P=0.003) compared with placebo (Figure 4.7). No difference was observed between the two caffeine trials (P=0.506). During the performance task RPE showed a main effect of time (P<0.05), but no main effect of trial (P=0.907) or trial x time interaction (P=0.098).

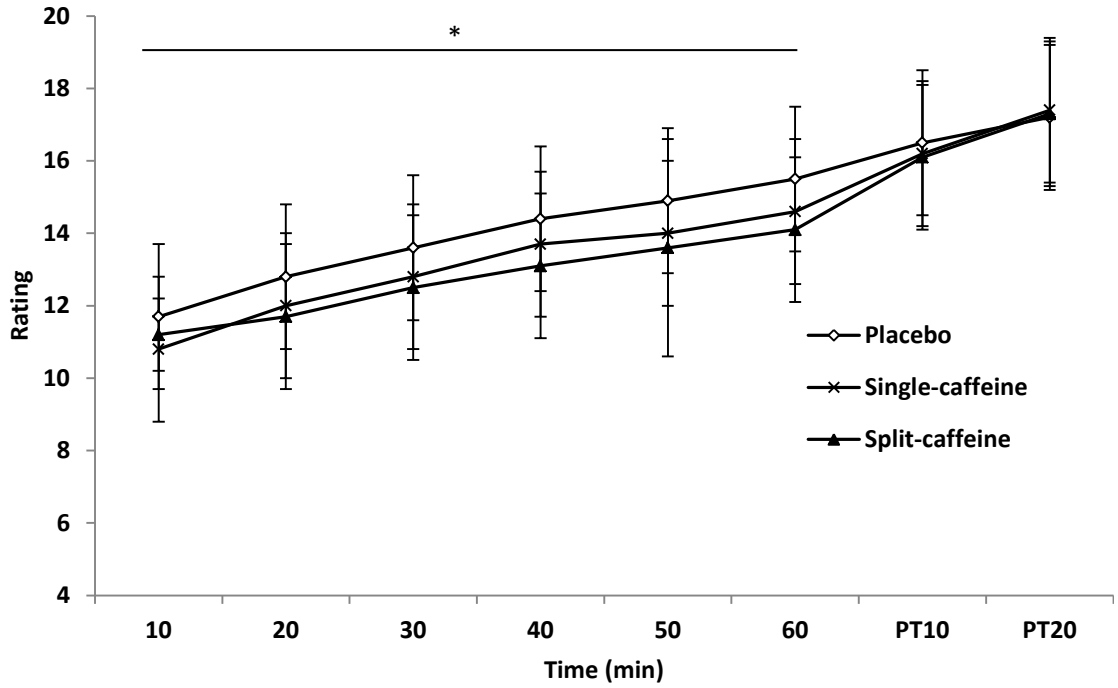

Figure 4.7 Perceived exertion during exercise. *denotes placebo greater than single-caffeine and split-caffeine (P<0.048). PT, performance test. Values are mean ± SD.

Thermal stress showed a main effect of time during the fixed-intensity exercise and performance task (P<0.05), but no main effect of trial (P=0.375) or interaction effect (P=0.832; Figure 3.8). Similarly, thermal stress showed a main effect of time during the performance task (P<0.05), but no main effect of trial (P=0.310) or trial x time interaction (P=0.092).

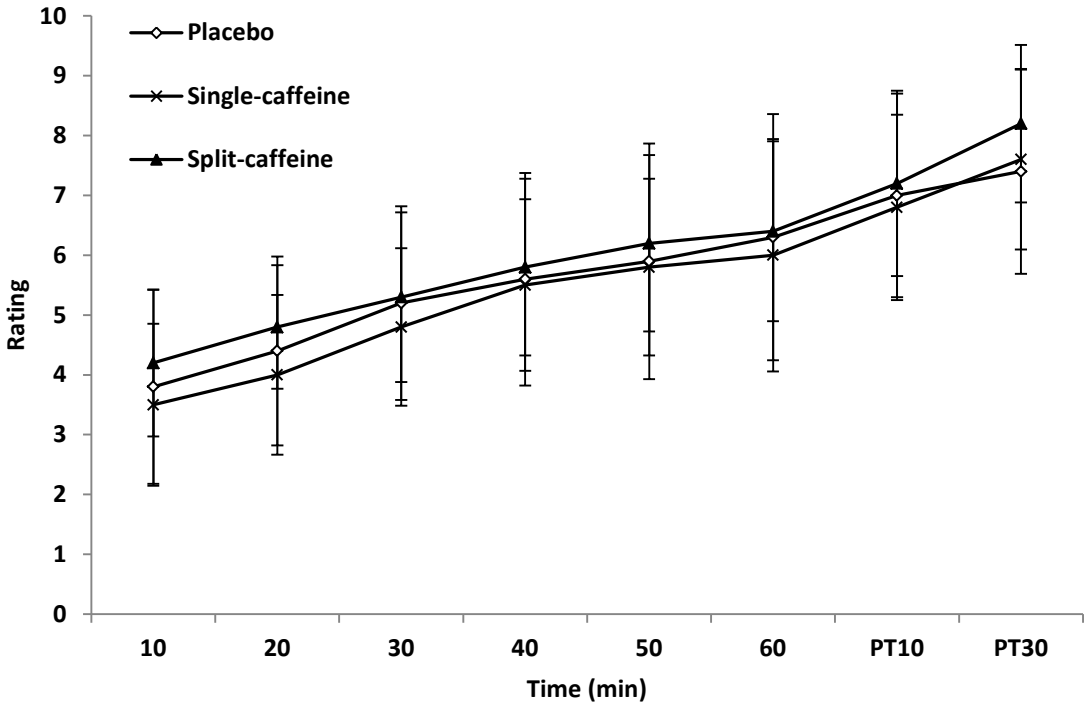

Figure 4.8 Perceived thermal stress during exercise. PT, performance test. Values are mean ± SD.

There was a main effect of trial for sweat rate (P<0.05). Higher values were recorded during the single-caffeine (20.8 ± 3.6 mL·min^{-1}; P=0.029) and split-caffeine trial (21.6 ± 4.4 mL·min^{-}1; P=0.001) compared with placebo (19.1 ± 4.7 mL·min^{-1}). No difference was observed between the two caffeine trials (P=0.213).

Pre-exercise heart rate was similar across trials (P=0.180; Figure 4.9). There was a main effect of time for heart rate during the fixed-intensity exercise (P<0.05), but no main effect of trial (P=0.486) or trial x time interaction (P=0.827). Heart rate showed a main effect of time (P<0.05), trial (P=0.007) and an interaction effect (P=0.031) during the performance task. Higher values were recorded from 5 to 20 min during the split-caffeine trial compared with single-caffeine (P<0.05). Additionally, higher values were recorded throughout the performance task during the split-caffeine trial compared with placebo (P<0.05; Figure 4.9).

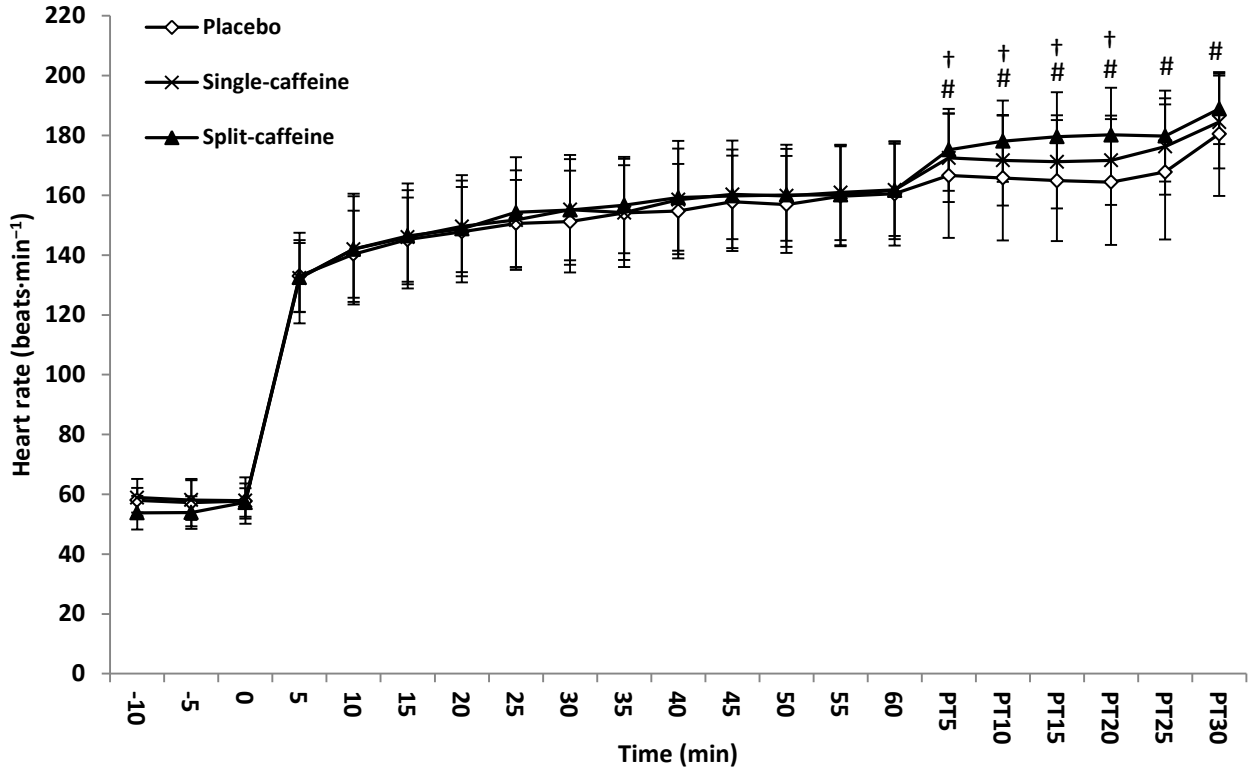

Figure 4.9 Heart rate during the experimental trials. Split-caffeine greater than single-caffeine and placebo is denoted as † and #, respectively. All notations are *P*<0.05. PT, performance test. Values are mean ± SD

Carbohydrate oxidation did not show a main effect of time (*P*=0.717), trial (*P*=0.475), nor an interaction effect (*P*=0.642; Table 4.1). There was a main effect of time for fat oxidation (*P*=0.026), but no main effect of trial (*P*=0.463) or interaction effect (*P*=0.591). No main effect of time (*P*=0.277), trial (*P*=0.492), or trial x time interaction (*P*=0.408) was observed for RER. Oxygen uptake showed a main effect of time (*P*<0.05), with the highest values at the end of the fixed-intensity exercise across all trials (*P*<0.05; Table 4.1). No main effect of trial (*P*=0.928) or interaction effect (*P*=0.971) occurred.

Table 4.1 Substrate oxidation and oxygen uptake during the fixed-intensity exercise

	Treatment	15	30	45	60
CHO ox (g·min^{-1})	**Placebo**	2.09 ± 0.64	2.23 ± 0.69	2.28 ± 0.69	2.32 ± 0.61
	Single-caffeine	2.28 ± 0.55	2.42 ± 0.57	2.25 ± 0.75	2.29 ± 0.61
	Split-caffeine	2.48 ± 0.52	2.47 ± 0.37	2.41 ± 0.38	2.42 ± 0.44
Fat ox (g·min^{-1})	**Placebo**	0.42 ± 0.19	0.42 ± 0.24	0.43 ± 0.19	0.45 ± 0.21
	Single-caffeine	0.37 ± 0.17	0.35 ± 0.16	0.43 ± 0.23	0.47 ± 0.22
	Split-caffeine	0.28 ± 0.23	0.34 ± 0.14	0.36 ± 0.15	0.41 ± 0.17
RER	**Placebo**	0.89 ± 0.05	0.90 ± 0.06	0.90 ± 0.05	0.90 ± 0.05
	Single-caffeine	0.91 ± 0.05	0.91 ± 0.04	0.90 ± 0.06	0.89 ± 0.05
	Split-caffeine	0.93 ± 0.05	0.92 ± 0.03	0.91 ± 0.03	0.90 ± 0.04
VO2 (L·min^{-1})	**Placebo**	2.40 ± 0.28	2.50 ± 0.29	2.56 ± 0.30	2.62 ± 0.33*
	Single-caffeine	2.43 ± 0.32	2.51 ± 0.36	2.55 ± 0.37	2.64 ± 0.38*
	Split-caffeine	2.42 ± 0.27	2.52 ± 0.28	2.53 ± 0.27	2.63 ± 0.27*

CHO ox, carbohydrate oxidation; Fat ox, fat oxidation; RER, respiratory exchange ratio; VO2, oxygen uptake. *denotes significantly greater (P<0.05) than all previous time points. Values are mean ± SD.

4.5 Discussion

The current study examined the performance and thermoregulatory responses to exercise in high ambient temperature after supplementation with 3 mg·kg^{-1} of caffeine administered either as a single-or split-dose (2 x 1.5 mg·kg^{-1}) before exercise. The results of the present investigation demonstrate that endurance performance was enhanced when caffeine was ingested both as a single-dose (d=0.42; P=0.029; 90.2% chance of benefit) and split-dose (d=0.67; P<0.05; 99.6% chance of benefit) compared with placebo. Furthermore, dividing the caffeine bolus further enhanced performance than single-dose administration (d=0.32; P=0.059; 78.5% chance of benefit). Additionally, neither caffeine-ingestion strategy adversely influenced core or skin temperature during exercise at a fixed work-rate (Figure 4.3).

The performance benefit observed during the single-caffeine trial confirms the results of a recent investigation (Pitchford et al. 2014). Participants in this study completed a predetermined amount of work 7% quicker than placebo when 3 mg·kg^{-1} of caffeine was ingested prior to cycle exercise in the heat. Moderate to large caffeine doses (i.e. 5-9 mg·kg^{-1}) provide less consistent performance benefits during exercise in warm conditions.

Caffeine at 6 mg·kg^{-1} enhanced performance during a 15 min performance task (Ganio et al. 2011) and increased force production of the quadriceps after prolonged exercise (Del Coso et al 2008). However, 6 mg·kg^{-1} had no effect on 30 min TT performance (Roelands et al. 2011) and 9 mg·kg^{-1} did not influence 15 min cycle performance (Cheuvront et al. 2009) or 21 km run time (Cohen et al. 1996). Hence, supplementation with single low-doses (~3 mg·kg^{-1}) may prove a better strategy to enhance performance in the heat than larger single-doses.

The mechanism of action of caffeine has not been fully elucidated. Microdialysis studies in rodents demonstrate that caffeine prolongs physical activity in association with increased synaptic dopamine concentrations (Solinas et al. 2002; Zheng et al. 2014). However, large doses (10-30 mg·kg^{-1}) administered via intraperitoneal injection are required to induce this response (Solinas et al. 2002; Zheng et al. 2014). Such doses are larger than necessary to enhance performance (3 mg·kg^{-1}; Desbrow et al. 2012) and higher than typically consumed by athletes during competition (Desbrow and Leveritt 2006). Serum prolactin was measured to provide an indirect assessment of central dopaminergic activity, with a decrease in prolactin suggesting an increase in dopamine (Ben-Jonathan and Hnasko 2001). The similar values across trials (Figure 4.5B) suggest caffeine failed to influence dopamine release. While only a peripheral measure, supplementation with L-DOPA, a drug which augments dopamine release in the human brain (Floel et al. 2008), attenuates circulating prolactin after prolonged exercise in warm conditions (Cordery et al. 2016). Importantly, a recent PET study demonstrated that oral caffeine supplementation (300 mg) failed to influence *in vivo* dopamine release in the human brain (Volkow et al. 2015). Alternatively, caffeine likely enhances performance through changes in adenosine-dopamine receptor-binding and intracellular signalling (Ferré, 2008; Lindskog et al. 2002).

An interesting finding of the present study was that dividing the caffeine bolus conferred an even greater performance benefit than single-dose administration, despite both trials providing 3 mg kg^{-1}. While this was not statistically significant (P=0.059), the small increase (d=0.32) represents a 78.5% chance of benefit, with a 0.3% likelihood of harm. Cox et al (2002) reported similar performance benefits when 6 mg·kg^{-1} caffeine was administered as a single dose before exercise compared with six 1 mg·kg^{-1} doses provided every 20 min during exercise. Conway et al (2003) also reported a similar performance benefit when 6

mg·kg^{-1} caffeine was ingested either as a single bolus or two 3 mg·kg^{-1} doses. The contrast between these studies and the present investigation might be due to differences in the ambient temperature, as prolonged exercise in the heat could provide conditions more suitable for repeated caffeine doses to provide an additional performance benefit.

Given the ergogenic effect of caffeine is due to central blockade of adenosine receptors (Ferré, 2008), perhaps the additive benefit during the split-caffeine trial is mediated by changes in adenosine receptor sensitivity during exercise. To date, no study has investigated the influence of heat stress and exercise on adenosine receptor function. However, a single bout of exercise increased the adenosine receptor-mediated response to insulin in rat soleus muscle (Langfort et al. 1993), while exercise and adenosine receptor antagonists interact to regulate antioxidant responses in rat cardiac tissue (Husain and Somani, 2005). Furthermore, single versus repeated injections of cocaine differentially influenced calcium signalling and protein phosphorylation in rat striatum (Kim et al. 2009). In striatal cells, the concentration of intracellular calcium regulates the function of the A_{2A}-D_2 heteromer (Navarro et al. 2014), a primary target of caffeine (Ferré, 2008). Thus, any change to calcium signalling within these neurons would likely influence adenosine receptor sensitivity and consequently the response to repeated caffeine doses.

Caffeine can influence the core temperature response to prolonged exercise in the heat. Supplementation with a single 6 mg·kg^{-1} dose induced a greater increase in core temperature than placebo during prolonged exercise at a fixed work-rate (Roelands et al. 2011). Additionally, a single 9 mg·kg^{-1} caffeine dose resulted in a modest increase in core (Cheuvront et al. 2009) and body temperature (Ely et al. 2011) during prolonged exercise. However, Ganio et al (2011) failed to report a caffeine-induced increase in core temperature after dividing a 6 mg·kg^{-1} bolus into two 3 mg·kg^{-1} doses. Therefore, the splitting of the caffeine bolus by Ganio et al (2011) might have distributed the thermogenic properties of the drug. Given there was no difference in core temperature between the two caffeine trials in the present study (Figure 4.3A), perhaps the diminished thermogenic properties from dividing the caffeine bolus only occur after intakes of moderate to large doses (i.e. 6-9 mg kg^{-1}).

Caffeine ingestion enhanced sweat-electrolytes losses during prolonged exercise in the heat (Del Coso et al. 2009). While higher sweat rates were recorded during both caffeine trials compared with placebo, this was likely due to the increased exercise intensity during the performance task and the resulting elevation in core temperature. When exercise is performed at a fixed-work rate, caffeine does not influence sweat rate in temperate or warm environmental conditions (Ganio et al. 2011). Even chronic supplementation (11 days) with low and moderate doses (3 and 6 $mg \cdot kg^{-1}$) did not influence fluid, electrolyte or renal indices of hydration (Armstrong et al. 2005). Therefore, body water losses attributable to caffeine appear to be of a similar magnitude as plain water (Maughan and Griffin, 2003).

In conclusion, the present study demonstrates that supplementation with a 3 mg kg^{-1} caffeine bolus ingested as a single-or split-dose before exercise improved endurance performance in high ambient temperature. Furthermore, dividing the caffeine bolus conferred a greater benefit than single-dose administration. This response appeared to be mediated by the gradual increase in serum caffeine during the split-dose trial, but more work is required to confirm these initial findings. Given that caffeine failed to influence thermoregulation during exercise, future studies should examine the thermoregulatory and performance effects of larger caffeine intakes (i.e. 6-9 mg kg^{-1}), divided into smaller doses. This will enable dosing strategies to be developed with the aim of enhancing endurance performance in the heat, without the previously reported deleterious effects on thermoregulation and fluid-balance.

Chapter 5

Effect of a Moderate Caffeine Dose on Endurance Cycle Performance and Thermoregulation During Prolonged Exercise in the Heat

5.1 Abstract

This study investigated the influence of a moderate caffeine dose on endurance cycle performance and thermoregulation during prolonged exercise in high ambient temperature. Eight healthy, recreationally active males (Mean ± SD; age: 22 ± 1 y; body mass: 71.1 ± 8.5 kg; VO_{2peak}: 55.9 ± 5.8 mL·kg^{-1}·min^{-1}; W_{max}: 318 ± 37 W) completed one VO_{2peak} test, one familiarisation trial and two experimental trials. After an overnight fast, participants ingested a placebo or a 6 mg·kg^{-1} caffeine dose 60 min before exercise. The exercise protocol consisted of 60 min of cycle exercise at 55% W_{max}, followed by a 30 min performance task (total kJ produced) in 30^{o}C and 50% RH. Performance was enhanced (Cohen's *d* effect size=0.22) in the caffeine trial (363.8 ± 47.6 kJ) compared with placebo (353.0 ± 49.0 kJ; *P*=0.004). Caffeine did not influence core (*P*=0.188) or skin temperature (*P*=0.577) during exercise. Circulating prolactin (*P*=0.572), cortisol (*P*=0.842) and the estimated rates of fat (*P*=0.722) and carbohydrate oxidation (*P*=0.454) were also similar between trial conditions. Caffeine attenuated perceived exertion during the initial 60 min of exercise (*P*=0.033), with no difference in thermal stress across trials (*P*=0.911). Caffeine supplementation at 6 mg·kg^{-1} improved endurance cycle performance in a warm environment, without differentially influencing thermoregulation than placebo during prolonged exercise at a fixed work-rate. Therefore, moderate caffeine doses which typically enhance performance in temperate environmental conditions also appear to benefit endurance performance in the heat.

5.2 Introduction

Caffeine is a well-established ergogenic aid commonly consumed by endurance athletes (Desbrow and Leveritt, 2006). Supplementation with low to moderate doses (3-6 $mg \cdot kg^{-1}$) consistently enhance performance in temperate environmental conditions (~$20^{o}C$), especially when exercise is performed for 30 min or longer (Ganio et al. 2009). However, few studies have investigated the potential for caffeine to improve endurance performance in the heat. Laboratory-based investigations demonstrate that caffeine does (Del Coso et al. 2008; Ganio et al. 2011), but not always (Chevront et al. 2009; Roelands et al. 2011), improve performance in high ambient temperature. Additionally, race performance in hot field conditions was not influenced by caffeine (Cohen et al. 1996). Hence, from the limited data available, it is unclear whether caffeine benefits endurance performance in the heat, despite a high prevalence of intake among athletes competing in warm environments (Desbrow and Leveritt, 2006).

The progressive impairment in endurance capacity with increasing ambient temperature is well-documented (Galloway and Maughan, 1997). Several explanations for this deterioration in performance have been proposed, including an increased physiological burden to dissipate heat via the skin and an increase in core temperature (Sawka et al. 2012). The resulting hyperthermia and increased brain temperature reduce central drive to continue exercise, thus precipitating the onset of fatigue (Nybo, 2010). During prolonged exercise in the heat, caffeine has elicited higher core temperatures than placebo (Chevront et al. 2009; Ely et al. 2011; Roelands et al. 2011). Consequently, these perturbations to thermoregulation might explain the lack of performance benefit in the heat after caffeine intake (Roelands et al 2011). Interestingly, larger caffeine doses (≥ 9 $mg \cdot kg^{-1}$) consistently induce elevations in core and body temperature during exercise in the heat (Chevront et al. 2009; Ely et al. 2011). Hence, the provision of smaller doses (6 $mg \cdot kg^{-1}$), which typically improve performance in temperate conditions (Desbrow et al. 2012; Ganio et al. 2009), might prove a more useful strategy to enhance performance in the heat. As lower caffeine intakes benefit endurance cycle performance in high ambient conditions (Pitchford et al. 2014; chapter 4), the influence of moderate caffeine doses warrant investigation.

Supplementation with 6 mg·kg^{-1} caffeine enhanced maximal voluntary contraction of the quadriceps after prolonged cycle exercise in a hot (36^{o}C) environment (Del-Coso et al. 2008). However, during exercise under the same environmental conditions, the same caffeine dose co-administered with carbohydrates elicited a higher core temperature than isolated carbohydrate intake (Del-Coso et al. 2008). To date, only two laboratory-based studies have examined the influence of 6 mg·kg^{-1} caffeine on endurance cycle performance without additional carbohydrates (Ganio et al. 2011; Roelands et al. 2011). Roelands et al (2011) reported no ergogenic effect of caffeine but an increase in core temperature during prolonged exercise at a fixed work-rate, while Ganio et al (2011) observed an improvement in endurance cycle performance but no thermogenic effects. Hence, it is unclear whether moderate caffeine doses influence endurance cycle performance or thermoregulation during prolonged exercise in high ambient temperature. Given the widespread intake of caffeine by athletes (Desbrow and Leveritt, 2006), it would be of interest to determine whether moderate doses which consistently enhance performance in temperate conditions (Desbrow et al. 2012; Ganio et al. 2009), also confer performance benefits in the heat.

Consequently, the aim of this study was to examine the performance and thermoregulatory responses to prolonged exercise in the heat following the ingestion of a 6 mg·kg^{-1} caffeine dose versus a placebo condition. Given the findings from chapter 4, it is hypothesised that caffeine will improve cycle performance

5.3 Methods

Eight healthy, low-caffeine consuming men (116 ± 46 mg·day^{-1}; age: 22 ± 1 y; body mass: 71.1 ± 8.5 kg; height: 1.74 ± 0.08 m; VO_{2peak}: 55.9 ± 5.8 mL·kg^{-1}·min^{-1}; W_{max}: 318 ± 37 W) took part in this investigation, which employed a double-blind, placebo controlled, randomised, cross-over design. All participants completed one maximal exercise test, one familiarisation trial and two experimental trials. The initial visit consisted of an incremental exercise test to volitional exhaustion conducted on an electronically braked cycle ergometer (Lode Corival, Groningen, Holland) to determine W_{max} and the power required to elicit 55% and 75% of W_{max}. This test was performed in temperate conditions (~20^{o}C). After 5-7 days, participants completed a familiarisation trial to minimise any learning or anxiety effects. This

visit was performed in 30°C and 50% RH and was identical to the experimental trials in all respects, although no treatment was administered.

The trial overview is illustrated in figure 5.1. The pre-trial dietary and activity standardisation requirements are outlined in chapter 3.5. No caffeine was permitted during the 24 hours before the familiarisation and experimental trials. On the evening before each trial, participants ingested a radio-telemetry pill (CoreTemp, HQ Inc, Palmetto, Florida, USA) to enable the measurement of core temperature. Upon arrival at the laboratory (7-9am), post-void nude body mass was recorded. After a brief period of seated rest (15 min), a 21-g cannula was introduced into an antecubital vein. A baseline venous sample was collected (7mL) before participants ingested a capsule containing 6 $mg \cdot kg^{-1}$ of anhydrous caffeine (Sigma-Aldrich, UK) or a placebo (BDH Ltd, Poole, UK) with 50 mL of plain water. All capsules were indistinguishable with regards to dimension, weight and colour. Next, a heart rate telemetry band was positioned (Polar Beat, Kempele, Finland) and skin surface thermistors (Grant Squirrel SQ800, Cambridgeshire, UK) were attached to four sites (chest, upper arm, thigh and calf) to calculate weighted mean skin temperature (Ramanathan, 1964). Following a 45 min period of seated rest in 20°C, core and skin temperature and heart rate were recorded at 5 min intervals. A second 7 mL venous sample was collected 60 min post-capsule ingestion while participants remained in a seated position.

Participants then entered the climatic chamber (Weiss-Gallenkamp, UK) maintained at 30°C and 50% RH and began 60 min of cycle exercise at a workload corresponding to 55% W_{max}. During this period, core and skin temperature and heart rate were recorded every 5 min. RPE (Borg, 1982) and perceived thermal stress (using a 21 point scale ranging from -10, unbearable cold, to +10, unbearable heat) were recorded every 10 min. Expired air samples (1 min) were collected every 30 min to calculate the rates of substrate oxidation during exercise (Peronnet and Massicotte, 1991). Participants were provided with 150 mL of plain water every 15 min and a third venous sample (7mL) was collected at 60 min while participants remained seated on the ergometer.

Subsequently, there was a 2-3 min delay while the ergometer was programmed for the performance task. Participants were instructed to produce as much work (kJ) as possible within 30 min. The initial workload was set at 75% W_{max}, but participants were free to adjust

their power output as desired from the outset. Core and skin temperature and heart rate were recorded every 5 min. A final 7 mL venous sample was collected immediately after the performance task while participants remained seated on the ergometer. Participants then returned to a temperate environment (20^{o}C) where the cannula, telemetry band and skin thermistors were removed. Nude body mass was then recorded. The change in body mass, corrected for fluid intake, was used to estimate sweat rate.

A small volume of whole blood (2 mL) was used to determine plasma glucose, Hb, and Hct. The remaining 5 mL was used to yield serum for the subsequent determination of cortisol and prolactin with ELISA (DRG diagnostic, Germany) and caffeine with reverse-phase HPLC (Holland et al. 1999) as described in chapter 3.7. The intra-assay CV for serum prolactin, cortisol, and caffeine was 4.8%, 4.1%, and 2.1%, respectively.

All data were analysed using IBM SPSS statistics version 22.0. To determine whether the outcome variables had a normal distribution, the Shapiro-Wilk test was used. Exercise performance, pre-exercise nude body mass, initial core temperature, fasting plasma glucose, and estimated sweat rates were examined using a paired *t*-test. Cohen's *d* effect size for differences in total work produced during the performance task was determined ([mean 1 - mean 2]/pooled SD) and interpreted as trivial (0-0.19), small (0.2-0.49), medium (0.5-0.79) or large (≥ 0.8) as previously described (Cohen, 1992). Variables measured throughout each trial were examined with a two-way (trial x time) repeated-measures ANOVA. The Greenhouse-Geisser correction was applied where the assumption of sphericity had been violated. Where a significant main effect or interaction was identified, Bonferroni adjusted paired *t*-tests for normally distributed data or Bonferroni adjusted Wilcoxon Signed Rank tests for non-normally distributed data were used. Data are presented as means ± SD throughout. Statistical significance was accepted at $P<0.05$.

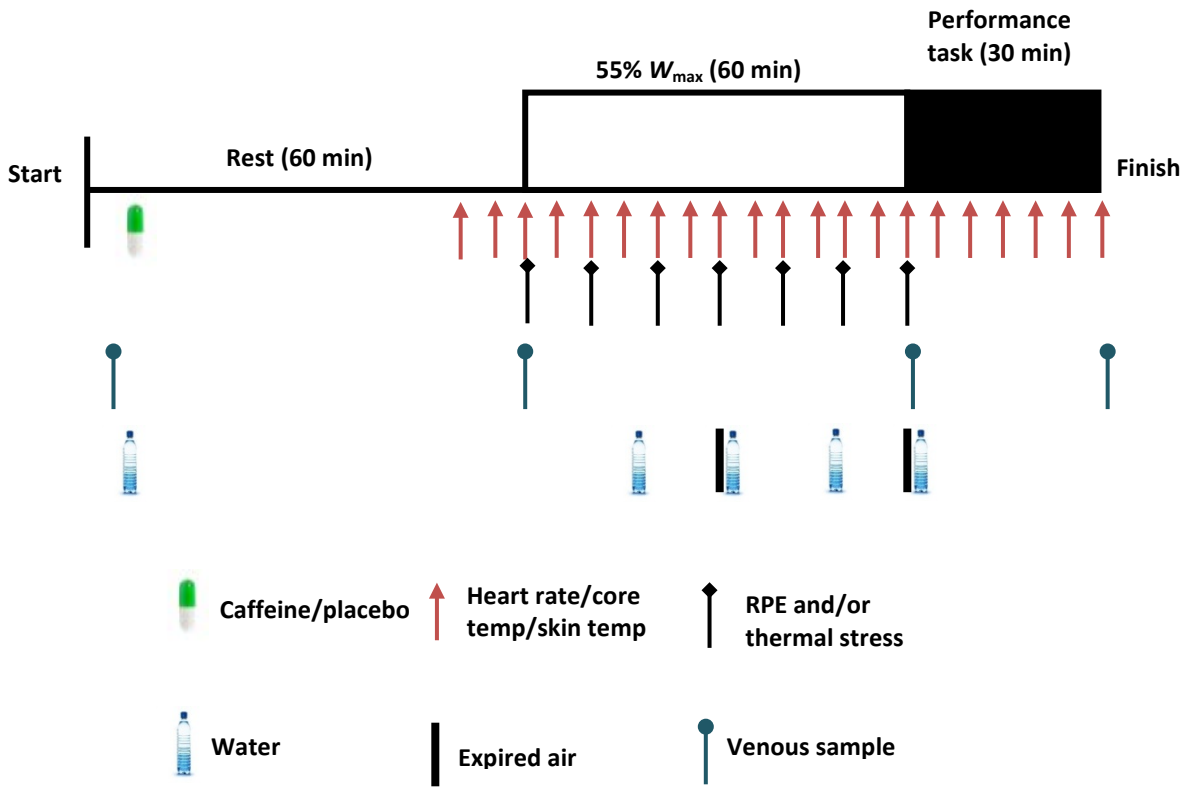

Figure 5.1 Trial overview

5.4 Results

Pre-exercise nude body mass (P=0.732), initial core temperature (P=0.279) and fasting plasma glucose (P=0.454) were not different between trials, suggesting that participants began each trial in a similar physiological state.

All eight participants completed both trials, no adverse effects were reported. There was a small increase (d=0.22) in total work produced during the caffeine trial (363.8 ± 47.6 kJ) compared with placebo (353.0 ± 49.0 kJ; P=0.004). This represents a percentage increase in performance of 3.0 ± 2.3% (range: -0.4 to 7.1%; Figure 5.2).

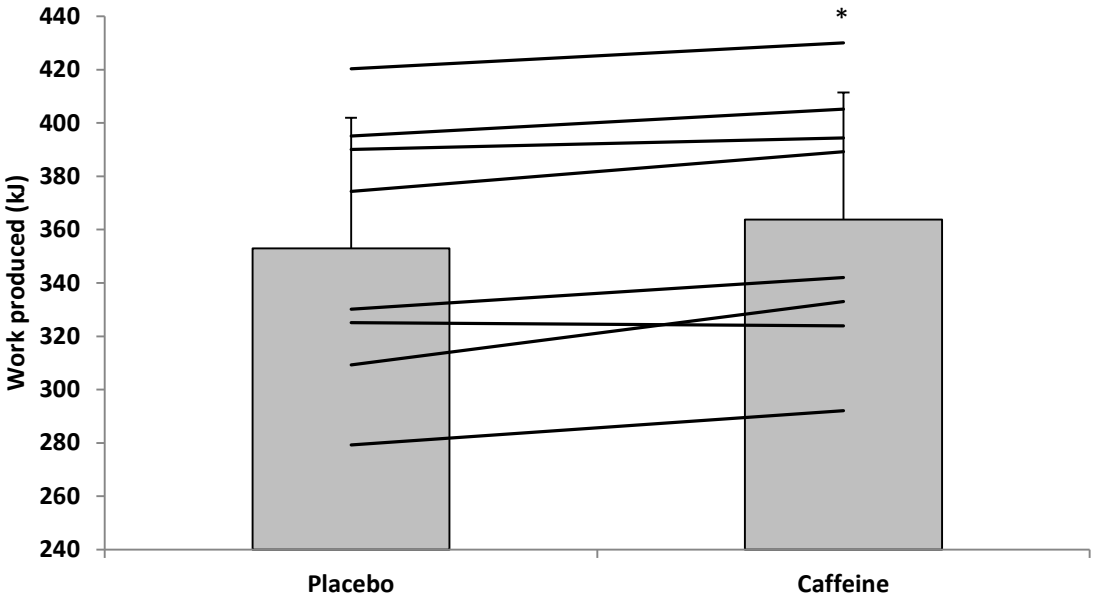

Figure 5.2 Mean (bars) and individual (lines) work produced (kJ) during the performance task. *denotes caffeine greater than placebo (*P*=0.004). Values are mean ± SD.

Pre-exercise core temperature was similar between trials (*P*=0.718; Figure 5.3A). During the initial 60 min of exercise core temperature showed a main effect of time (*P*<0.05), but no main effect of trial (*P*=0.188) or trial x time interaction (*P*=0.112). Core temperature showed main effects of time (*P*<0.05) and trial (*P*=0.006), as well as an interaction effect (*P*=0.005) during the performance task. Higher values were recorded from 20 to 30 min during the caffeine trial compared with placebo (*P*<0.05; Figure 5.3A). Pre-exercise skin temperature was similar between trials (*P*=0.429; Figure 5.3B). During the initial 60 min of exercise, there was a main effect of time (*P*<0.05), but no main effect of trial (*P*=0.648) or trial x time interaction (*P*=0.219). Similarly, during the performance task skin temperature showed a main effect of time (*P*<0.05), but no main effect of trial (*P*=0.970) or interaction effect (*P*=0.311; Figure 5.3B).

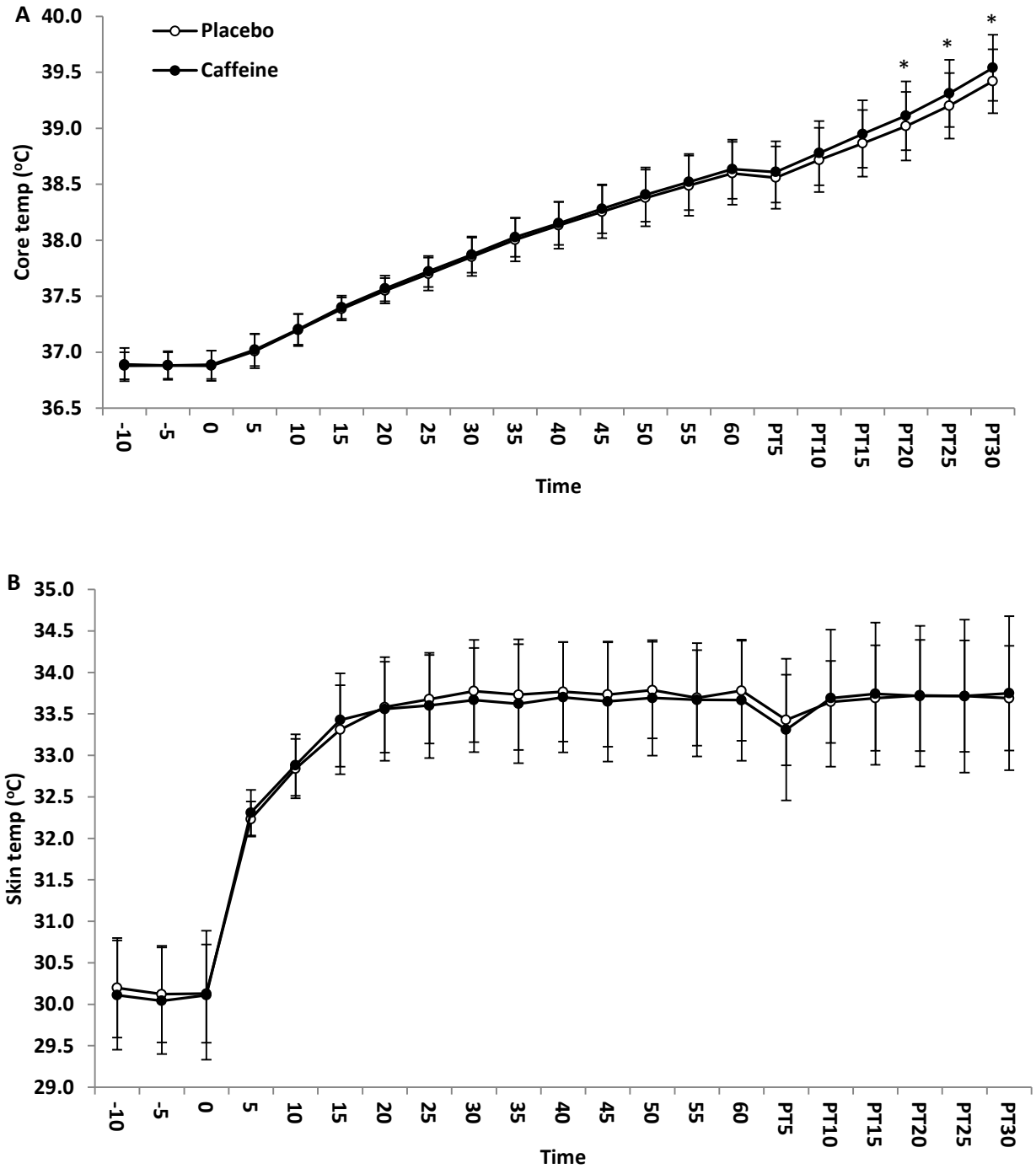

Figure 5.3 Core (A) and skin (B) temperature during the experimental trials. *denotes caffeine greater than placebo (P<0.05). PT, performance test. Values are mean ± SD.

Pre-exercise heart rate was not different between trials (P=0.240; Figure 5.4). There was a main effect of time for heart rate during the initial 60 min of exercise (P<0.05), but no main effect of trial (P=0.644) or trial x time interaction (P=0.320). During the performance task heart rate showed a main effect of time (P<0.05) and trial (P=0.011), but no interaction effect (P=0.904; Figure 5.4).

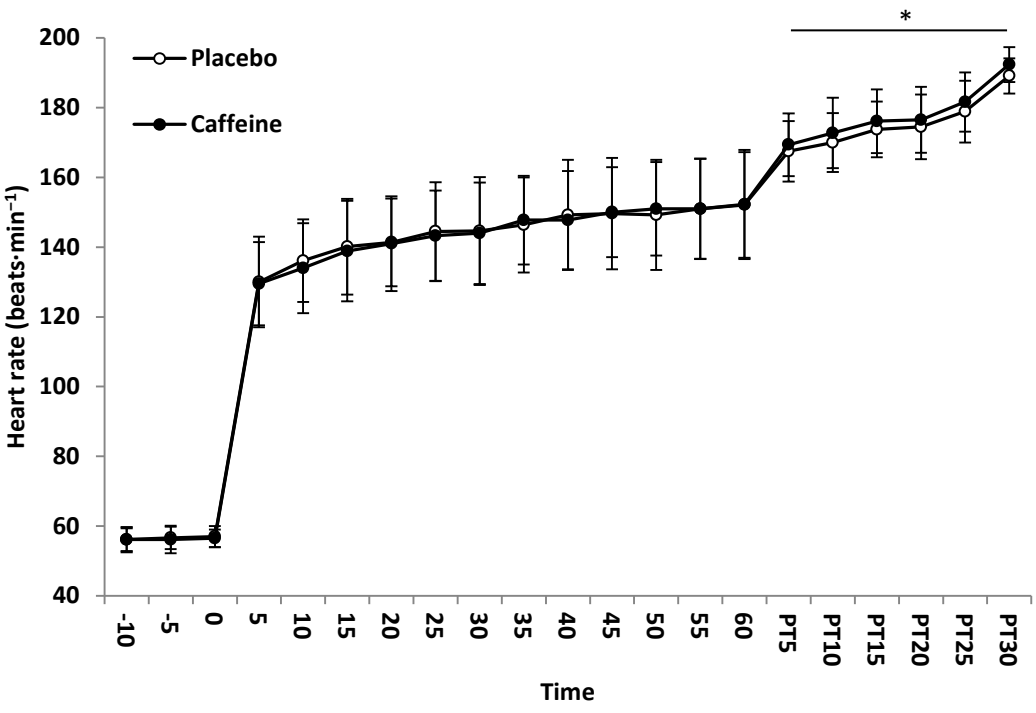

Figure 5.4 Heart rate during the experimental trials. *denotes caffeine greater than placebo (P=0.011). PT, performance test. Values are mean ± SD.

During exercise RPE showed main effects of time (P<0.05) and trial (P=0.033), but no interaction effect (P=0.662; Figure 5.5).

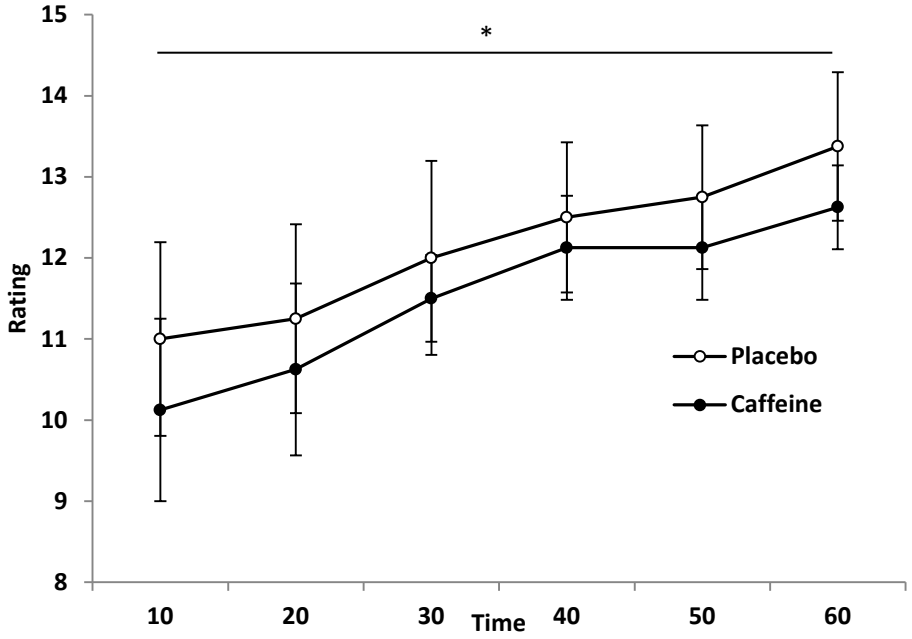

Figure 5.5 Perceived exertion during the fixed-intensity exercise. *denotes placebo greater than caffeine (*P*=0.033). Values are mean ± SD.

Perceived thermal stress showed a main effect of time (*P*<0.05), but no main effect of trial (*P*=0.829) or interaction effect (*P*=0.253; Figure 5.6).

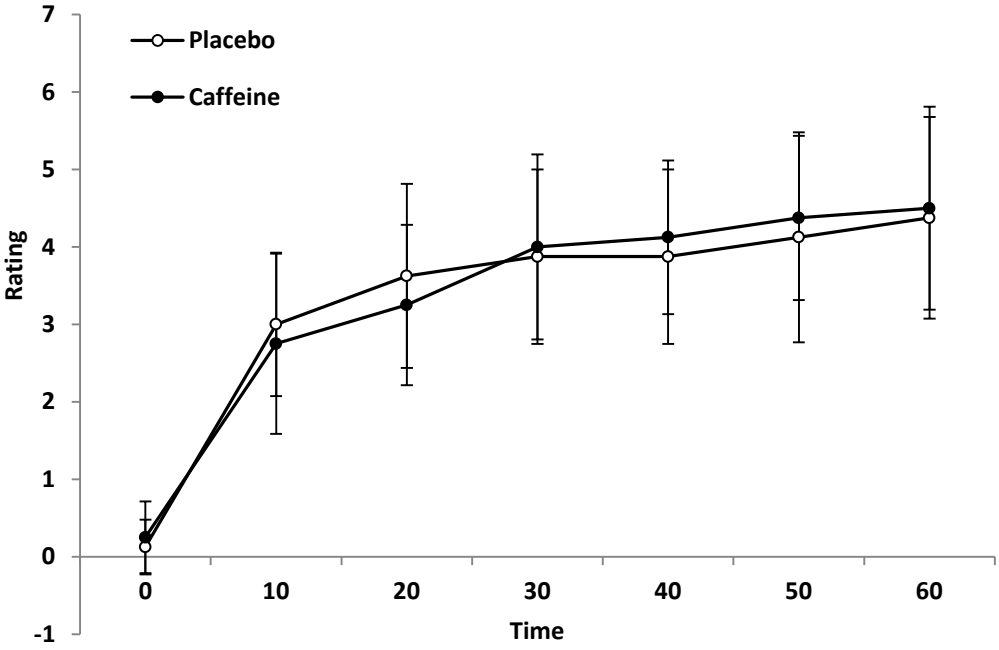

Figure 5.6 Perceived thermal stress at rest and during the fixed-intensity exercise. Values are mean ± SD.

Caffeine concentrations remained below the limit of quantification during the placebo trial and for the baseline sample during the caffeine trial. Circulating concentrations peaked 60 min post-caffeine ingestion, with values remaining constant throughout exercise (Figure 5.7). No pair-wise differences were identified from 60 to 150 min post-ingestion (*P*>0.279).

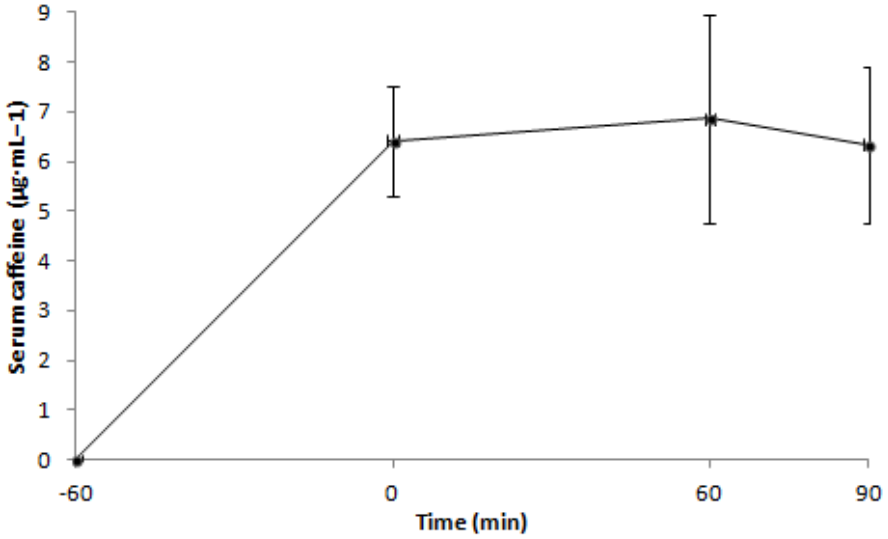

Figure 5.7 Serum caffeine concentrations. Values are mean ± SD.

Serum cortisol showed a main effect of time (*P*<0.05), but no main effect of trial (*P*=0.842) or trial x time interaction (*P*=0.148; Figure 5.8).

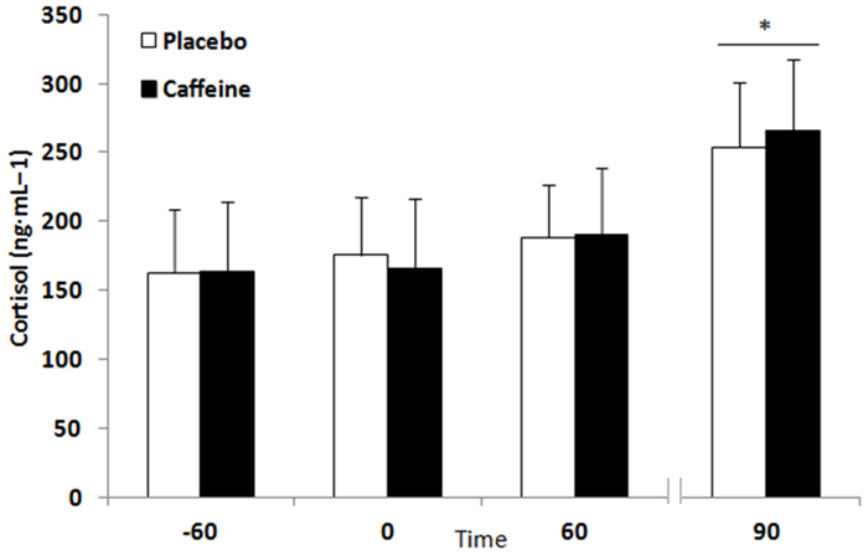

Figure 5.8 Circulating cortisol during the experimental trials. *denotes a significant difference from the -60 value (*P*<0.05). Values are mean ± SD.

There was a main effect of time for serum prolactin (P<0.05), but no main effect of trial (P=0.572) or interaction effect (P=0.551; Figure 5.9).

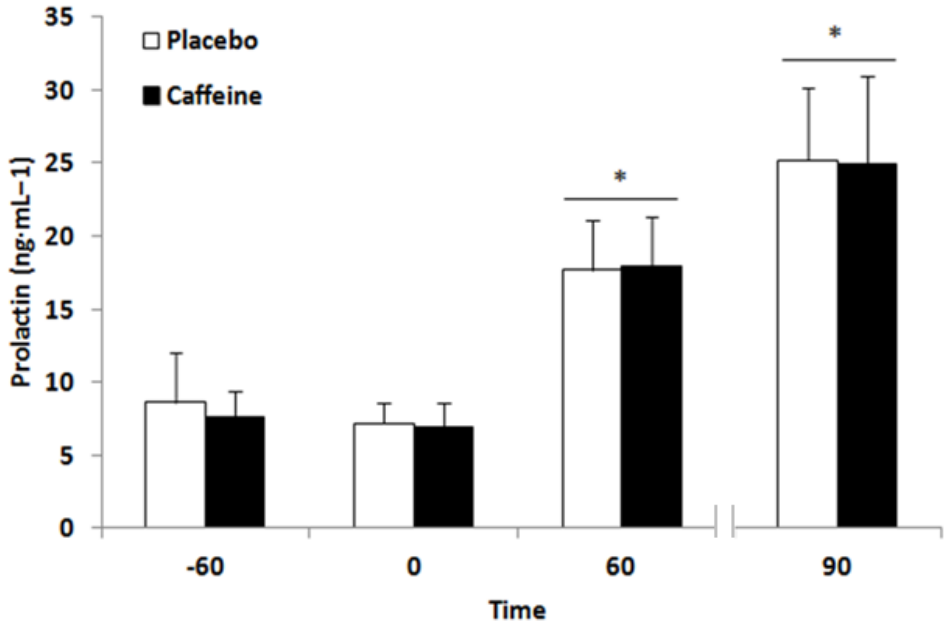

Figure 5.9 Circulating prolactin during the experimental trials. *denotes a significant difference from the -60 value (P<0.05). Values are mean ± SD.

Plasma glucose showed a main effect of time (P<0.05), but no main effect of trial (P=0.068) or trial x time interaction (P=0.176; Figure 5.10). Similarly, the percentage change to blood and plasma volumes both showed main effects of time (P<0.05), but no main effects of trial (P>0.056) or interactions effects (P>0.111) occurred.

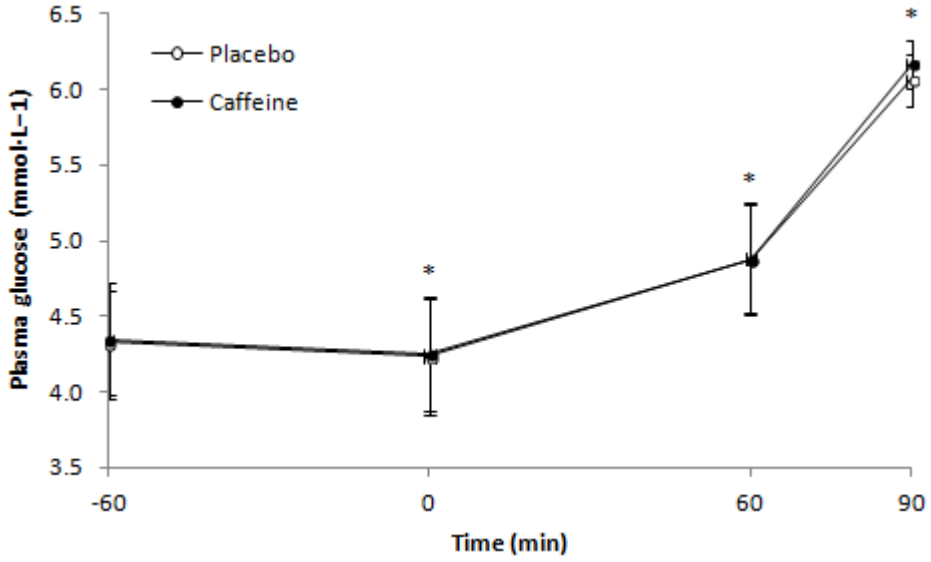

Figure 5.10 Plasma glucose during the experimental trials. *denotes a significant difference (*P*<0.05) from -60. Values are mean ± SD.

There were no main effect of time (*P*>0.363), trial (*P*>0.454), or interaction effects (*P*>0.410) for fat and carbohydrate oxidation or RER (Table 5.1). Oxygen uptake showed a main effect of time (*P*=0.001), but no main effect of trial (*P*=0.361) or trial x time interaction (*P*=0.188; Table 5.1).

Table 5.1 Substrate oxidation and oxygen uptake during the fixed-intensity exercise

	Treatment	30	60
CHO ox (g·min^{-1})	**Placebo**	2.27 ± 0.29	2.24 ± 0.31
	Caffeine	2.28 ± 0.30	2.34 ± 0.42
Fat ox (g·min^{-1})	**Placebo**	0.27 ± 0.10	0.32 ± 0.13
	Caffeine	0.28 ± 0.14	0.28 ± 0.21
RER	**Placebo**	0.93 ± 0.02	0.92 ± 0.02
	Caffeine	0.93 ± 0.03	0.93 ± 0.04
VO2 (L·min^{-1})	**Placebo**	2.22 ± 0.30	2.30 ± 0.31*
	Caffeine	2.26 ± 0.32	2.30 ± 0.30*

CHO ox, carbohydrate oxidation; Fat ox, fat oxidation; RER, respiratory exchange ratio; VO2, oxygen uptake. *denotes significantly greater (*P*<0.05) than 30 min. Values are mean ± SD.

Estimated sweat rates were slightly higher during the caffeine trial (25.7 ± 4.8 mL·min^{-1}) than placebo (24.5 ± 4.1 mL·min^{-1}; *P*=0.034).

5.5 Discussion

This study investigated the performance and thermoregulatory effects of a 6 mg·kg^{-1} caffeine dose during prolonged exercise in the heat. This caffeine dose consistently improves endurance performance in temperate environmental conditions (Desbrow et al. 2012; Ganio et al. 2009), yet there are conflicting reports when exercise is performed in the heat (Ganio et al. 2011; Roelands et al. 2011). In the study by Roelands et al (2011), caffeine failed to enhance performance but increased core temperature during exercise. Conversely, Ganio et al (2011) reported enhanced 15 min cycle TT performance with no difference in core temperature versus placebo. The results of the present study agree with the latter findings, as caffeine provided a small, but significant ergogenic effect (Figure 5.2), with no clear difference in core or skin temperature between trials (Figure 5.3). These findings also support those of chapter 4 and suggest that caffeine intakes of 3-6 mg·kg^{-1} are ergogenic to endurance cycle performance in the heat.

Several studies failed to report performance improvements in the heat after caffeine ingestion (Chenvront et al. 2009; Cohen et al. 1996; Roelands et al. 2011), attributing this response to an elevation in core temperature during exercise (Roelands et al. 2011). However, even large doses of caffeine (9 mg·kg^{-1}) result in only mild thermogenic effects (Chevront et al. 2009; Ely et al. 2011), which is typically undetected by participants (Ely et al. 2011). In addition, five days of controlled caffeine intake (3 and 6 mg·kg^{-1}) did not influence core temperature during exercise in the heat versus placebo (Roti et al. 2006). Alternatively, some researchers suggest that a high environmental temperature might negate the efficacy of caffeine (Chenvront et al. 2009). This study reported no performance benefit in 40^{o}C when 9 mg·kg^{-1} caffeine was ingested before a short exercise protocol (30 min at 50% VO_{2peak} plus a 15 min performance task). The lower environmental temperature and/or caffeine dose employed in the present study might account for these divergent findings. Additionally, 21 km race time in the heat was not influenced by caffeine intakes of 5 or 9 mg·kg^{-1} (Cohen et al. 1996). However, participants in this study became ~4% dehydrated

during exercise, thus it is unknown if caffeine would have enhanced performance if fluid-balance was maintained. When hydration status is controlled across temperate and hot environmental conditions, caffeine still improves cycle performance (Ganio et al. 2011).

The ergogenic effect of caffeine was previously attributed to changes in fat metabolism during exercise, resulting in a glycogen sparring effect (Costill et al. 1978). However, there is compelling evidence caffeine enhances performance via direct actions within the central nervous system (Fredholm et al. 1999). Caffeine increases synaptic dopamine concentrations in exercising rats, although large doses (10-30 $mg \cdot kg^{-1}$) are required to induce this response (Solanis et al. 2002). Using positron emission topography, a low dose of caffeine (300 mg) failed to influence *in vivo* dopamine release in the human brain (Volkow et al. 2015). Attenuated prolactin concentrations suggest an increase in dopamine (Ben-Jonathan and Hnasko, 2001), but similar values were observed across trials (Figure 5.9). Alternatively, caffeine influences key neuronal signaling proteins which mediate increases in physical activity (Lindskog et al. 2002) and potentiates adenosine-dopamine receptor binding in striatum (Ferré, 2008). A reduced perception of effort is a common response to caffeine intake, which might account for approximately 29% of its ergogenic effect (Doherty and Smith, 2005). Participants in the present study reported lower RPE values during the initial hour of exercise after caffeine (Figure 5.5), which is likely mediated by a reduced activity of cortical premotor and motor areas (de Morree et al. 2014).

Supplementation with 6 $mg \cdot kg^{-1}$ caffeine enhanced sweat-electrolyte losses in 37^{o}C (Del-Coso et al. 2009), while 3 $mg \cdot kg^{-1}$ augmented sweat rates during submaximal cycle exercise in 24^{o}C (Kim et al. 2011). In the present study, greater sweat rates were observed during the caffeine trial versus placebo over the entire 90 min period (25.7 ± 4.8 $mL \cdot min^{-1}$ vs 24.5 ± 4.1 $mL \cdot min^{-1}$). However, this small difference likely reflects the higher work rate during the performance task in the caffeine trial and the concomitant elevation in core temperature (Figure 4.3A). During prolonged exercise at a fixed work-rate, caffeine failed to differentially influence fluid-balance, sweat rate and serum osmolality in cool and warm environmental conditions compared with placebo (Ganio et al. 2011). Additionally, there were no differences in fluid, electrolyte, or renal indices of hydration after 5 days of controlled caffeine intake (3 and 6 $mg \cdot kg^{-1}$) versus placebo (Armstrong et al. 2005).

In conclusion, the provision of a 6 $mg \cdot kg^{-1}$ caffeine dose before prolonged exercise in 30°C and 50% RH improved endurance cycle performance in non-heat acclimatised participants, without differentially influencing thermoregulation than placebo. There appeared to be a developing trend for core temperature during the initial 60 min of exercise (interaction effect, P=0.112), suggesting that a longer period of fixed-intensity exercise might enable caffeine to elicit a greater increase in core temperature than placebo during exercise under these conditions. However, the difference at the end of the preload was small (0.03°C, Figure 4.3A), which was also undetected by participants (Figure 5.6). These data, together with previous reports (Ganio et al. 2011), suggest that moderate caffeine doses which typically benefit endurance performance in temperate conditions (Desbrow et al. 2012; Ganio et al. 2009), also improve endurance cycle performance in the heat.

Chapter 6

Chronic Ingestion of a Low Dose of Caffeine Induces Tolerance to the Performance Benefits of Caffeine

6.1 Abstract

This study examined effects of four weeks caffeine supplementation on endurance performance. Eighteen low-habitual caffeine consumers (<75 mg·day^{-1}) were randomly assigned to ingest caffeine (1.5 – 3.0 mg·kg^{-1}day^{-1}; titrated) or placebo for 28 days. Groups were matched for age, body mass, $\dot{V}O_{2peak}$ and W_{max} (P>0.05). Before supplementation, all participants completed one $\dot{V}O_{2peak}$ test, one practice trial and two experimental trials (acute 3 mg·kg^{-1} caffeine [precaf] and placebo [testpla]). During the supplementation period a second $\dot{V}O_{2peak}$ test was completed on day 21 before a final, acute 3 mg·kg^{-1} caffeine trial (postcaf) on day 29. Trials consisted of 60 min cycle exercise at 60% $\dot{V}O_{2peak}$ followed by a 30 min performance task. All participants produced more external work during the precaf trial than testpla, with increases in the caffeine (383.3 ±75 kJ vs. 344.9 ± 80.3 kJ; Cohen's d effect size=0.49; P=0.001) and placebo (354.5 ± 55.2 kJ vs. 333.1 ± 56.4 kJ; d=0.38; P=0.004) supplementation group, respectively. This performance benefit was no longer apparent after four weeks of caffeine supplementation (precaf: 383.3 ± 75.0 kJ vs. postcaf: 358.0 ± 89.8 kJ; d=0.31; P=0.025), but was retained in the placebo group (precaf: 354.5 ± 55.2 kJ vs. postcaf: 351.8 ± 49.4 kJ; d=0.05; P>0.05). Circulating caffeine, hormonal concentrations and substrate oxidation did not differ between groups (all P>0.05). Chronic ingestion of a low dose of caffeine develops tolerance in low-caffeine consumers. Therefore, individuals with low-habitual intakes should refrain from chronic caffeine supplementation to maximise performance benefits from acute caffeine ingestion.

6.2 Introduction

Acute caffeine (1,3,7-trimethylxanthine) supplementation approximately 60 min before exercise improves endurance performance in laboratory-based studies (Burke, 2008). The same occurs in the field (Berglund and Hemmingsson, 1982), leading to its widespread use by athletes during competition (Desbrow and Leveritt, 2006). To determine optimum conditions by which caffeine improves performance, factors such as dose (Desbrow et al. 2012), source (Hodgson et al. 2013), and the timing of intake (Cox et al. 2002) have been investigated. However, habituation to chronic caffeine intake has received less attention (Bell and McLellan, 2002). This is important from a practical standpoint given the high prevalence of daily caffeine intake in the general population (Fitt et al. 2013) and by athletes during competition (Desbrow and Leveritt, 2006).

Caffeine likely improves exercise performance through its role as a non-selective adenosine receptor antagonist (Fredholm et al. 1999). A prominent role for the adenosine A_1 receptor in mediating the acute performance enhancing effects of caffeine has been demonstrated (Snyder et al. 1981). However, more recent studies with adenosine A_{2A} receptor knockout mice confirmed that central blockade of this adenosine receptor isoform is largely responsible for the performance enhancing properties of the drug (El Yacoubi et al. 2000). Chronic caffeine intake influences the concentration of A_1 and A_{2A} receptors in several brain regions (Johansson et al. 1993; Svenningsson et al. 1999). This includes A_{2A} expression in the striatum (Svenningsson et al. 1999), a sub-cortical region essential for coordinating voluntary actions (Tepper et al. 2008). Therefore, it is possible that habituation influences performance benefits typical of acute caffeine supplementation. Data from animal studies support this hypothesis, as chronic exposure to caffeine in the drinking water of rats resulted in tolerance to the performance benefit of a subsequent acute caffeine dose (Karcz-Kubicha et al. 2003). Although these findings have been confirmed in other animal models (Quarta et al. 2004), the doses administered have been large (i.e. 130 $mg \cdot kg \cdot day^{-1}$) and much greater than those typically consumed by the general population (Fitt et al. 2013). Whether the same tolerance develops after habituation to doses typically consumed by the general population is not clear.

The magnitude of performance benefit after an acute 5 mg·kg^{-1} caffeine dose was less pronounced in individuals already habituated to caffeine (>300 mg·day^{-1}) than their caffeine-naive counterparts (Bell and McLellan, 2002). Similar metabolic responses have occurred after an acute caffeine dose in comparisons of low-and high-habitual caffeine users (Bangsbo et al. 1992). However, studies which have employed a period of controlled caffeine intake failed to report any change in metabolism or performance following the habituation protocol. For example, sub-chronic intake (5 days) both of low (3 mg·kg^{-1}) and moderate (6 mg·kg^{-1}) caffeine doses did not influence thermoregulatory or cardiovascular responses during exercise in the heat (Roti et al. 2006). Furthermore, TT performance was similar when individuals received an acute 3 mg·kg^{-1} caffeine dose subsequent to either a four-day habituation (3 mg·kg^{-1}day^{-1}) or withdrawal period (Irwin et al. 2011). Therefore, perhaps a greater duration of supplementation is required before the performance benefit of an acute caffeine dose becomes compromised. To date, no study has systematically evaluated a prolonged period of controlled caffeine intake and its influence on endurance performance. Hence, the aim of this study was to examine the effect of a four-week period of controlled caffeine supplementation on endurance performance.

6.3 Methods

Eighteen healthy, recreationally active men (age: 21 ± 2 y; body mass: 74.1 ± 8.6 kg; stature: 1.75 ± 0.06 m; $\dot{V}O_{2peak}$: 51.4 ± 8.7 ml·kg^{-1}·min^{-1}; W_{max}: 289 ± 46 W) were recruited and completed this study. Habitual caffeine intake was assessed (Addicot et al. 2008) to ensure intake did not exceed 75 mg·day^{-1}. This cut-off point was chosen as it equates to approximately one cup of caffeinated instant coffee (Fitt et al. 2013) and is similar to previous studies (Bell and McLellan, 2002).

The experimental design is illustrated in figure 6.1. All participants attended the laboratory on six occasions. During the initial visit each participant undertook an incremental exercise test to volitional exhaustion on an electronically braked cycle ergometer (Lode Corival, Groningen, the Netherlands) to determine $\dot{V}O_{2peak}$ and the power output required to elicit 60% and 75% $\dot{V}O_{2peak}$. After this visit, each participant completed one familiarisation trial. This was undertaken to ensure that all participants were accustomed to procedures, to

minimise order effects from learning or anxiety and ensure attainment of a maximal effort during the performance task.

After these initial tests, each participant completed one acute caffeine trial (precaf) and one placebo trial (testpla), separated by 5-7 days. Thereafter, participants were randomly assigned to ingest daily doses of caffeine (BDH Ltd, Poole, UK) or starch (placebo: BHD Ltd, Poole, UK) for 28 days. Both supplementation groups were matched for age, height, body mass, $\dot{V}O_{2peak}$ and W_{max} (P>0.05). During the first seven days of supplementation, the caffeine group ingested half of the prescribed caffeine dose (1.5 mg·kg^{-1}) in their morning capsule (7-9 am) followed by a placebo capsule (250 mg starch) in the afternoon (1-3 pm). From days 8 to 28, the caffeine group received the full 3 mg·kg^{-1} dose, equally divided between the morning and afternoon capsules. This titrated approach minimised negative influences of caffeine on daily activities in caffeine-naive individuals (e.g. jitteriness, disturbed sleep etc). The placebo group followed the same pattern of intake, but received starch (250 mg) in both capsules. Participants were instructed to ingest the capsules at the same time of day throughout the supplementation period and compliance was verified by telephone contact, email and in person. Both the placebo and caffeine capsules were visually identical and blinded by an external party not involved in any stage of data collection. A second incremental exercise test was completed on the morning of day 21, before the ingestion of any capsules. This followed the same procedure as the initial visit and was undertaken to account for any potential change in $\dot{V}O_{2peak}$ before the final single-blind acute 3 mg·kg^{-1} caffeine trial on day 29 (postcaf).

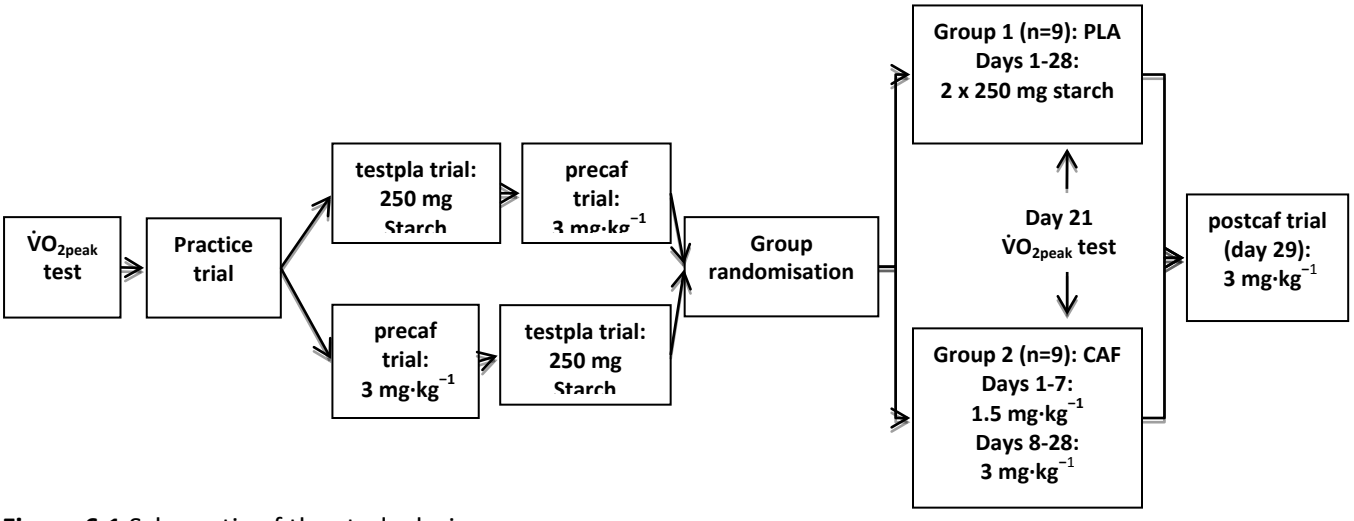

Figure 6.1 Schematic of the study design

The order of the testpla and precaf trials and assignment to either supplementation group was via a double-blind, randomised design. No strenuous exercise, alcohol, or caffeine ingestion was permitted during the 24 h before each trial. However, the caffeine provided in the capsules was permitted during the 24 h before the postcaf trial (caffeine group). No additional dietary caffeine was permitted during the supplementation period in either group and participants were provided with a list of commonly consumed caffeinated foods and drinks to help achieve this. Participants were also instructed to maintain their usual dietary and exercise patterns throughout the supplementation period.

The trial overview is depicted in figure 6.2. Upon arrival at the laboratory, post-void nude body mass was recorded. To enable repeated blood sampling during each trial, a 21 g cannula was inserted into an antecubital vein after 15 min of seated rest. A baseline venous sample was collected (7mL) before participants ingested a capsule containing either 3 $mg \cdot kg^{-1}$ of anhydrous caffeine (precaf and postcaf; BDH Ltd, Poole, UK) or starch (testpla; BDH Ltd, Poole, UK). Next, a heart rate telemetry band was positioned (Polar Beat, Kempele, Finland). After 60 min of seated rest, a second venous sample (7mL) was drawn before participants cycled for 60 min at an intensity equivalent to 60% $\dot{V}O_{2peak}$. Heart rate and RPE (Borg, 1982) were recorded every 5 and 10 min, respectively. Expired air samples (1 min) were collected into Douglas bags every 15 min to determine the rates of fat and carbohydrate oxidation (Peronnet and Massicotte, 1991). After each sample was collected, participants were provided with 100 mL of plain water. A third 7 mL blood sample was collected immediately after the fixed-intensity exercise while participants remained seated on the ergometer.

Subsequently, there was a 2-3 min delay while the ergometer was set for the performance task. Performance was assessed as the maximum amount of external work (kJ) that could be completed in 30 min. The initial intensity was set at 75% $\dot{V}O_{2peak}$, but participants were free to adjust the workload as from the outset. Heart rate was recorded every 5 min and RPE at 10 and 20 min, respectively. A final 7 mL blood sample was collected upon completion of exercise while participants remained seated on the ergometer. After this, the cannula was removed.

Whole blood (2 mL) was used to determine plasma glucose, Hb, and Hct. The remaining 5 mL was used to yield serum for the subsequent determination of cortisol and prolactin with ELISA (DRG diagnostic, Germany) and caffeine with reverse-phase HPLC (Holland et al. 1999). Chapter 3.7 contains additional information regarding blood collection and analysis. The intra-assay CV for serum prolactin, cortisol, and caffeine was 4.9%, 5.3%, and 2.9%, respectively.

All data were analysed using IBM SPSS statistics version 21.0. Normality was assessed with the Shapiro Wilk test. Between-group comparisons of self-reported habitual caffeine intake, height, body mass, age, $\dot{V}O_{2peak}$ and W_{max} were determined with *t*-tests for independent samples. Repeated measurements of body mass, $\dot{V}O_{2peak}$ and W_{max} were analysed using a two-way (group x time) mixed-design factorial ANOVA. Exercise performance and fasting plasma glucose were analysed using a two-way (group x trial) mixed-design factorial ANOVA. Variables measured throughout each trial were analysed using a three-way (group x trial x time) mixed-design factorial ANOVA. Where a main effect or interaction occurred, Bonferroni adjusted paired *t*-tests for normally distributed data or Wilcoxon Signed Rank tests for non-normally distributed data were used. Between-group comparisons during the testpla, precaf and postcaf trials were determined with *t*-tests for independent samples. In addition to null-hypothesis testing, magnitude-based inferences were made to examine whether the observed differences in total external work produced were meaningful (Hopkins, 2000). The magnitude of the smallest worthwhile change in performance was set at 3% (~12 kJ), based on the findings of Jenkins et al (2008). Cohen's *d* effect size (ES) examined the magnitude of individual differences in total external work produced ([mean 1 - mean 2]/pooled SD) and were interpreted as trivial (0-0.19), small (0.2-0.49), medium (0.5-0.79) or large (>0.8) as previously described (Cohen, 1992). Data are presented as means ± SD, unless otherwise stated. Statistical significance was accepted at $P<0.05$.

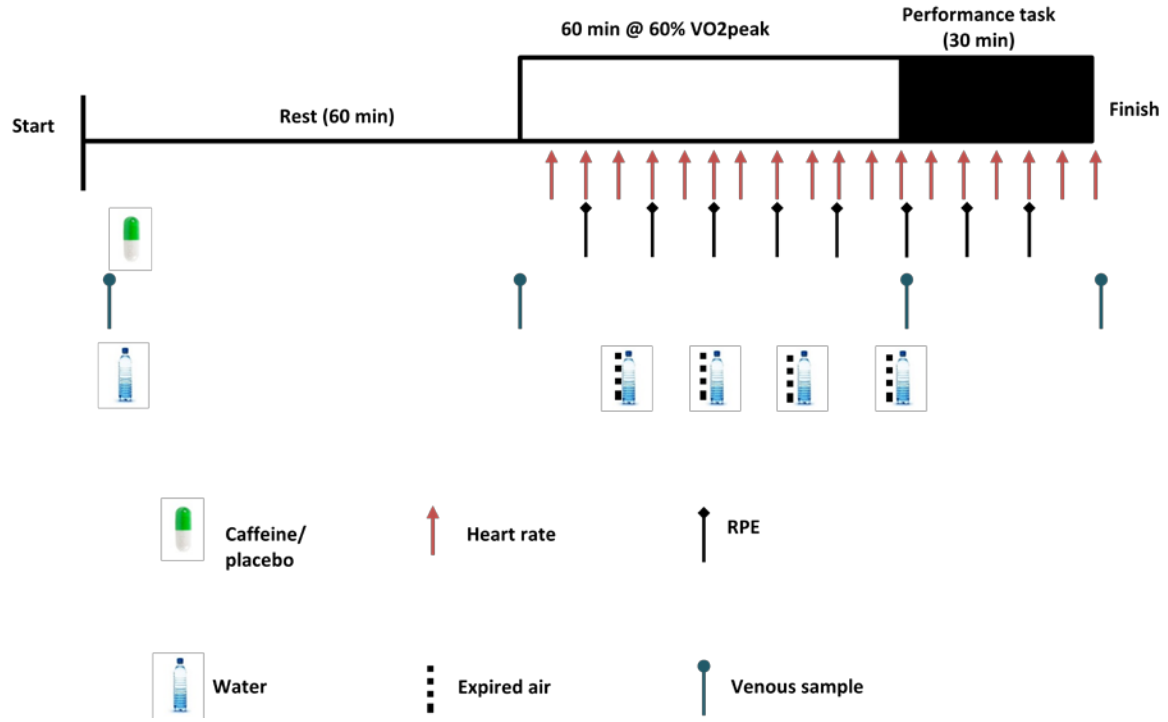

Figure 6.2 Trial overview

6.4 Results

Self-reported habitual caffeine intake was similar between groups (placebo: 66 ± 6 mg·day^{-1} vs. caffeine: 60 ± 8 mg·day^{-1}; *P*=0.076) There were no between-group differences for baseline measures of age (placebo: 21 ± 2 y; caffeine: 21 ± 2 y; *P*=0.710), height (placebo: 1.75 ± 0.06 m; caffeine: 1.76 ± 0.08 m; *P*=0.781), body mass (placebo: 73.3 ± 7.4 kg; caffeine: 74.8 ± 10.1 kg; *P*=0.708), $\dot{V}O_{2peak}$ (placebo: 51.6 ± 9.6 ml·kg^{-1}·min^{-1}; caffeine: 51.2 ± 8.4 ml·kg^{-1}·min^{-}1; *P*=0.860) or W_{max} (placebo: 286 ± 47 W; caffeine: 296 ± 55 W; *P*=0.667). Day 21 body mass (placebo: 73.1 ± 6.8 kg; caffeine: 74.8 ± 10.2 kg), $\dot{V}O_{2peak}$ (placebo: 51.0 ± 9.2 ml·kg^{-1}·min^{-1}; caffeine: 50.6 ± 8.3 ml·kg^{-1}·min^{-1}) and W_{max} (placebo: 282 ± 43 W; caffeine: 289 ± 47 W) was similar to baseline between both supplementation groups (trial x group interactions, *P*>0.646).

Performance during the testpla trial was similar between the caffeine (344. 9 ± 80.3 kJ) and placebo (333.1 ± 56.4 kJ) groups (*d*=0.17; *P*=0.723; Figure 6.3). Compared with testpla, total work produced during the precaf trial increased 12.0 ± 7.4% in the caffeine group (383.3 ±

75 kJ vs. 344. 9 ± 80.3 kJ; *d*=0.49; *P*=0.001) and 6.7 ± 4.2% in the placebo group (354.4 ± 55.2 kJ vs. 333.1 ± 56.4 kJ; *d*=0.38; *P*=0.004; Figure 6.3). Based on a smallest worthwhile change in performance of 12 kJ, these within-group increases represent an 'almost certainly beneficial' (caffeine group) and 'probably beneficial' (placebo group) effect on performance, respectively (Table 6.1).

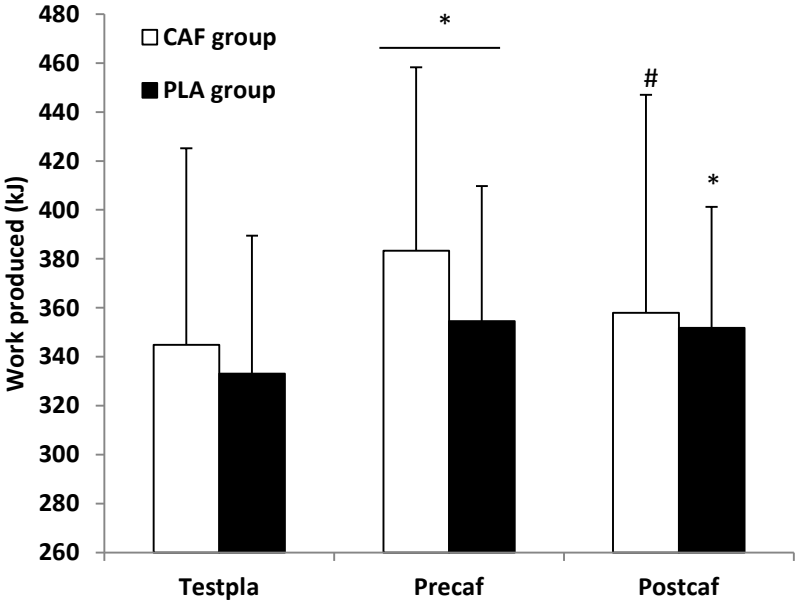

Figure 6.3 Total work produced (kJ) during the performance task. Trial x group interaction (*P*=0.017). * and # denote a within-group difference (*P*<0.05) compared with testpla and precaf, respectively. Values are mean ± SD.

Chronic caffeine supplementation resulted in a 7.3 ± 6.3% decrease in performance during the postcaf trial compared with precaf (358 ± 89 kJ vs. 383.3 ± 75 kJ; *d*=-0.31; *P*=0.025; Figure 6.3), with eight of nine participants demonstrating impaired performance following the chronic supplementation period (Figure 6.4A). This diminished response represents a 'probably harmful' effect on performance (Table 6.1). Total work produced during the postcaf trial and tetspla was not statistically different (358 ± 89 kJ vs. 344.9 ± 80.3 kJ; *d*=0.16; *P*=0.188). However, inferences suggest the difference between these trials represents a 'possibly beneficial' effect (Table 6.1). Hence, chronic caffeine supplementation might have not completely eliminated the performance benefit of caffeine (i.e. postcaf vs. testpla; Table 6.1).

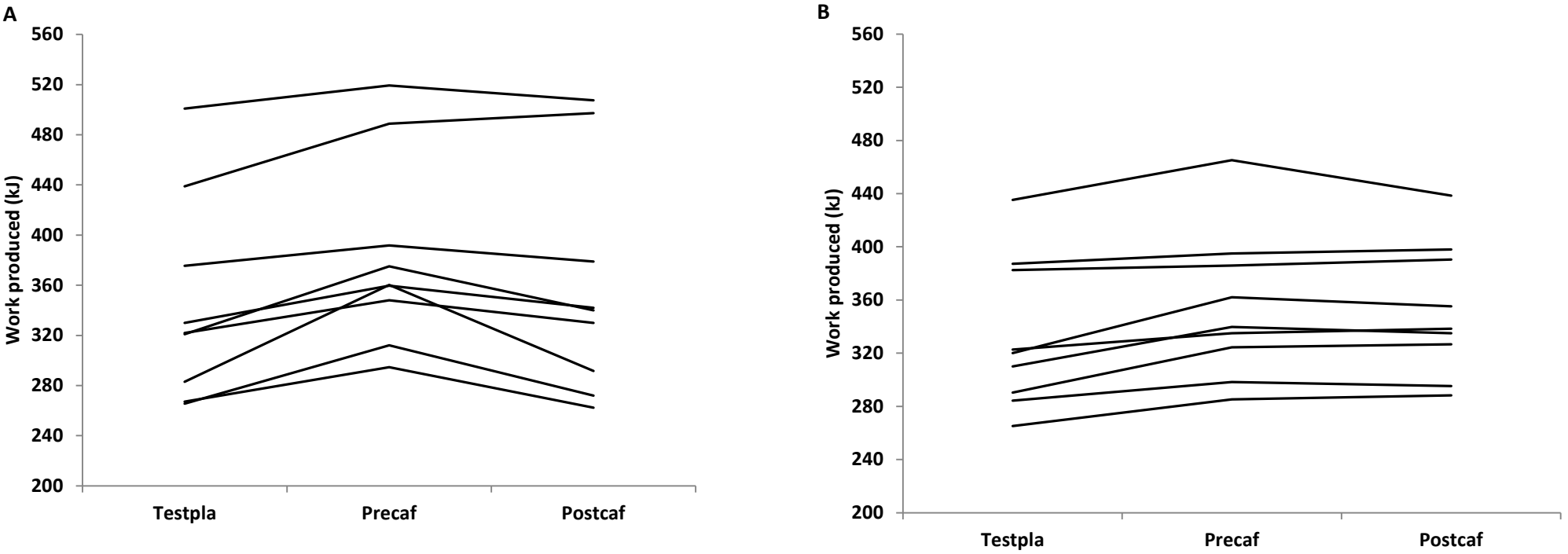

Figure 6.4 Individual responses during the performance task by participants in the Caffeine (A) and Placebo (B) supplementation group, respectively.

Participants in the placebo group produced 6.1 ± 2.4% more work during the postcaf trial than testpla (351.8 ± 49.4 kJ vs. 333.1 ± 56.4; *d*=0.33; *P*=0.004; Figure 6.3), with this increase representing a 'probably beneficial' effect on performance (Table 6.1). Accordingly, there was no difference between the precaf and postcaf trials (354.4 ± 55.2 kJ vs. 351.8 ± 49.4 kJ; *d*=0.05; *P*>0.05).

There were no significant between-group differences during the precaf (28.7 ± 74.8 kJ; *d*=0.44; *P*=0.368) or postcaf (6.2 ± 90.7 kJ; *d*=0.09; *P*=0.858) trials (Figure 6.3; Table 6.1).

Table 6.1 Differences in total work produced (kJ) during the experimental trials within and between supplementation groups.

Treatment comparison	Mean ± SD difference and 95% confidence interval	*d*	Qualitative outcome (beneficial/trivial/harmful)
CAF			Almost certainly beneficial (100/0/0)
Precaf-testpla	38.4 ± 19.9 (18.4 to 58.4)	0.49	Possibly beneficial (55/44/1)
Postcaf-testpla	13.1 ± 18.2 (-5.2 to 31.3)	0.16	Probably harmful (0/9/91)
Postcaf-precaf	-25.3 ± 21.9 (-47.3 to -3.4)	-0.31	Probably beneficial (94/6/0)
PLA			Probably beneficial (91/9/0)
Precaf-testpla	21.4 ± 13.1 (8.3 to 34.7)	0.38	Unclear (50/0/50)
Postcaf-testpla	18.7 ± 11.9 (6.8 to 30.6)	0.33	Unclear (50/26/24)
Postcaf-precaf	-2.8 ± 9.8 (-12.7 to 7.1)	-0.05	Unclear (70/19/11)
Testpla			Unclear (43/26/30)
CAF-PLA	11.8 ± 89.7 (-58.3 to 81.9)	0.17	
Precaf			
CAF-PLA	28.7 ± 74.8 (-37.7 to 95.2)	0.44	
Postcaf			
CAF-PLA	6.2 ± 90.7 (-68.1 to 80.5)	0.09	

PLA, placebo group; CAF, caffeine group; *d*, Cohen's *d* effect size. Qualitative outcome numbers indicate the percentage chance the true value is beneficial, trivial or harmful based on a 12 kJ difference in work produced during the performance task. An effect was deemed unclear when the percentage chances of benefit and harm were >5%

The order of the experimental trials was correctly guessed by two participants in each supplementation group. Furthermore, three participants in each supplementation group correctly guessed whether they received the caffeine or placebo treatment during the habituation period. Therefore, blinding can be considered successful as these odds are less than what could occur purely by chance.

Circulating caffeine, cortisol, prolactin, and glucose values recorded during exercise are shown in table 6.2. Acute caffeine supplementation increased serum concentrations during the precaf and postcaf trials, peaking 60 min after ingestion and remaining greater throughout exercise than baseline and testpla (trial x time interaction, $P<0.05$). There were no changes in serum caffeine concentrations during testpla, with values remaining close to baseline throughout exercise in both groups. The habituation protocol did not influence caffeine metabolism ($P=0.605$). Serum cortisol increased progressively throughout exercise ($P<0.05$), peaking at the end of the performance task in both groups. No influence from trial ($P=0.535$) or supplementation group ($P=0.628$) occurred. Similarly, prolactin concentrations increased during exercise ($P<0.05$), but the rate of increase was similar across trials ($P=0.498$) and between groups ($P=0.649$). The greatest concentrations were at the end of the performance task across all trials in both groups ($P<0.05$). Neither cortisol ($P=0.552$) nor prolactin ($P=0.965$) were influenced by the habituation protocol. Fasting plasma glucose was similar across all three trials in both supplementation groups ($P=0.465$). During exercise, plasma concentrations increased steadily ($P<0.05$), with similar values across trials ($P=0.096$) and between groups ($P=0.443$). Compared with baseline, both blood and plasma volumes were reduced during exercise ($P<0.05$). No influence of trial ($P>0.135$) or group ($P>0.649$) occurred.

Table 6.2 Circulating caffeine, cortisol, prolactin, and glucose concentrations during the experimental trials.

	PLA				CAF			
Variable	**-60**	**0**	**60**	**90**	**-60**	**0**	**60**	**90**
Caffeine (μg·mL^{-1})								
Testpla	0.06 ± 0.07	0.06 ± 0.07	0.06 ± 0.07	0.07 ± 0.06	0.13 ± 0.07	0.08 ± 0.10	0.10 ± 0.08	0.05 ± 0.08
Precaf	0.09 ± 0.07	3.54 ± 0.59*$^{\#}$	3.17 ± 0.44*$^{\#}$	2.97 ± 0.23*$^{\#}$	0.28 ± 0.29	3.48 ± 0.57*$^{\#}$	3.40 ± 0.53*$^{\#}$	3.03 ± 0.56*$^{\#}$
Postcaf	0.10 ± 0.09	3.54 ± 0.65*$^{\#}$	3.22 ± 0.44*$^{\#}$	2.97 ± 0.55*$^{\#}$	0.49 ± 0.37	3.69 ± 0.60*$^{\#}$	3.26 ± 0.53*$^{\#}$	3.09 ± 0.66*$^{\#}$
Cortisol (ng·mL^{-1})								
Testpla	131.55 ± 37.22	125.29 ± 59.77	153.22 ± 75.59	211.17 ± 90.96	115.47 ± 14.78	85.30 ± 33.50	163.73 ± 20.75*	236.10 ± 51.18*
Precaf	142.13 ± 26.85	118.00 ± 50.96	177.90 ± 86.66	227.32 ± 90.89	136.25 ± 34.27	104.55 ± 26.11	159.76 ± 46.14	225.63 ± 48.25
Postcaf	146.42 ± 33.79	122.48 ± 36.89	185.70 ± 63.54	249.50 ± 71.88	121.87 ± 42.89	80.30 ± 38.35	168.10 ± 42.36	234.73 ± 38.28*
Prolactin (ng·mL^{-1})								
Testpla	8.13 ± 2.68	7.80 ± 3.16	10.01 ± 2.80	19.65 ± 4.43*	7.83 ± 3.86	7.84 ± 3.02	9.99 ± 2.79	20.53 ± 4.99*
Precaf	7.91 ± 1.78	7.43 ± 1.46	10.39 ± 2.13	19.42 ± 3.18*	7.89 ± 3.65	7.57 ± 3.31	10.23 ± 2.10	20.03 ± 5.22*
Postcaf	7.59 ± 2.50	8.78 ± 3.27	10.37 ± 1.16*	19.25 ± 3.69*	8.33 ± 3.31	7.94 ± 3.66	9.79 ± 3.06	19.68 ± 5.06*
Glucose (mmol·L^{-1})								
Testpla	4.17 ± 0.27	4.18 ± 0.38	4.45 ± 0.51	4.71 ± 0.82	4.26 ± 0.28	4.21 ± 0.35	4.50 ± 0.39	5.03 ± 0.57
Precaf	4.10 ± 0.30	4.10 ± 0.35	4.52 ± 0.51	4.99 ± 1.03	4.19 ± 0.42	4.21 ± 0.35	4.49 ± 0.32	5.35 ± 0.77
Postcaf	4.18 ± 0.22	4.22 ± 0.17	4.70 ± 0.48	5.06 ± 0.75	4.41 ± 0.39	4.25 ± 0.25	4.57 ± 0.37	5.32 ± 0.76

PLA, Placebo group; CAF, Caffeine group. *denotes a within-trial significant difference (P<0.05) compared with -60. $^{\#}$denotes a significant difference (P<0.05) compared with the corresponding time point in the testpla trial. There were no significant trial x group (P>0.552) or trial x time x group (P>0.512) interactions for any variable. Values are mean ± SD.

Exercise caused a progressive increase in heart rate throughout the fixed-intensity exercise (*P*<0.05). This increase remained similar across trials (*P*=0.169) and between supplementation groups (*P*=0.984; Table 6.3). Similarly, heart rate increased during the performance task (*P*<0.05), but this increase was independent of trial (*P*=0.891) and group (*P*=0.887). Within-group differences in mean heart rate occurred across trials. The greatest values were during the precaf trial in both groups (Table 6.3). No between-group differences were apparent (*P*>0.274).

Table 6.3 Mean heart rate during the experimental trials.

	testpla	**precaf**	**postcaf**	***P***
HR (beats·min^{-1}), fixed				
PLA	146 ± 7	145 ± 7	145 ± 8	
CAF	145 ± 6	144 ± 7	146 ± 7	0.312
HR (beats·min^{-1}), PT				
PLA	167 ± 13	172 ± 12*	172 ± 12*	
CAF	169 ± 9	177 ± 5*	171 ± 9†	0.034

PLA, Placebo group; CAF, Caffeine group; HR, heart rate; Fixed, values recorded during the fixed-intensity exercise; PT, values recorded during the performance task. *P* values are derived from trial x group interactions. *denotes a within-group significant difference (*P*<0.05) compared with testpla. †denotes a within-group comparison (*P*=0.061) to precaf. Values are mean ± SD.

There was a steady increase in RPE during the fixed-intensity exercise (P<0.05), but this response was not influenced by trial (*P*=0.265) or group (*P*=0.441; Figure 6.5). Similarly, RPE increased throughout the performance task (*P*<0.05), but this response was independent of trial (*P*=0.174) and group (*P*>0.05; Figure 6.5).

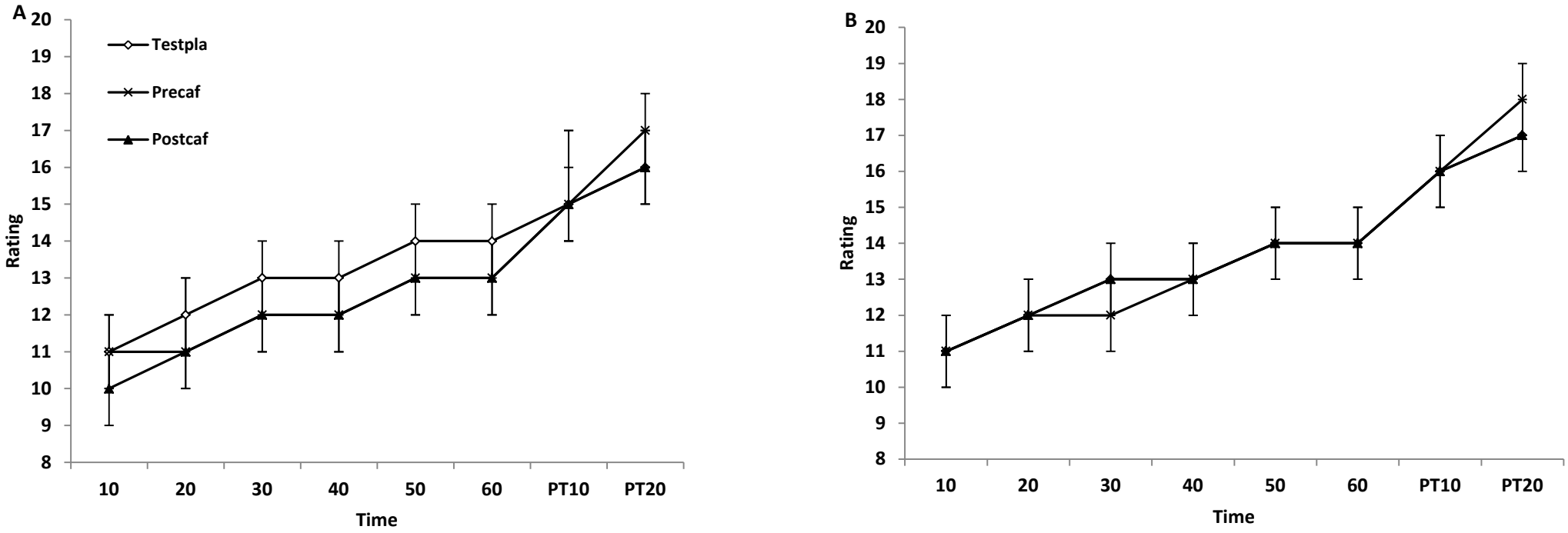

Figure 6.5 Perceived exertion during exercise in the placebo (A) and caffeine (B) supplementation group, respectively. PT, performance task. Values are mean ± SD.

Rates of carbohydrate oxidation decreased (P=0.026) while rates of fat oxidation increased (P<0.05) during the fixed-intensity exercise. Neither of these were influenced by trial (P>0.784) or group (P>0.328; Table 6.4). Furthermore, RER values decreased (P<0.05) while $\dot{V}O2$ increased (P<0.05) during exercise. No influence from trial (P>0.691) or group (P>0.189) occurred (Table 6.4).

Table 6.4 Substrate oxidation and oxygen uptake during the fixed-intensity exercise.

	PLA				CAF			
Variable	**15**	**30**	**45**	**60**	**15**	**30**	**45**	**60**
CHO ox (g·min^{-1})								
Tetspla	2.11 ± 0.38	2.10 ± 0.45	1.93 ± 0.59	1.96 ± 0.32	2.36 ± 0.40	2.22 ± 0.48	2.25 ± 0.49	2.15 ± 0.44
Precaf	2.08 ± 0.42	2.12 ± 0.44	2.07 ± 0.56	2.00 ± 0.68	2.45 ± 0.46	2.34 ± 0.40	2.43 ± 0.45	2.26 ± 0.56
Postcaf	2.01 ± 0.48	1.81 ± 0.27	2.04 ± 0.47	2.00 ± 0.29	2.36 ± 0.77	2.23 ± 0.62	2.17 ± 0.63	1.87 ± 0.72
Fat ox (g·min^{-1})								
Tetspla	0.33 ± 0.15	0.38 ± 0.20	0.45 ± 0.22	0.46 ± 0.14	0.25 ± 0.15	0.32 ± 0.22	0.33 ± 0.16	0.37 ± 0.15
Precaf	0.34 ± 0.18	0.36 ± 0.19	0.38 ± 0.21	0.43 ± 0.26	0.22 ± 0.15	0.30 ± 0.12	0.29 ± 0.19	0.35 ± 0.17
Postcaf	0.35 ± 0.16	0.46 ± 0.12	0.40 ± 0.19	0.46 ± 0.16	0.28 ± 0.31	0.32 ± 0.21	0.38 ± 0.26	0.49 ± 0.35
RER								
Tetspla	0.91 ± 0.03	0.90 ± 0.04	0.88 ± 0.06	0.88 ± 0.03	0.93 ± 0.03	0.92 ± 0.04	0.91 ± 0.04	0.90 ± 0.03
Precaf	0.91 ± 0.04	0.91 ± 0.04	0.90 ± 0.05	0.89 ± 0.06	0.94 ± 0.03	0.92 ± 0.03	0.92 ± 0.04	0.91 ± 0.04
Postcaf	0.90 ± 0.05	0.88 ± 0.02	0.90 ± 0.04	0.89 ± 0.03	0.93 ± 0.07	0.91 ± 0.05	0.90 ± 0.06	0.88 ± 0.07
VO2 (L·min^{-1})								
Tetspla	2.23 ± 0.36	2.33 ± 0.35	2.34 ± 0.28	2.38 ± 0.25	2.25 ± 0.31	2.30 ± 0.35	2.34 ± 0.31	2.35 ± 0.34
Precaf	2.22 ± 0.34	2.30 ± 0.31	2.31 ± 0.32	2.36 ± 0.31	2.26 ± 0.36	2.34 ± 0.32	2.39 ± 0.32	2.39 ± 0.34
Postcaf	2.19 ± 0.24	2.27 ± 0.28	2.32 ± 0.21	2.42 ± 0.23	2.31 ± 0.29	2.31 ± 0.22	2.38 ± 0.19	2.38 ± 0.26

PLA, Placebo group; CAF, Caffeine group. CHO ox, carbohydrate oxidation; Fat ox, fat oxidation; RER, respiratory exchange ratio; VO2, oxygen uptake. There were no significant trial x group (P>0.472), time x group (P>0.189), trial x time (P>0.784) or trial x time x group (P>0.142) interactions for any variable. Values are mean ± SD.

6.5 Discussion

This study examined whether four-weeks of controlled caffeine intake could influence endurance performance in a group of recreationally active men with low-habitual caffeine intakes. The results of the present investigation demonstrate that chronic supplementation with a titrated low dose of caffeine developed tolerance to the ergogenic effect a subsequent acute caffeine dose. While these results contrast with previous studies that have examined effects of sub-chronic caffeine supplementation (Irwin et al. 2011), this is the first study to investigate the effects of a prolonged period of controlled caffeine intake typical of the general population (Fitt et al. 2013). This suggests that supplementation protocols in previous studies (Irwin et al. 2011) were too short to influence mechanisms that develop tolerance.

Previous research demonstrated caffeine prolonged exercise capacity due to enhanced fat oxidation late in exercise with a subsequent sparring of muscle glycogen (Costill et al. 1978). The results of the present study are contrary to this as substrate oxidation was not influenced either by acute or chronic caffeine supplementation (Table 6.4). Alternatively, chronic caffeine intake could influence caffeine metabolism (Svenningsson et al. 1999). This might lead to an increase in the concentrations of paraxanthine and theophylline, caffeine's primary metabolites (Svenningsson et al. 1999). As these possess a greater affinity for adenosine receptors than caffeine (Fredholm et al. 1999), this could result in enhanced development of tolerance. However, caffeine concentrations were similar between the precaf and postcaf trials in the caffeine group (Table 6.2), suggesting the habituation protocol did not influence caffeine metabolism. Although paraxanthine and theophylline concentrations were not measured, these methylxanthines do not penetrate the blood-brain-barrier with the same efficacy as caffeine (Svenningsson et al. 1999). Therefore, any subtle change in the peripheral concentrations of these metabolites attributable to the chronic supplementation protocol is unlikely to explain the development of tolerance.

Serum cortisol and prolactin were assessed as these are indirect indicators of central noradrenergic (Tsigos and Chrousos, 2002) and dopaminergic (Ben-Jonathan and Hnasko, 2001) activity, respectively. Chronic caffeine supplementation did not influence the circulating concentrations of these hormones (Table 6.2), suggesting that neurotransmitter

release along these neural pathways does not explain the development of tolerance. Direct analysis of neurotransmitter release with microdialysis (Acquas et al. 2002; De Luca et al. 2007) and PET (Volkow et al. 2015) also support this hypothesis. Although high acute caffeine doses increase striatal dopamine release (i.e. 30 $mg \cdot kg^{-1}$; Solinas et al. 2002), lower doses (i.e. 0.25-5 $mg \cdot kg^{-1}$), typically consumed by the general population (Fitt et al. 2013), have not influenced dopamine release both in rat (Acquas et al. 2002; De Luca et al. 2007) and human (Volkow et al. 2015) striatum. Therefore, an alternative mechanism is likely responsible. Chronic caffeine supplementation has been associated with changes in A_{2A} expression across several brain regions (Svenningsson et al. 1999). However, a cross-tolerance to the A_1 receptor probably plays a more important role in mediating the development of tolerance (Karcz-Kubicha et al. 2003). This could involve a functional change in the striatal A_1/A_{2A} heteromer (Ciruela et al. 2006), while others have reported changes in A_1 receptor expression throughout the brain after chronic caffeine supplementation (Johansson et al. 1993). A recent PET study demonstrated that almost half of *in vivo* cerebral A_1 receptors were occupied by caffeine when participants received an intravenous dose of 4.3 $mg \cdot kg^{-1}$, which corresponded to a plasma concentration of ~8 $\mu g \cdot mL^{-1}$ (Elmenhorst et al. 2012). Participants in the present study were habituated to daily doses of 3 $mg \cdot kg^{-1}$ from days 8 to 28, resulting in serum concentrations of approximately 3.5 $\mu g \cdot mL^{-1}$ (Table 6.2). Based on these observations, it could be that the 3 $mg \cdot kg^{-1}$ caffeine dose administered in the present study resulted in the occupation of approximately a quarter of cerebral A_1 receptors. This suggests supplementation with larger daily caffeine doses (i.e. 6-9 $mg \cdot kg^{-1}$), which will ultimately occupy more A_1 receptors, results in accelerated and/or total development of tolerance.

The influence of caffeine habituation of participants is often overlooked in many studies, despite evidence which demonstrates that this influences performance after acute supplementation (Bell and McLellan, 2002). To minimise this confounder, all participants in the present study were low caffeine consumers before participation. Differences in habitual caffeine consumption are associated with single nucleotide polymorphisms (SNP's) in the ADORA2A gene encoding for the A_{2A} receptor (Cornelis et al. 2007). This study reported that individuals with the homozygous recessive (TT) genotype consumed less caffeine than their homozygous dominant (CC) counterparts (Cornelis et al. 2007). Recently, TT carriers

performed better during a short performance task (10 min) than CC carriers when supplemented with an acute 5 $mg \cdot kg^{-1}$ caffeine dose (Loy et al. 2015). Perhaps this could explain the small between-group difference in total external work produced during the precaf trial (28.7 ± 74.8 kJ; ES=0.44), with more TT carriers present in the caffeine group. However, genotype determination was not undertaken in the present study, which limits the extent to which this relationship can be inferred.

Well-trained individuals produce more reliable performance data during cycle-based TT than their recreationally active counterparts (Zavorsky et al. 2007). However, recreationally active individuals produced a CV of 1.7% (Zavorsky et al. 2007) and 0.7% (Fleming and James, 2014) during cycle-and running-based TT's, respectively. Furthermore, similar performance tests to that in the present study elicit a CV of approximately 3% (Jeukendrup et al. 1996; Sewell and McGregor, 2008). This variability is less than the percentage increase in performance during the precaf trials (caffeine: 12.0 ± 7.4%; placebo: 6.7 ± 4.2%) and the percentage decrease in performance during the postcaf trial compared with precaf in the caffeine group (-7.3 ± 6.3%). Therefore, neither the participant group nor the performance test used in the present study adversely influenced the validity of the performance data.

Ideally, the study design would have incorporated a post-supplementation placebo trial, hence providing a direct comparison with the postcaf trial after the chronic supplementation protocol. It was deemed difficult to implement as timing both trials to occur at the end of the supplementation period was not possible. For example, two randomised trials, undertaken seven days apart, means the supplementation period before the postcaf trial would be twenty-eight days for half the participants and thirty-five days for the remaining participants. Importantly, peak power output and maximal oxygen uptake were similar between the two $\dot{V}O_{2peak}$ tests. Furthermore, heart rate and oxygen uptake during the fixed-intensity exercise was similar during all three trials. This suggests participants maintained similar fitness throughout the study period and exercise intensity was matched before the performance task during each of the experimental trials. Hence, any influence on performance during the postcaf trial in either supplementation group is likely due to participants receiving caffeine or placebo during the chronic supplementation period.

In conclusion, the present findings demonstrate that chronic ingestion of a titrated low dose of caffeine results in the development of tolerance in a group of healthy, recreationally active males with low-habitual caffeine intakes. This occurred despite no difference before or after the supplementation protocol in circulating caffeine, hormonal concentrations or substrate oxidation. The influence of chronic caffeine intake should be examined in well-trained individuals with low-habitual caffeine intakes. In addition, futures studies should identify when the tolerance to caffeine occurs and examine whether supplementation with larger daily doses (i.e. 6-9 $mg \cdot kg^{-1}$) influences the rate and extent of the development of tolerance.

Chapter 7

Octopamine Supplementation Does Not Influence Endurance Cycle Performance

7.1 Abstract

Octopamine is a naturally occurring compound found in low concentrations in plasma and throughout the central nervous system. It has previously been demonstrated to increase locomotor activity in rats and induce lipolysis by stimulating β_3 adrenoreceptors. Hence, the aim of this study was to examine the influence of octopamine supplementation on endurance performance and exercise metabolism. Ten healthy, recreationally active men (Mean ± SD; age: 24 ± 2 years; body mass: 78.4 ± 8.7 kg; VO_{2peak}: 50.5 ± 6.8 ml·kg^{-1}·min^{-1}) completed one VO_{2peak} test, one familiarisation trial and two experimental trials. After an overnight fast, participants ingested either a placebo or 150 mg of octopamine 60 min before exercise. Trials consisted of 30 min of cycle exercise at 55% W_{max}, followed by a 30 min performance task. Performance was similar between the experimental trials (placebo: 352.8 ± 39 kJ; octopamine: 350.9 ± 38.3 kJ; Cohen's *d* effect size=0.05; *P*=0.380). Substrate oxidation and circulating concentrations of free fatty acids, prolactin and cortisol were similar between trial conditions (all *P*>0.05). There were also no differences across trials for heart rate or perceived exertion during exercise (both *P*>0.05). The present results indicate that acute octopamine supplementation fails to influence endurance performance, which could be due to the low serum concentrations observed. Future studies should examine the influence of larger doses during exercise both in temperate and high environmental conditions.

7.2 Introduction

The prevalence of dietary supplement use is becoming increasingly widespread among athletes (Knapik et al. 2016) and university students (Lieberman et al. 2015). This has contributed to an increase in global sales, which was estimated at approximately £73 billion in 2012 (Nutrition Business Journal, 2014). One of the primary reasons athletes consume supplements is to enhance performance (Lieberman et al. 2015), yet only a few appear to be ergogenic. Of these, caffeine has been consistently demonstrated to improve performance in laboratory-based studies (Burke, 2008), which is likely due to its ability to stimulate the CNS (Fredholm et al. 1999). This led to other drugs which possess stimulant-like properties to be investigated for their ergogenic potential (Earnest et al. 2004). However, some of these may be unsafe, especially when taken in combination with other stimulants (Williams, 2006). In addition, many supplements contain deleterious compounds not declared on the label (Maughan, 2005), while the use of potentially dangerous pharmacological agents by endurance athletes have increased in recent years (Machnik et al. 2009). Given these observations, it would be of interest to identify a safe and novel supplement capable of improving endurance performance.

Octopamine is a naturally occurring amine structurally similar to the neurotransmitter noradrenaline (Farooqui, 2012). It was first isolated from the salivary glands of the octopus (Erspamer, 1948) and is synthesised from the amino acid tyrosine with tyramine as an intermediate (Brandau and Axelrod, 1972). The function of octopamine has been well characterised in invertebrates, where it modulates signal transduction processes through the activation of octopamine receptors (Farooqui, 2012). Vertebrates, including humans, are absent of these receptors, which led to the suggestion that endogenous octopamine exerts no major role in human physiology (Farooqui, 2012). However, low circulating concentrations are present in plasma (D'Andrea et al. 2010), leading octopamine to being classified as one of the primary trace amines (Burchett and Hicks, 2006). A unique group of G protein-coupled receptors known as trace amine-associated receptors (TAAR) have been identified in recent years (Borowsky et al. 2001; Bunzow et al. 2001). Importantly, octopamine has been shown to bind to the TAAR1 subtype (Borowsky et al. 2001), a receptor which modulates the release of monoamines from presynaptic terminals in the brain (Liberles, 2015). This confirms previous reports of the presence of octopamine in

mammalian nerve tissues and brain (Ibrahim et al. 1985). Furthermore, octopamine has been suggested to play a role in the pathogenesis of migraine and the early stages of Parkinson's disease (D'Andrea et al. 2010; D'Andrea et al. 2012). Therefore, octopamine may, in part, modulate normal and abnormal neurophysiological processes (Burchett and Hicks, 2006) and possess stimulant-like properties capable of influencing exercise performance (Stohs, 2014; WADA, 2015). Given that caffeine influences the CNS (Fredholm et al. 1999) and enhances performance in temperate (Burke, 2008; chapter 6) and warm (chapters 4 and 5) ambient conditions, it would be of interest to determine the performance effects of octopamine following acute supplementation.

Octopamine was studied as a therapeutic agent to treat hypotensive disorders, with doses of 450-600 $mg \cdot day^{-1}$ resulting in mild increases in systolic blood pressure without the presence of adverse effects (Kuske, 1969; Ziegelmayer, 1972). Subsequent studies demonstrated the ability of octopamine to activate β_3 adrenoreceptors and stimulate lipolysis (Carpéné et al. 1999), suggesting octopamine could influence fat metabolism. Furthermore, intracerebroventricular administration of octopamine increased locomotor activity in rats (Jagiełło-Wójtowicz, 1979). Despite these observations, no human study has examined the influence of octopamine on exercise performance or substrate metabolism. Therefore, the aim of the study was to determine whether an acute oral dose of octopamine could influence endurance performance and/or exercise metabolism in a group of healthy volunteers.

7.3 Methods

Ten healthy, recreationally active men (age: 24 ± 2 y; body mass: 78.4 ± 8.7 kg; height: 1.81 ± 0.07 m; VO_{2peak}: 50.5 ± 6.8 $ml \cdot kg^{-1} \cdot min^{-1}$; W_{max}: 295 ± 41 W) participated in this study, which employed a double-blind, randomised, counter-balanced, cross-over design. All participants completed one incremental maximal exercise test, one familiarisation trial and two experimental trials. The initial visit consisted of incremental cycle exercise to volitional exhaustion on an electronically braked cycle ergometer (Lode Corival, Groningen, Holland) to determine W_{max} and the power output required to elicit 55% and 75% of W_{max}. Following

this, participants completed a familiarisation trial. All visits to the laboratory were separated by 5-7 days.

The trial overview is illustrated in figure 7.1. The pre-trial dietary and activity standardisation requirements are outlined in chapter 3.5. No additional dietary caffeine (i.e. above habitual intake) was permitted during the 24 hours prior to the familiarisation and experimental trials. Participants arrived at the laboratory in the morning (7-9am) and post-void nude body mass was recorded. After 15 min of seated rest, a 21-g cannula was inserted into an antecubital vein to enable repeated blood sampling. A baseline venous sample (12 mL) was collected before participants ingested a capsule containing either 150 mg of octopamine (Blackburn Distributions, Lancashire, UK) or 150 mg of starch (placebo; BDH Ltd, Poole, UK) with 50 mL of plain water. The purity of octopamine was certified at >99% (HFL Sport Science, Fordham, UK; Ref: LGC255966). The 150 mg dose was chosen to avoid hypertensive effects previously reported after oral intakes of 450-600 mg in hypotensive patients (Kuske, 1969; Ziegelmayer, 1972). All capsules were visually identical and blinded by an external party not involved in any stage of data collection. Following ingestion of the capsules, participants rested in a comfortable environment for 60 min; this timeframe is sufficient to elicit peak octopamine concentrations in the blood (Hengstmann et al. 1974). After the rest period a second venous sample (12 mL) was collected before participants began cycle exercise for 30 min at a workload corresponding to 55% W_{max}. During this period heart rate and RPE (Borg, 1982) were recorded every 5 and 10 min, respectively. Expired air samples (1 min) were collected at 15 and 30 min to determine the rates of substrate oxidation (Peronnet and Massicotte, 1991). After each sample, participants were provided with 100 mL of plain water. At 30 min a third venous sample (12 mL) was collected while participants remained seated on the ergometer.

Subsequently, there was a 2-3 min delay while the ergometer was set up for the performance task. Participants were instructed to complete as much work (kJ) as possible within 30 min. Participants began exercise at a workload corresponding to 75% W_{max}, but were free to adjust the intensity of exercise as desired from the outset. Heart rate was recorded every 5 min and RPE at 10 and 20 min, respectively. A final venous sample (12 mL) was collected upon completion of exercise while participants remained seated on the ergometer. After this, the cannula was removed.

Whole blood (2 mL) was used to determine plasma glucose, Hb, and Hct. Further details regarding handling and analysis are described in chapter 3.7. An additional 5 mL was used to yield serum for the subsequent determination of cortisol and prolactin with ELISA (DRG diagnostic, Germany) and octopamine with reverse-phase HPLC (Wood and Hall, 2000). The remaining 5 mL was used for the subsequent determination of FFA by colorimetric methods (described in chapter 3.7). The intra-assay CV for serum prolactin, cortisol, octopamine, and FFA's was 5.1%, 4.0%, 2.5%, and 3.6%, respectively.

All data were analysed using IBM SPSS statistics version 21.0. Normality was assessed with the Shapiro Wilk test. To evaluate differences in exercise performance, pre-exercise nude body mass, and fasting plasma glucose across trial conditions, a paired *t*-test was employed. Cohen's *d* effect size for differences in total work produced during the performance task was also determined ([mean 1 - mean 2]/pooled SD) and interpreted as trivial (0-0.19), small (0.2-0.49), medium (0.5-0.79) or large (>0.8) as previously described (Cohen, 1992). Variables measured throughout each trial were analysed using a two-way (trial x time) repeated-measures ANOVA. Where the assumption of sphericity had been violated, the degrees of freedom were corrected with a Greenhouse-Geisser as appropriate. Main effects and interactions were followed up with Bonferroni adjusted paired *t*-tests for normally distributed data or Bonferroni adjusted Wilcoxon Signed Rank tests for non-normally distributed data. Data are presented as means ± SD throughout. Statistical significance was accepted at $P<0.05$.

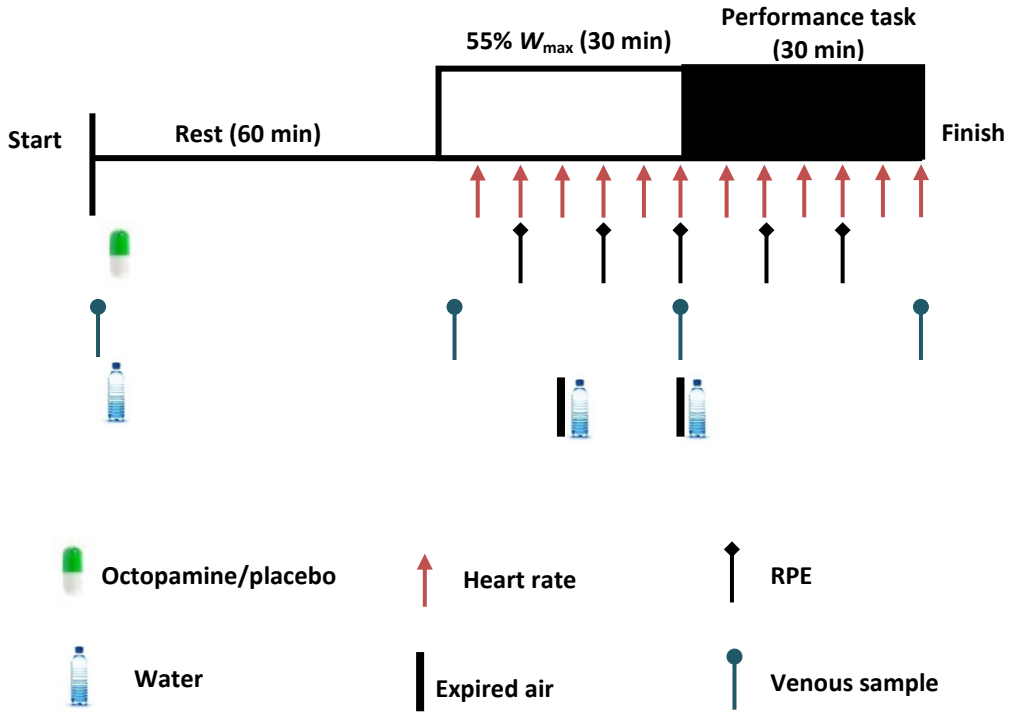

Figure 7.1 Trial overview

7.4 Results

Mean environmental temperature was similar between trials (placebo: 20.0 ± 0.8 °C; octopamine: 20.0 ± 0.8 °C; *P*=0.903). There were no differences across trials for pre-exercise nude body mass (placebo: 78.6 ± 8.8 kg; octopamine: 78.7 ± 8.9 kg, *P*=0.602) or fasting plasma glucose (placebo: 4.4 ± 0.5 mmol·L^{-1}; octopamine: 4.4 ± 0.5 mmol·L^{-1}, *P*=0.483), suggesting participants began each trial in a similar physiological state.

All ten participants completed both experimental trials, no adverse effects were reported. There was no clear difference in total work produced during the performance task, with mean values of 352.8 ± 39 kJ and 350.9 ± 38.3 kJ recorded during the placebo and octopamine trials, respectively (*d*=0.05; *P*=0.380; Figure 7.2).

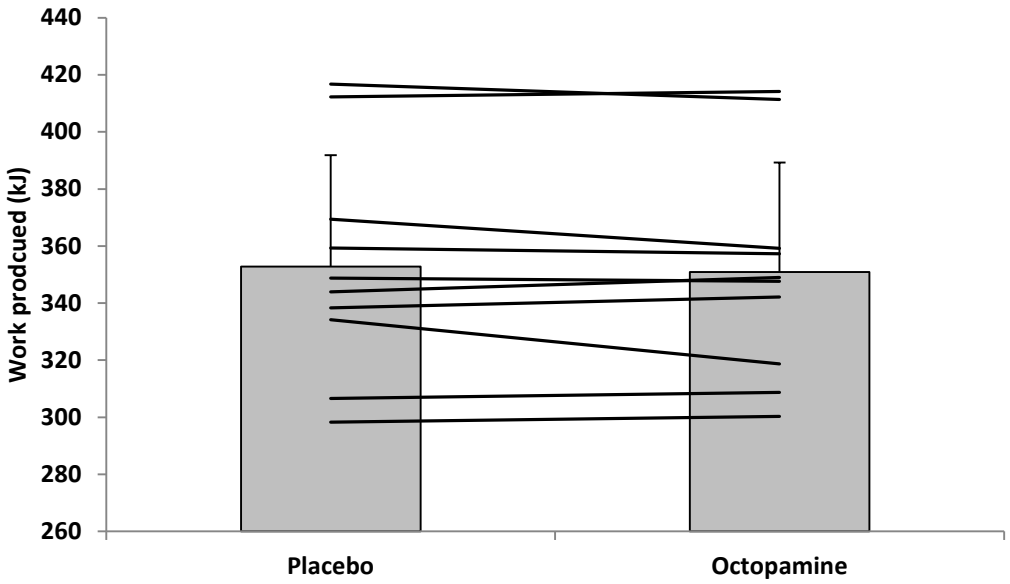

Figure 7.2 Mean (bars) and individual (lines) work produced (kJ) during the performance task. Values are mean ± SD.

Serum octopamine concentrations remained below the limit of detection for all time points during the placebo trial and for the baseline sample during the octopamine trial. During the octopamine trial, serum concentrations increased throughout exercise ($P<0.05$; Figure 7.3). No pair-wise differences were identified from 60 to 120 min post-ingestion ($P>0.725$).

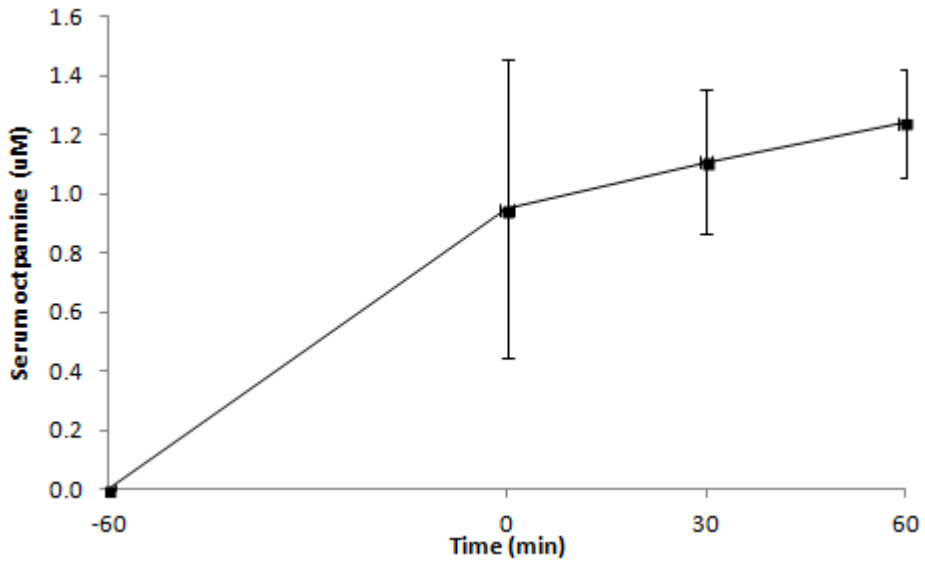

Figure 7.3 Serum octopamine concentrations. Values are mean ± SD.

Circulating cortisol showed a main effect of time (P<0.05), but no main effect of trial (P=0.334) or a trial x time interaction (P=0.080; Figure 7.4A). There was a main effect of time for serum prolactin (P<0.05), with higher values recorded at 30 and 60 min compared with baseline (P<0.08; Figure 7.4B). No main effect of trial (P=0.833) or interaction effect was observed (P=0.288).

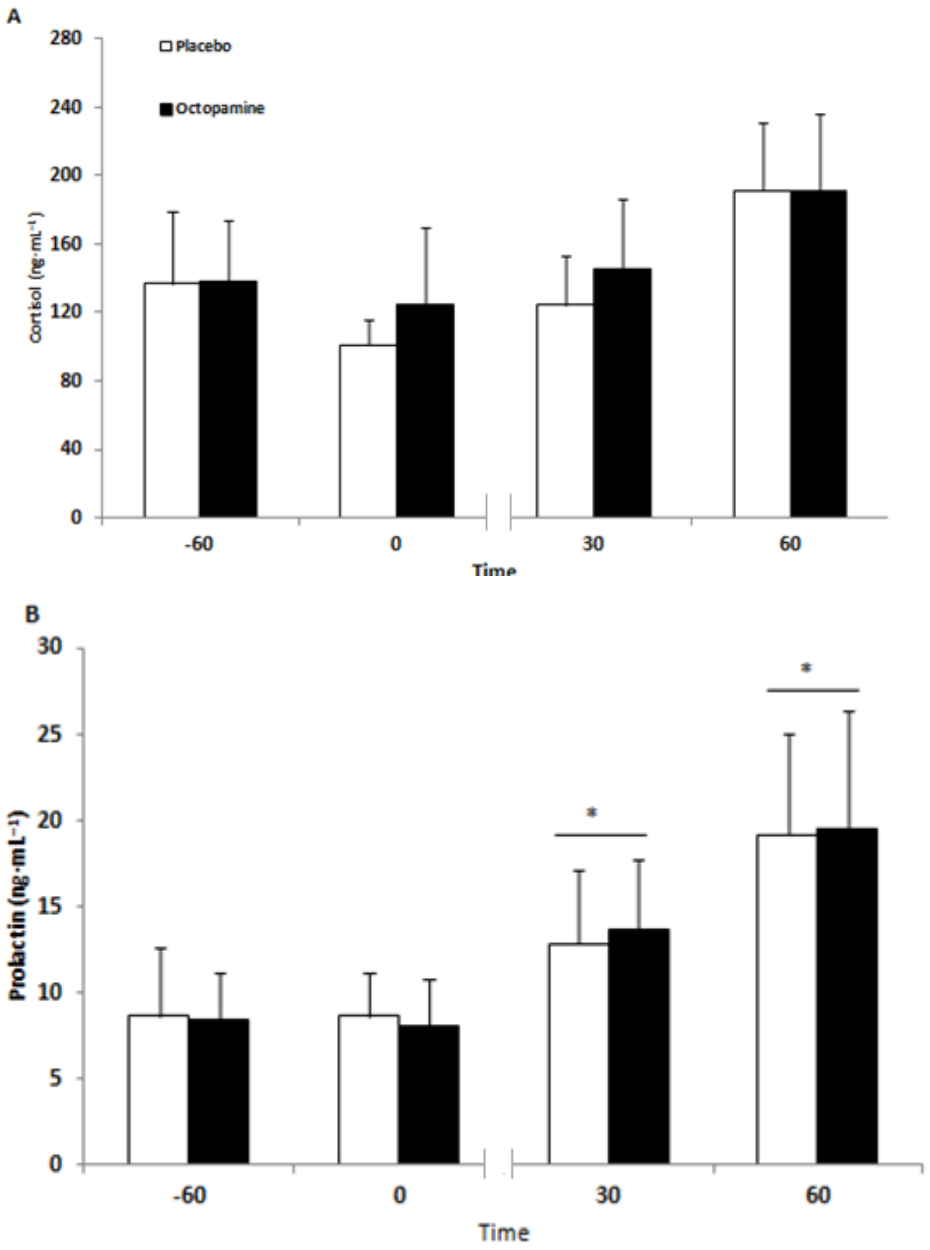

Figure 7.4 Circulating cortisol (A) and prolactin (B) during the experimental trials. *denotes a significant difference (P<0.05) from -60. Values are mean ± SD.

FFA concentrations remained similar compared with baseline during both trials, with no main effect of time (P=0.783), trial (P=0.351) or trial x time interaction (P=0.412; Figure 7.5A) apparent. Glucose concentrations showed a main effect of time (P<0.05), with higher values at 30 and 60 min compared with baseline (P<0.05; Figure 7.5B). No main effect of trial (P=0.240) or a trial x time interaction (P=0.704) occurred. There was a main effect of time for blood and plasma volume (P<0.05), but no main effects of trial (P>0.231) or interaction effects (P>0.504).

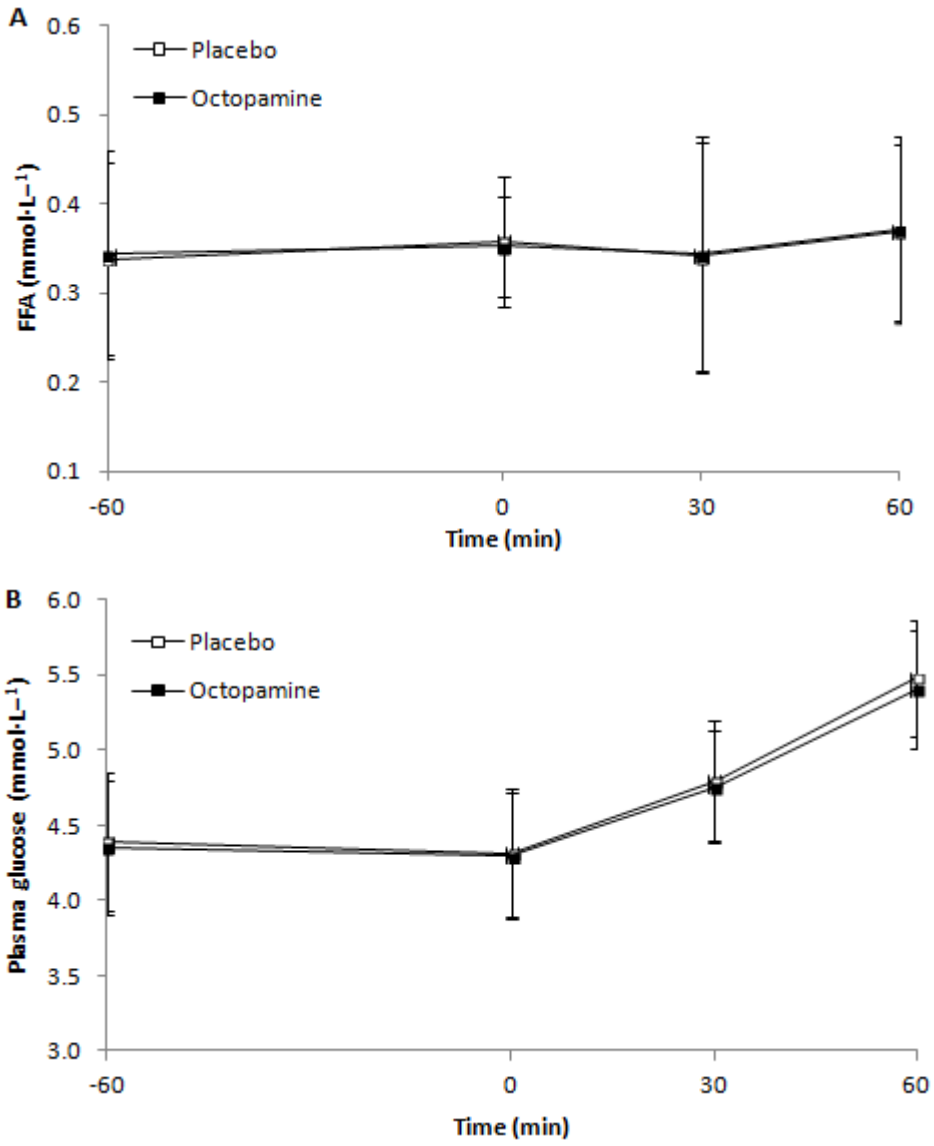

Figure 7.5 Plasma FFA's (A) and glucose (B) during the experimental trials. *denotes a significant difference (P<0.05) from -60. Values are mean ± SD.

For carbohydrate oxidation there was no main effect of trial (*P*=0.661), time (*P*=0.148) or an interaction effect (*P*=0.419). There was a main effect of time for fat oxidation (*P*=0.026), but no main effect of trial (*P*=0.597) or trial x time interaction (*P*=0.387; Table 7.1). Oxygen uptake showed a main effect of time (*P*=0.001), with higher values at 30 min compared with 15 min (*P*<0.05; Table 7.1). No main effect of trial (*P*=0.927) or trial x time interaction (*P*=0.382) was observed. For RER there was no main effect of trial (*P*=0.775), time (*P*=0.121) or an interaction effect (*P*=0.366; Table 7.1).

Table 7.1 Substrate oxidation and oxygen uptake during the fixed-intensity exercise

	Treatment	**15**	**30**
CHO ox (g·min^{-1})	**Placebo**	2.46 ± 0.35	2.46 ± 0.37
	Octopamine	2.44 ± 0.38	2.51 ± 0.33
Fat ox (g·min^{-1})	**Placebo**	0.19 ± 0.08	0.23 ± 0.08
	Octopamine	0.20 ± 0.04	0.21 ± 0.05
RER	**Placebo**	0.95 ± 0.02	0.94 ± 0.02
	Octopamine	0.94 ± 0.01	0.94 ± 0.01
VO2 (L·min^{-1})	**Placebo**	2.22 ± 0.30	2.29 ± 0.31*
	Octopamine	2.21 ± 0.31	2.29 ± 0.30*

ValuesCHO ox, carbohydrate oxidation; Fat ox, fat oxidation; RER, respiratory exchange ratio; VO2, oxygen uptake. *denotes significantly greater (*P*<0.05) than 15 min. Values are mean ± SD.

Heart rate showed a main effect of time during the fixed-intensity exercise (*P*<0.05) and during the performance task (*P*<0.05). No main effects of trial (*P*>0.240) or interaction effects (*P*>0.168) were observed (Figure 7.6).

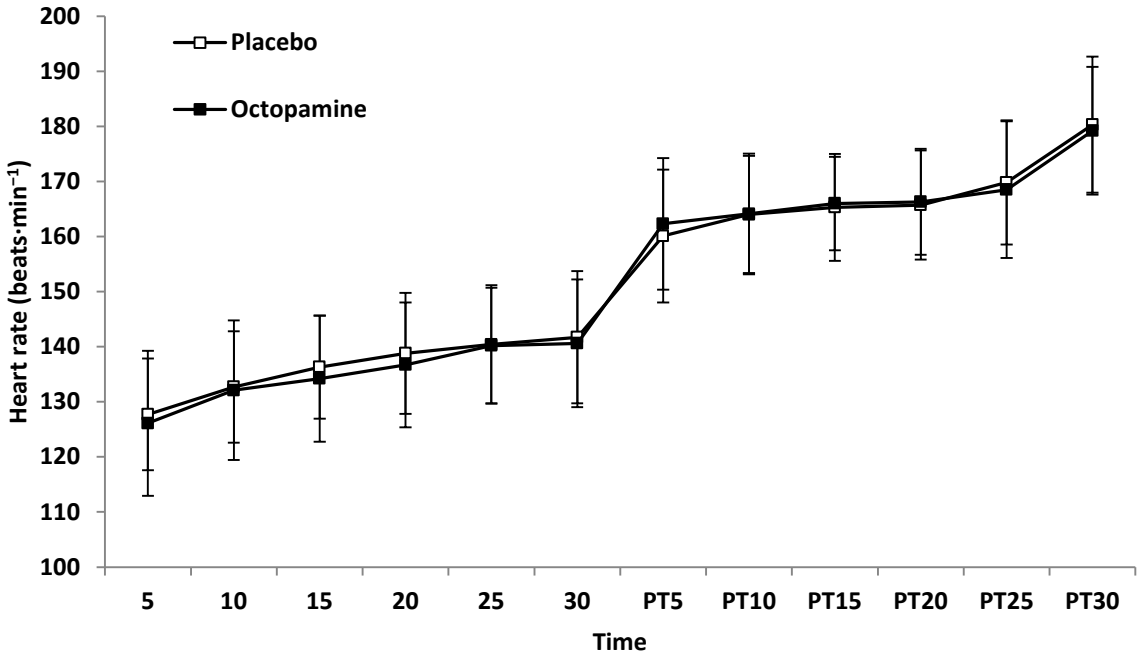

Figure 7.6 Heart rate during the experimental trials. PT, performance test. Values are mean + SD.

There was a main effect of time for RPE during the fixed-intensity exercise (P=0.01) and during the performance task (P<0.05). No main effects of trial (P>0.177) or trial x time interactions (P>0.241) occurred (Figure 7.7).

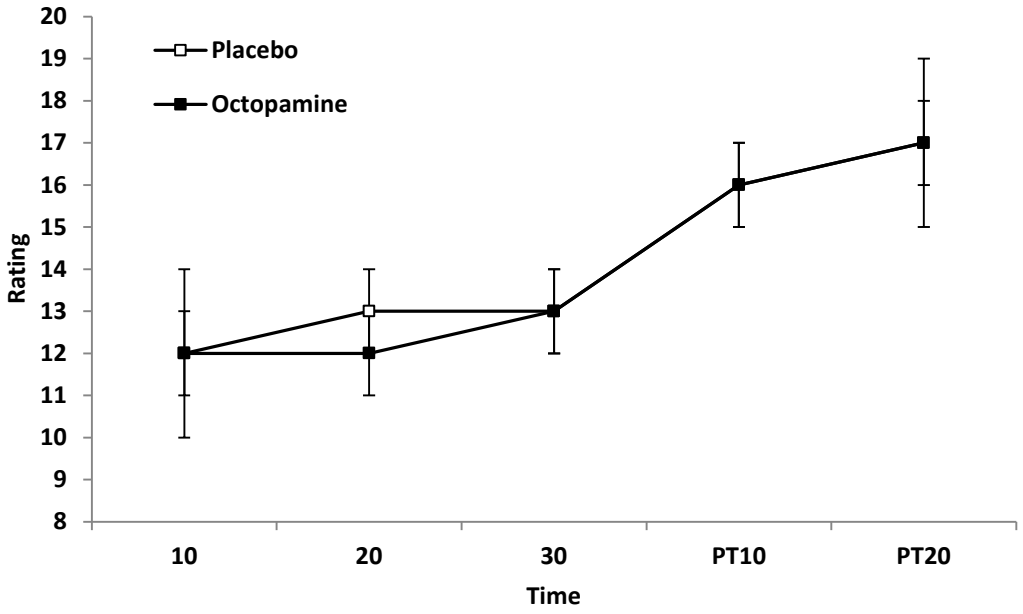

Figure 7.7 Perceived exertion during exercise. PT, performance test. Values are mean + SD.

7.5 Discussion

The present study was the first to examine whether octopamine supplementation could influence endurance performance or exercise metabolism in a group of healthy, recreationally active male participants. The results of the present investigation demonstrate that an acute 150 mg dose of octopamine failed to enable participants to increase the amount of work produced during a 30 min performance task compared with a placebo condition. Furthermore, there were no differences between trials in the estimated rates of fat and carbohydrate oxidation or in the peripheral concentrations of FFA's, prolactin and cortisol.

While the mechanism of action of octopamine is well established in invertebrates (Farooqui, 2012), its precise function in humans remains elusive (Burchett and Hicks, 2006: Stohs, 2014). However, low concentrations have been observed in plasma (D'Andrea et al. 2010) and throughout the central nervous system (Burchett and Hicks, 2006; Ibrahim et al. 1985 Liberles, 2015). Previous work demonstrated that octopamine can bind to TAAR1 (Borowsky et al. 2001), a receptor which modulates neurotransmitter release across several brain regions (Liberles, 2015). However, the EC_{50} values for TAAR1 from human, rat and mouse transfected-cell lines are in the range of 2-20 µM (Lindemann et al. 2005). These values are greater than the serum concentrations reported in the present study (0.95 to 1.24 µM; Figure 7.3), suggesting a larger dose of octopamine may be required to influence this receptor. Furthermore, octopamine is rapidly metabolised after oral ingestion, with eleven times more conjugated octopamine present in the urine compared with intravenous infusion (Hengstmann et al. 1974). This might explain the contrast between the present study and a previous animal model (Jagiełło-Wójtowicz, 1979), as octopamine was directly introduced into the brain of rats and therefore not subjected to extensive hepatic first-pass metabolism. Moreover, endurance performance in the heat is influenced by pharmacological manipulation of central catecholamines (Roelands and Meeusen, 2010). Hence, the provision of a larger dose of octopamine coupled with a high ambient temperature could provide conditions by which octopamine might enhance performance; this hypothesis warrants investigation in future studies.

Previous research demonstrated that octopamine can selectively and potently bind to β_3 adrenoreceptors and stimulate lipolysis in mammalian fat cells (Carpéné et al. 1999), while expressing little affinity for the α-adrenergic receptors (Brown et al. 1988). These observations suggest that octopamine supplementation might influence fat metabolism in humans. However, no differences were observed between the two trials in the estimated rates of fat and carbohydrate oxidation or in the peripheral concentrations of FFA's. While these findings contrast with previous *in vitro* data (Carpéné et al. 1999), the doses required to induce lipolysis in these experiments ranges from 10 µM to 1 mM (Carpéné et al. 1999; Visentin et al. 2001). Therefore, observations from *in vitro* models may not translate to the effects observed after oral intake in humans. Furthermore, even chronic ingestion (4 weeks) of a dose approximately seven times greater than the present study (15.3 $mg \cdot kg^{-1}$) failed to induce higher FFA, glycerol or triglyceride concentrations in rats (Bour et al. 2003). For an 80 kg human, this would correspond to a daily dose of approximately 1,200 mg, which is twice the dose previously demonstrated to induce hypertensive effects (Kuske, 1969; Ziegelmayer, 1972). Hence, low-dose octopamine supplementation is unlikely to influence fat metabolism in humans.

The present study was the first to examine whether octopamine could enhance endurance performance or exercise metabolism. It was demonstrated that an acute oral dose failed to influence performance, substrate oxidation or the peripheral concentrations of FFA's, cortisol and prolactin. These finding are likely due to the low serum concentrations observed. Therefore, future studies should examine whether supplementation with larger doses (i.e. 300-400 mg), yet still below those reported to induce hypertensive effects (Kuske, 1969; Ziegelmayer, 1972), can influence performance. Given the training status of the participants in the present study (recreationally active), it would be of interest to investigate the influence of octopamine in well-trained individuals. Furthermore, as central catecholaminergic neurotransmission can modulate endurance performance in the heat (Roelands and Meeusen, 2010), the influence of a high ambient temperature on the ergogenic potential of octopamine should be investigated. Nevertheless, the results of the present study may be of interest to the WADA, given octopamine is currently on the list of prohibited substances, meaning its use is banned in competition by athletes.

Chapter 8

General Discussion

8.1 Overview of experimental chapters

Few studies have investigated the performance and physiological effects of caffeine during prolonged exercise in the heat. Furthermore, despite the observed differences in endurance performance and metabolic responses in low-and high-habitual caffeine users, no study had examined these effects following a prolonged period of controlled caffeine intake typical of the general population. Finally, despite reports from *in vitro* models demonstrating stimulant-like and lipolytic effects of octopamine, these responses have not been examined during prolonged exercise in humans. Therefore, the aim of the work described in this thesis was to examine the performance and physiological effects of caffeine and octopamine supplementation during prolonged cycle exercise. The main objectives were as follows.

- To investigate the performance and physiological responses following acute supplementation with low (Chapter 4) and moderate (Chapter 5) caffeine doses during prolonged exercise in the heat
- To examine the influence of a prolonged period of controlled caffeine intake on endurance cycle performance and exercise metabolism (Chapter 6)
- To determine the performance and metabolic effects of acute octopamine supplementation during prolonged exercise (Chapter 7)

The main findings of the current thesis are presented in table 8.1 and the results of each investigation are discussed relative to the outcome variables in chapters 8.2-8.6.

Table 8.1 Summary of experimental protocols and main findings from experimental chapters

Chapter	Participants	Experimental protocol	Acute dosing protocol	Main outcomes
4	Ten recreationally active males (VO_{2Peak}: 49.0 ± 3.4 $mL \cdot kg^{-1} \cdot min^{-1}$)	60 min preload @ 60% VO_{2Peak} + 30 min TT in 30°C and 50% RH	1 x 1.5 $mg \cdot kg^{-1}$ caffeine 60 min and immediately before exercise (split-dose) 1 x 3 $mg \cdot kg^{-1}$ caffeine 60 min before exercise (single-dose)	• Both caffeine trials enhanced performance versus placebo (*d*>0.42; *P*<0.029). The split-dose trial further enhanced performance than the single-dose protocol (*d*=0.32; *P*=0.059) • Thermoregulatory and metabolic responses during the preload were similar across trials (*P*>0.05) • RPE was lower during the preload after caffeine intake versus placebo (*P*<0.048). No difference was observed between the two caffeine trials (*P*=0.506)
5	Eight recreationally active males (VO_{2Peak} : 55.9 ± 5.8 $mL \cdot kg^{-1} \cdot min^{-1}$)	60 min preload @ 55% W_{max} + 30 min TT in 30°C and 50% RH	1 x 6 $mg \cdot kg^{-1}$ caffeine 60 min before exercise	• Caffeine enhanced performance (*d*=0.22; *P*=0.004) • Caffeine did not influence thermoregulation, blood variables or substrate oxidation rates (*P*>0.05) • Caffeine reduced RPE during the preload (*P*=0.033)
6	Eighteen recreationally active males (VO_{2Peak} : 51.4 ± 8.7 $ml \cdot kg^{-1} \cdot min^{-1}$)	Two groups (n=9) completed 3 trials: • Two before chronic caffeine (28 days of 1.5-3 $mg \cdot kg \cdot day^{-1}$) or placebo (Starch: 250 mg·day) ingestion (precaf and testpla) • One on day 29 (postcaf) • 60 min preload @ 60% VO_{2Peak} + 30 min TT in approx. 20°C and 50% RH	1 x 3 $mg \cdot kg^{-1}$ caffeine 60 min before exercise (precaf and postcaf)	• Chronic caffeine intake developed tolerance to an acute dose (*d*=0.31; *P*=0.025) • No change in blood variables, substrate oxidation rates or RPE during the preload
7	Ten recreationally active males (VO_{2Peak} : 50.5 ± 6.8 $ml \cdot kg^{-1} \cdot min^{-1}$)	30 min preload @ 55% W_{max} + 30 min TT in aprox. 20°C and 50%RH	1 x 150 mg octopamine 60 min before exercise	• Octopamine did not enhance performance (*d*=0.05; *P*=0380) • No change in blood variables, substrate oxidation or RPE during exercise (*P*>0.05)

Note: All studies provided a placebo trial for comparison. VO_{2Peak}, peak oxygen uptake; TT, time-trial; RH, relative humidity; RPE, rating of perceived exertion; precaf; pre-supplementation caffeine trial; testpla, pre-supplementation placebo trial; poscaf, post-supplementation caffeine trial; *d*, Cohen's *d* effect size.

8.2 Endurance performance

While caffeine supplementation consistently enhances performance during prolonged exercise in temperate environmental conditions (20^{o}C; Burke, 2008), the influence of a high ambient temperature on the ergogenic effect of caffeine has received less attention (Armstrong et al. 2007). The studies in chapters 4 and 5 investigated whether low-to-moderate caffeine doses (3-6 mg·kg^{-1}), which consistently enhance performance in temperate conditions (Burke, 2008; Ganio et al. 2009b), also confer performance benefits in the heat (30^{o}C and 50% RH). Despite some researchers suggesting that a high ambient temperature negates the capacity for caffeine to enhance performance in the heat (Cheuvront et al. 2009; Roelands et al. 2011), the results of chapters 4 and 5 demonstrate that total work produced during a 30 min performance task was increased after caffeine intake versus a placebo condition. These findings support previous studies which demonstrated a beneficial effect of caffeine intake (3-6 mg·kg^{-1}) when exercise was performed in the heat (Del-Coso et al. 2008; Cureton et al. 2007; Ganio et al. 2011; Pitchford et al. 2014).

The results of chapter 4 demonstrate that 3 mg·kg^{-1} caffeine, administered either as a single-or split-dose (2 x 1.5 mg·kg^{-1}) prior to exercise, improved endurance cycle performance. The performance benefit during the single-caffeine trial supports the results of Pitchford et al (2014), whereby trained cyclists completed an energy-based TT 7% quicker after ingesting 3 mg·kg^{-1} caffeine. To date, these are the only studies to investigate the ergogenic effect of 3 mg·kg^{-1} caffeine and suggest that even low doses are sufficient to benefit performance in the heat. An interesting finding of chapter 4 was that dividing the caffeine bolus appeared to confer an even greater performance benefit than single-dose administration (*d*=0.32; *P*=0.059; 78.5% likelihood of benefit). This additive benefit is not observed when exercise is performed in temperate conditions (Conway et al. 2003; Cox et al. 2002), suggesting the addition of a high ambient temperature might provide an environment more suitable for repeated caffeine doses to further enhance performance. The precise mechanism mediating this response is not clear, but may reflect a change in adenosine receptor sensitivity or function during exercise. Acute bouts of exercise can influence the adenosine receptor-mediated response to insulin (Langforf

et al. 1993), while exercise and adenosine receptor antagonists interact to regulate antioxidant responses in cardiac tissue (Husain and Somani, 2005). In rats, repeated versus single injections of cocaine differentially influenced calcium signalling in striatum (Kim et al. 2009). Intracellular calcium regulates the function of the striatal A_{2A}/D_2 heteromer (Navarro et al. 2014), a primary target of caffeine (Ferré, 2008). Hence, calcium-mediated changes in striatal A_{2A}/D_2 signalling might result in enhanced motor cortex excitability and thus stronger efferent projections to the motor neurons innervating the locomotor muscles during the split-caffeine trial, thereby enabling a higher power output during the performance task. However, this is purely speculative.

The results of chapter 5 demonstrate that 6 $mg \cdot kg^{-1}$ caffeine administered 60 min prior to exercise is sufficient to enhance performance in the heat. This supports the findings of Ganio et al (2011), who examined the ergogenic effect of 6 $mg \cdot kg^{-1}$ caffeine in cool (11^{o}C) and high (33^{o}C) ambient conditions in the same participant group. These authors reported improvements in 15 min TT performance after caffeine supplementation independent of the ambient temperature. Furthermore, Del-Coso et al (2008) reported improvements in VA after exercise when 6 $mg \cdot kg^{-1}$ caffeine was ingested either without fluid, with water to replace 97% of sweat loss, or with the same volume of a 6% carbohydrate-electrolyte drink, versus these treatments without caffeine. To date, the only study which failed to report an ergogenic effect of 6 $mg \cdot kg^{-1}$ caffeine was that of Roelands et al (2011). These authors supplemented participants with caffeine 60 min before prolonged exercise and reported similar performance times during an energy-based TT (approx. 30 min). These data clearly contrast with the results of chapter 5 and those of Ganio et al (2011), which could be due to differences in the core temperature response during exercise (discussed in chapter 8.3). Alternatively, performance benefits after caffeine intake can be highly variable during exercise in temperate conditions (Jenkins et al. 2008), which may also occur when exercise is performed in the heat. Therefore, more non-responders to caffeine may have been present in the study of Roelands et al (2011) than in Ganio et al (2011) and chapter 5. Finally, the contrasting findings between studies (Ganio et al. 2011; Roelands et al. 2011) might be due to the dosing strategy employed.

Whereas Roelands et al (2011) administered caffeine as a single bolus before exercise, Ganio et al (2011) administered a 3 $mg \cdot kg^{-1}$ caffeine dose 90 min before and 45 min during exercise.

Despite the widespread intake of caffeine (Desbrow and Leveritt, 2006; Fitt et al. 2013; Fredholm et al. 1999), few studies have investigated the influence of habituation on exercise performance (Bell and McLellan, 2002; Dodd et al. 1991; Irwin et al. 2011; Roti et al. 2006). The performance benefit of an acute 5 $mg \cdot kg^{-1}$ caffeine dose was less pronounced in regular caffeine users versus their caffeine naive counterparts (Bell and McLellan, 2002), while chronic caffeine intake influenced the metabolic response to steady-state exercise (Bangsbo et al. 1992). However, a four-day period of controlled caffeine intake failed to influence the ergogenic effect of a subsequent acute caffeine dose (Irwin et al. 2011), suggesting the habituation protocol was too short to influence mechanisms that develop tolerance. Accordingly, chapter 6 investigated whether a more prolonged period of controlled caffeine intake could influence endurance cycle performance. In contrast with previous studies (Irwin et al. 2011; Roti et al. 2006), chronic caffeine intake resulted in the development of tolerance. The ergogenic effect of an acute 3 $mg \cdot kg^{-1}$ caffeine dose was less pronounced after the habituation period, while performance was unchanged in the placebo group.

Several mechanisms are proposed to explain the development of tolerance. In rats, long-term caffeine treatment is associated with an increased expression of adenosine A_1 and A_{2A} receptors in several brain regions (Johansson et al. 1993; Svenninggsson et al. 1999). However, changes in adenosine receptor density does not affect the binding potency of an antagonist (Holtzman et al. 1991), while tolerance to caffeine can occur without any change in adenosine receptor expression (Georgiev et al. 1993). There is evidence to suggest that chronic caffeine intake (14 days) attenuates the caffeine-mediated increase in striatal dopamine release (Quarta et al. 2004), although very high doses were administered (130 $mg \cdot kg \cdot day^{-1}$). Chronic exposure to caffeine in the drinking water of rats resulted in partial tolerance to the performance benefit of a selective A_1 but not an A_{2A} antagonist (Karcz-Kubicha et al. 2003). These data suggest a cross-tolerance to the A_1 receptor plays an important role in the development of tolerance and that the residual ergogenic effects of caffeine is mostly due to A_{2A} blockade. The lack of

tolerance after repeated administration of a selective A_{2A} antagonist (Halldner et al. 2000) provides further support for this hypothesis. The cross-tolerance to A_1 blockade may involve a functional change in the striatal A_1/A_{2A} heteromer in the presence of increased extracellular adenosine (Ferré et al. 2008). Chronic exposure to caffeine augments circulating adenosine (Conway et al. 1997), which occupies a greater concentration of A_{2A} receptors in this heteromer (Ciruela et al. 2006). This inhibits A_1 function through an intramembrane interaction, thus attenuating caffeine-mediated blockade of the A_1 receptor (Ciruela et al. 2006; Ferré et al. 2008). The accumulation of cerebral adenosine following chronic caffeine administration (Conway et al. 1997) impairs running performance in rats (Davis et al. 2003), likely through a decrease in neuronal firing rates (Fredholm et al. 1999). Therefore, tolerance to caffeine may result in a decrease in central motor output during exercise due to enhanced adenosinergic neurotransmission in the motor-related areas of the brain where A_1 receptors are densely expressed (Fredholm et al. 1999; Table 2.3). Under these conditions, a larger dose of caffeine will be required to tonically antagonise the same concentration of adenosine receptors. However, this hypothesis has yet to be investigated, and no study has demonstrated that elevated cerebral adenosine impairs endurance performance in humans.

Despite octopamine being placed on the WADA list of prohibited substances (WADA, 2015) and data from animal models (Jagiełło-Wójtowicz, 1979) and cell-lines (Borowsky et al. 2001) suggesting stimulant-like properties, no study had investigated the performance effects of octopamine in humans. Therefore, chapter 7 investigated the whether an acute 150 mg dose of octopamine administered 60 min before prolonged exercise could benefit endurance performance. Compared with a placebo condition, octopamine supplementation did not enable participants to produce more work during a 30 min performance task. Therefore, at the dose prescribed, it seems octopamine is not ergogenic to endurance cycle performance in temperate conditions.

Humans do not possess specific octopamine receptors, yet the identification of the TAAR's has identified a potential central role for octopamine (Borowsky et al. 2001; Bunzow et al. 2001). While the precise function and distribution of these receptors is still being elucidated,

octopamine binds to TARR1 with highest affinity (Borowsky et al. 2001). This receptor is located throughout the brain (Borowsky et al. 2001; Linderman et al. 2008; Xie et al. 2007) and is interconnected with the monoamine neurotransmitter systems (Pei et al. 2016; Xie et al. 2007; Xie and Miller, 2009). Specifically, TARR1 has been implicated in dopamine release and reuptake inhibition (Miller, 2011). However, due to the high turnover rate of octopamine via monoamine oxidase, cerebral concentrations are several hundred-fold lower than classical monoamine neurotransmitters (Berry, 2004). As such, octopamine is unlikely to directly influence the firing rate of postsynaptic neurons (Lindermann and Hoener, 2005). In support, Berry (2004) argues that physiologically relevant doses of octopamine do not change the electrical activity of a postsynaptic neuron, but instead modify the action of a coexisting neurotransmitter. This was demonstrated for the trace amine tyramine on rat mid-brain GABA neurons (Federici et al. 2005). Furthermore, octopamine is rapidly conjugated after oral ingestion, resulting in a larger concentration of inactive by-products several hours post-ingestion (Hengstmann et al. 1974). Given these observations, it seems the combination of high cerebral turnover rates, extensive metabolism in the periphery, and the provision of a relatively low dose precluded any performance effect of octopamine. Therefore, while octopamine possesses stimulant-like properties *in vitro* (Borowsky et al. 2001; Xie and Miller, 2009), these effects doe not translate to a performance benefit following low dose oral ingestion in humans.

8.3 Thermoregulation

There is evidence to suggest that caffeine elevates core temperature during prolonged exercise in the heat (Ely et al. 2011; Cheuvront et al. 2009; Roelands et al. 2011), although this response is not always observed (Ganio et al. 2011; Pitchford et al. 2014). While larger caffeine doses (>9 $mg \cdot kg^{-1}$) induce consistent elevations in core and body temperature (Cheuvront et al. 2009; Ely et al. 2011), the influence of lower doses (3-6 $mg \cdot kg^{-1}$) are less clear (Ganio et al. 2011; Pitchford et al. 2014; Roelands et al. 2011). Given these divergent findings, core and skin temperature was measured at rest and during prolonged exercise following low (chapter 4) and moderate (chapter 5) caffeine intakes.

The results of chapter 4 demonstrate that 3 mg·kg^{-1} caffeine, whether administered as a single 3 mg·kg^{-1} bolus or 2 x 1.5 mg·kg^{-1} doses before exercise, does not influence core or skin temperature during 60 min of fixed-intensity exercise versus a placebo condition. The core temperature data supports the results of a recent cycle TT study conducted in a hot, dry environment (35^{o}C and 25% RH; Pitchford et al. 2014). However, these authors did not include a period of fixed-intensity exercise before the TT, thus changes in self-selected power output could have masked subtle changes in core temperature during exercise. Furthermore, skin temperature was not measured, which is suggested to play a key role in the development of fatigue during prolonged exercise in the heat (Cheuvront et al. 2010). Therefore, chapter 4 was the first study to measure core and skin temperature during exercise at a fixed work-rate. The results suggest that, unlike larger caffeine doses (Cheuvront et al. 2009; Ely et al. 2011), intakes of 3 mg·kg^{-1} do not adversely influence thermoregulation when endurance exercise is performed in the heat.

Similar to chapter 4, the core temperature data from chapter 5 suggests that moderate caffeine doses (6 mg·kg^{-1}) do not influence thermoregulation during prolonged exercise in the heat versus placebo. Ganio et al (2011) also reported that 6 mg·kg^{-1} caffeine did not alter core or skin temperature responses to exercise in cool (11^{o}C) and warm (33^{o}C) ambient conditions. To date, the only study to report a caffeine-induced increase in core temperature in the heat without carbohydrates was that of Roelands et al (2011). These authors reported a greater increase in core temperature during 60 min of fixed-intensity exercise after supplementation with 6 mg·kg^{-1} caffeine compared with placebo. The difference in core temperature responses between this study and those of chapter 5 are not clear, but it should be noted that the caffeine-induced increase in core temperature in the study of Roelands et al (2011) did not impair subsequent TT performance. Furthermore, Del-Coso et al (2009) reported a 0.2-0.3^{o}C increase in core temperature during 120 min of fixed-intensity exercise in 36^{o}C when 6 mg·kg^{-1} caffeine was ingested with a 6% carbohydrate-electrolyte solution versus the drink without caffeine. However, these authors did not observe any difference in core temperature when caffeine and water was co-ingested versus isolated water intake. The results of chapter 5 support these latter findings, as participants received caffeine and water without additional

carbohydrates during the experimental trials. Finally, similar to the performance differences discussed in chapter 8.2, the contrasting thermoregulatory findings between studies (Ganio et al. 2011; Roelands et al. 2011) might be due to the dosing strategy employed (i.e. single-versus split-dose administration). The similar core temperature values observed during the 60 min preload in the single-and split-caffeine trials in chapter 4 provide some support for this hypothesis. However, further research is required.

8.4 Hormonal concentrations

Serum cortisol and prolactin were measured during each investigation as these hormones provide an indirect assessment of central noradrenergic (Tsigos and Chrousos, 2002) and dopaminergic (Ben-Jonathan and Hnasko, 2001) activity, respectively. Cerebral noradrenaline and dopamine are implicated in regulating arousal, motivation, reinforcement and reward (Girault and Greengard, 2004; Sara and Bouret, 2012), thus influencing the activity of one or both of these neurotransmitters would be expected to influence performance.

Despite data from animals models demonstrating that caffeine administration increases dopamine release in striatal (Borycz et al. 2007; Solanis et al. 2002; Quarta et al. 2004) and non-striatal (Zheng et al. 2014) regions, the lack of change in circulating prolactin in chapters 4, 5, and 6 suggest that caffeine ingestion doses not influence dopamine release in humans. The findings from this thesis support those of two previous PET studies conducted on resting human participants following acute supplementation with 200-300 mg caffeine (Kaasinen et al. 2004; Volkow et al. 2015). The difference between human and animal models might be explained by several factors: 1) the doses administered in previous animal models (30 $mg \cdot kg^{-1}$; Solanis et al. 2002) are much higher than necessary to enhance endurance performance in humans (3-6 $mg \cdot kg^{-1;}$ Desbrow et al. 2012), 2) the route of administration (i.e. oral ingestion versus intraperitoneal injection), 3) differences in adenosine receptor distribution and binding affinities between species (Fredholm et al. 1999; Fredholm et al. 2011), and 4) rodents and humans differ in their thermoregulatory physiology (Gordon, 1990), thus limiting the extrapolation of the findings obtained from one system to another (chapters 4 and 5).

Furthermore, while caffeine ingestion was shown to increase noradrenaline turnover in rats (Miñana and Grisolía, 1986), more recent studies using microdialysis techniques have failed to report any change in cerebral noradrenaline after caffeine administration (Zheng et al. 2014; Zheng et al. 2016). The lack of change in circulating cortisol in chapters 4, 5 and 6 support these latter observations. However, no human PET study has investigated the influence of caffeine intake on central noradrenaline release.

Similar to the findings discussed above, octopamine supplementation did not influence peripheral prolactin concentrations in chapter 7. Despite the link between octopamine, TAAR1 and central dopaminergic activity *in vitro* (Pei et al. 2016; Xie et al. 2007; Xie and Miller, 2009), doses of 2-20 μM are typically required to influence TAAR1 on mammalian transfected cell-lines (Lindemann et al. 2005). These concentrations are much larger than the serum levels observed during the experimental trials (0.95 to 1.24 μM). Similarly, octopamine supplementation did not influence serum cortisol, suggesting no effect on central noradrenaline release. While one rodent study reported a decrease in GABAeric neurotransmission following octopamine administration (Jagiełło-Wójtowicz and Chodkowska, 1984), no animal or human studies have directly assessed cerebral noradrenaline activity after acute octopamine supplementation.

8.5 Substrate oxidation

Whole-body fat and carbohydrate oxidation rates and RER were estimated during 60 min (chapters 4, 5 and 6) or 30 min (chapter 7) of fixed-intensity exercise before the performance task.

Despite promising results form early studies (Costill et al. 1978; Essig et al. 1980; Ivy et al. 1979), there is little doubt that caffeine exerts little to no influence on skeletal muscle metabolism during exercise (Graham et al. 2008). This has been shown with stable-isotopic tracer (Hulston and Jeukendrup, 2008) and muscle biopsy techniques (Graham et al. 2008). There was no difference in fat and carbohydrate oxidation or RER values across trials in chapters 4 and 5, suggesting the addition of a high ambient temperature exerts no influence on

caffeine's effects on substrate metabolism during prolonged exercise. In support, Ganio et al (2011) also reported no effect of caffeine on RER during exercise in cool and warm environmental conditions. Furthermore, the results of chapter 6 suggest that neither acute (3 $mg \cdot kg^{-1}$) nor chronic (1.5-3 $mg \cdot kg^{-1}$) supplementation of caffeine induced any change in estimated substrate oxidation rates. While previous studies and the findings from this thesis strongly suggest that caffeine does not influence substrate metabolism during exercise, the prevalence of specific SNP's across individuals may partly explain some small differences reported (Graham et al. 2008). However, these findings are from investigations conducted in a temperate environment, and no study has assessed the influence of different SNP's on substrate metabolism following a prolonged period of controlled caffeine intake.

Observations from *in vitro* models suggest that octopamine can selectively and potently bind to β_3 adrenoreceptors and stimulate lipolysis in mammalian fat cells (Carpéné et al. 1999), while expressing little affinity for the α-adrenergic receptors (Brown et al. 1988). Thus, octopamine supplementation could influence fat metabolism in humans. However, no differences were observed across trials for fat and carbohydrate oxidation or circulating FFA concentrations. Similar to TAAR1 activity discussed in chapter 8.4, the contrasting findings between studies is likely due to the low serum levels of octopamine; *in vitro* models typically require concentrations of 10 µM to 1 mM to induce lipolysis (Carpéné et al. 1999; Visentin et al. 2001). Furthermore, when rats were administered with daily injections of 15.3 $mg \cdot kg^{-1}$ octopmaine, no change in circulating FFA's, glycerol or triglyceride concentrations were observed (Bour et al. 2003). Therefore, based on these observations, it is unlikely that low oral doses of octopamine influence fat metabolism in humans. However, the influence of larger acute doses and chronic supplementation has not been investigated.

8.6 Perceptual variables

Caffeine consistently attenuates RPE during prolonged exercise in temperate conditions (Doherty and Smith, 2005), while other stimulant-based compounds such as reuptake inhibitors can influence perceptual variables during endurance exercise in temperate and warm

environmental conditions (Roelands and Meeusen, 2010). Therefore, RPE was recorded during each of the investigations in this thesis. Furthermore, perceived thermal stress was measured when caffeine was ingested prior to exercise in the heat (chapters 4 and 5).

During self-paced cycle exercise in the heat, caffeine intake enables participants to maintain a higher power output despite a similar RPE (Pitchford et al. 2014). This suggests a reduced relative perception of effort during exercise. Furthermore, caffeine intake reduces absolute RPE values during exercise at a fixed-work rate in temperate conditions (Doherty and Smith, 2005). Chapter 4 was the first study to investigate whether 3 $mg \cdot kg^{-1}$ caffeine could reduce RPE during exercise at a fixed work-rate in warm ambient conditions and demonstrates that even low doses are sufficient to attenuate perception of effort during prolonged exercise. A similar response was observed in chapter 5 when a 6 $mg \cdot kg^{-1}$ caffeine dose was ingested before exercise. However, in chapter 6, an acute 3 $mg \cdot kg^{-1}$ caffeine dose did not influence RPE responses during 60 min of fixed-intensity exercise before or after a chronic supplementation period, despite an increase in performance. While the RPE responses during the preload were not different across trials, absolute RPE values were also similar during the performance task. Therefore, when caffeine enhanced performance (precaf trial in both groups and postcaf trial in the placebo group) the relative perception of effort was lower (i.e. higher work-rate for the same RPE). Conversely, when there was a reduction in performance (i.e. postcaf versus precaf trial in the caffeine group) the relative perception of effort increased (i.e. lower work-rate for the same RPE). In contrast to the above observations, octopamine supplementation did not influence RPE values during the fixed-intensity exercise or the performance task (chapter 7).

Perception of effort during exercise is believed to reflect changes in central motor command (de Morree et al. 2012). Specifically, RPE is centrally generated from forwarding neural signals, or corollary discharges, from motor to sensory areas of the cerebral cortex (Marcora, 2009). Therefore, when inducing muscle fatigue with epidural anaesthesia (Kjaer et al. 1999; Smith et al. 2003), it is not surprising that the increase in central motor drive required to exercise at the same power output is perceived as increased effort. Thus, using this model, reducing RPE would be expected to enhance performance. The results of a recent brain imaging study support this

hypothesis, as 6 mg·kg^{-1} caffeine reduced the activity of pre motor and motor areas of the cerebral cortex in association with a reduction in RPE, despite a similar power output versus placebo (de Morree et al. 2014). The results of this thesis also support these findings, and suggest that low (chapter 4) and moderate (chapter 5) caffeine doses are capable of reducing RPE during fixed-intensity exercise with a subsequent increase in endurance performance, even when exercise is performed in the heat. Furthermore, it was suggested that adenosine accumulation in the pre-supplementary area of the motor cortex could partly explain the higher RPE values reported during a mentally-engaging task (Pageaux et al. 2013; Pageaux et al. 2014; Pageaux et al. 2015). Given that chronic caffeine intake increases cerebral adenosine concentrations (Conway et al. 1997), the increased relative perception of effort reported after chronic caffeine intake in chapter 6 (i.e. postcaf versus precaf trial) may be partly due to enhanced adenosinergic neurotransmission and a corresponding increase in central motor command (thus increasing RPE). Finally, the similar RPE values and lack of performance benefit reported after octopamine supplementation in chapter 7 would suggest this purported stimulant-based compound did not affect the neural substrates of perception of effort during exercise.

Perceived thermal stress was also recorded during the investigations in chapters 4 and 5. In contrast to the RPE data, caffeine supplementation did not influence perceived thermal stress during the fixed-intensity exercise. This is not surprising given that core and skin temperature values were similar across trials in both investigations. However, higher core temperatures were achieved during the performance task after caffeine intake (due to increased work-rate), with similar thermal stress responses versus placebo in both studies. This was also demonstrated for the dual dopamine-noradrenaline reuptake inhibitor bupropion (Watson et al. 2005a), with these authors suggesting attenuated afferent feedback from peripheral tissues during exercise. While the PO/AH receives input from numerous systems during exercise, including peripheral and spinal thermoreceptors, as well as skin, core and blood temperature (Boulant, 2000), the increased afferent feedback during the performance task (i.e. elevated core temperature due to increased work-rate) did not adversely influence performance. These data suggest that the conscious self-regulation of power output during exercise, and not

afferent feedback from peripheral systems, is more important in determining endurance cycle performance (Marcora, 2009).

8.7 Limitations and directions for future research

While the studies contained within thesis have provided novel findings relating to the performance and physiological effects of caffeine and octopamine during prolonged exercise, several limitations should be addressed. Firstly, all studies were performed on recreationally active participants and therefore it is not clear if these results would translate to the effects observed in well-trained cyclists; this should be explored in future studies. Secondly, each investigation required participants to exercise in a fasted state. While this isolates the performance and physiological effects of the intervention without other potential confounding factors (i.e. carbohydrates), this is not how individuals would nutritionally prepare for an endurance event on race day. Therefore, future studies should investigate a more sport-specific nutrition strategy and how this influences the performance and physiological effects of caffeine and octopamine supplementation. Thirdly, all investigations were performed in a laboratory environment. While this enables a tighter control of potential confounding factors (i.e. changes in temperature), this environment is far different from a field setting where other variables are likely to influence performance (i.e. pacing by other competitors). Therefore, field studies are necessary to truly elucidate the performance effects of caffeine and octopamine. Fourthly, each investigation employed a self-paced performance task as the performance measure. While this is a sensitive and reliable test (Sewell and McGregor, 2008), a time to exhaustion protocol also provides a valid measure of performance (Laursen et al. 2007). Therefore, future studies should employ different exercise models to further explore the performance effects of caffeine and octopamine.

The results of chapters 4 and 5 demonstrate performance benefits of caffeine for individuals not accustomed to exercise in the heat. While it seems logical to suggest that these effects would translate to heat-acclimatised individuals due to the additional physiological mechanisms in place to attenuate the detrimental effects of heat strain (i.e. plasma volume expansion,

increase in sweat rate, lower resting core temperature etc), this has yet to be investigated. Furthermore, a limitation of the investigation in chapter 6 was that the post-supplementation caffeine trial (postcaf) was not performed after 24-hours of caffeine withdrawal in accordance with the previous experimental trials (testpla and precaf). However, this was unlikely to have adversely influenced the performance data, as several days of caffeine withdrawal are required to re-sensitise individuals to the acute effects of the drug (Fisher et al. 1986). Furthermore, the experimental protocol in chapter 6 could not determine when the tolerance to caffeine occurred, or the mechanisms which mediated this response. Therefore, future studies could include more frequent trials (i.e. every 7-days) and employ direct measurements of brain function similar to previous studies (de Morree et al. 2014) to determine the neurophysiological responses to acute caffeine intake before and after chronic supplementation. Furthermore, as the study in chapter 6 examined low-caffeine consumers, the influence of a prolonged period of controlled caffeine intake should be investigated in participants with varied habitual caffeine intakes. Finally, the study in chapter 7 examined the influence of a low dose of octopamine in temperate conditions. Therefore, larger doses of octopamine should be investigated in temperate and high ambient conditions.

8.8 Practical applications

Despite the limitations mentioned above, the results from the studies contained within this thesis have several important implications. Firstly, the results from chapters 4 and 5 suggest that acute supplementation with low-to-moderate caffeine doses are sufficient to benefit endurance performance in the heat, without adversely influencing thermoregulation or cardiovascular function during exercise. Furthermore, caffeine appears to attenuate perceived exertion during prolonged fixed-intensity exercise. These responses support the results of studies conducted in temperate conditions (Burke, 2008; Doherty and Smith, 2005) and suggest that heat strain does not negate the efficacy of caffeine supplementation. An interesting finding of chapter 4 was that dividing the 3 $mg \cdot kg^{-1}$ caffeine bolus in to 2 x 1.5 $mg \cdot kg^{-1}$ doses appeared to confer an additional performance benefit (*d*=0.32), although this did not quite reach

significance (*P*=0.059). These data suggest that ingesting repeated low-doses of caffeine may potentiate the efficacy of the same total caffeine dose when exercise is performed in the heat.

Chapter 6 demonstrated a performance decrement to an acute 3 $mg \cdot kg^{-1}$ caffeine dose following long-term supplementation in low-habitual caffeine consumers. Therefore, the negative influence of habitation on endurance performance may only be relevant to a small portion of individuals, given the high prevalence of caffeine intake in the general population (Fitt et al. 2013) and by athletes during competition (Desbrow and Leveritt, 2006). Nonetheless, individuals with low-habitual intakes should refrain from chronic caffeine supplementation in order to maximise the ergogenic effect attained from acute supplementation.

The findings reported in chapter 7 suggest that low doses of octopamine do not influence endurance performance or exercise metabolism. While the influence of larger doses (>150 mg) are unknown, individuals should refrain from ingesting this compound until more studies have examined the effects of larger doses across different ambient conditions in recreationally active and well-trained participants.

8.9 Conclusions

The work presented in this thesis has attempted to further explore the ergogenic and physiological effects of caffeine during prolonged exercise, while aiming to elucidate a potential ergogenic role for octopamine. Acute supplementation with low and moderate caffeine doses benefits endurance cycle exercise in the heat, without augmenting core temperature versus placebo. Therefore, caffeine doses which consistently enhance improve performance in temperate conditions (Burke, 2008; Ganio et al. 2009b) also benefit endurance performance in the heat. Chronic supplementation with a titrated low-dose of caffeine induces the development of tolerance in low-habitual caffeine users. These findings confirm the results when comparing low-and high-habitual caffeine users (Bell and McLellan, 2002), but contrast with studies which employed sub-chronic supplementation protocols (Irwin et al. 2011; Roti et al. 2006). Therefore, relatively short habituation protocols do not appear to influence the

mechanisms which develop tolerance. Finally, an acute 150 mg dose of octopamine did not influence endurance performance, substrate oxidation, circulating hormonal concentrations or RPE. Therefore, at the dose prescribed, octopamine does not appear to benefit endurance performance or alter the metabolic or perceptual response to prolonged cycle exercise in temperate conditions.

Key points:

1) A single and divided low dose of caffeine improves endurance cycle performance in the heat without adversely influencing thermoregulation during exercise. A greater performance benefit may be attained form dividing the caffeine bolus, although further work is required to confirm this observation.

2) A moderate caffeine dose improves endurance cycle performance in the heat without augmenting core temperature during prolonged exercise at a fixed work-rate.

3) Chronic supplementation with a titrated low dose of caffeine results in the development of tolerance in low-habitual caffeine users.

4) An acute oral dose of octopamine does not influence endurance cycle performance, estimated substrate oxidation rates or the peripheral concentrations of FFA's, cortisol and prolactin.

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Appendix

Perceived thermal stress scale

-10 Cold impossible to bear

-9

-8 Very cold, shivering hard

-7

-6 Cold, light shivering

-5

-4 Most areas of the body feel cold

-3

-2 Some areas of the bold feel cold

-1

0 Neutral

1

2 Some areas of the body feel warm

3

4 Most areas of the body feel hot

5

6 Very hot, uncomfortable

7

8 Extremely hot, close to limit

9

10 Heat impossible to bear