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
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**THE ROLE OF CENTRAL CATECHOLAMINES IN
PERFORMANCE DURING PROLONGED EXERCISE IN
WARM CONDITIONS**

By

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A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy
of Loughborough University

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Abstract

Performance during prolonged exercise capacity diminishes with increasing temperatures. The onset of fatigue under these conditions is not adequately explained by peripheral mechanisms. Recently, drugs which inhibit the reuptake of dopamine and noradrenaline in the brain have been found to improve exercise performance in warm conditions. The aim of this thesis was to further explore and characterise the role of these neurotransmitters during prolonged exercise in warm conditions by manipulating their reuptake or synthesis.

The first series of experiments were designed to further investigate the effects of bupropion, a dopamine and noradrenaline reuptake inhibitor, which has been found to improve performance in warm conditions. To explore gender differences in response to acute bupropion administration, the effects of bupropion on prolonged exercise performance in warm conditions in women was investigated in Chapter 3. The results of this study suggest that during the follicular phase of the menstrual cycle, acute administration of bupropion improves exercise performance. To determine whether there are any dose-dependent effects of bupropion, the experiment in Chapter 4 was designed to test three different doses of bupropion. Exercise performance was only improved for the maximal dose, suggesting a threshold for the performance effects of bupropion.

Catecholamine precursors do not appear to improve exercise performance as consistently as reuptake inhibitors. In agreement with previous studies, the dopamine precursor L-DOPA did not affect exercise performance in warm conditions in Chapter 5. In Chapter 6 the effect of the atypical antidepressant nutritional supplement S-adenosylmethionine was investigated for its role in the synthesis of dopamine and noradrenaline. S-adenosylmethionine appeared to negatively influence cognitive function, increased skin temperature and circulating prolactin concentrations, but no effects on exercise performance were observed.

Keywords: central nervous system, heat strain, central fatigue, dopamine, noradrenaline, serotonin.

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List of Abbreviations

Abbreviations are defined in the text in the first instance.

AAAD	Aromatic L-amino acid decarboxylase
ACC	Anterior cingulate cortex
ACTH	Adrenocorticotrophic hormone
ADHD	Attention deficit hyperactivity disorder
AMPT	Alpha-methyl-para-tyrosine
ANOVA	Analysis of Variance
AUC	Area under the curve
BCAA	Branched-chain amino acid
cAMP	Cyclic adenosine monophosphate
CCT	Cytidine 5'-triphosphate: phosphocholine cytidyltransferase
C_{max}	Maximum plasma concentration
CNS	Central nervous system
CO₂	Carbon dioxide
COMT	Catechol-O-methyltransferase
CRF	Corticotropin-releasing factor
CV	Coefficient of variation
DAT	Dopamine transporter
EEG	Electroencephalogram
ELISA	Enzyme-linked immunosorbent assay
ERN	Error-related negativity
FSH	Follicle stimulating hormone
GH	Growth hormone
Hb	Haemoglobin
Hct	Haematocrit
HPA	Hypothalamic-pituitary adrenal axis
HPLC	High performance liquid chromatography
K₂EDTA	Potassium ethylenediaminetetraacetic acid
L-DOPA	L-3,4-dihydroxyphenylalanine

LH	Luteinising hormone
LNAAs	Large neutral amino acid
MAO	Monoamine oxidase
MRI	Magnetic resonance imaging
MTHF	Methyltetrahydrofolate
NAc	Nucleus accumbens
NAT	Noradrenaline transporter
NTS	Nucleus tractus solitarius
O₂	Oxygen
PET	Positron emission tomography
PFC	Prefrontal cortex
PVN	Paraventricular nucleus of the hypothalamus
RPE	Rating of perceived exertion
RVIP	Rapid visual information processing
SAM	S-adenosylmethionine
SD	Standard deviation
STPD	Standard temperature and pressure for dry gas
T_{core}	Core temperature
TH	Tyrosine hydroxylase
TIDA	Tuberoinfundibular dopamine
TMS	Transcranial magnetic stimulation
TPH	Tryptophan hydroxylase
T_{skin}	Weighted mean skin temperature
TT	Time trial
VO_{2max}	Maximum oxygen uptake
VO_{2peak}	Peak oxygen uptake
VTA	Ventral tegmental area
W	Watt
W_{max}	Maximum workload
W_{out}	Workload of final stage

Chapter 1

General Introduction

1.1 - Central Versus Peripheral Fatigue

Fatigue during exercise has been defined as “the inability to maintain the required or expected power output that leads to a loss of performance in a given task” (Edwards, 1981) and “any exercise-induced reduction in the ability to exert muscle force or power, regardless of whether or not the task can be sustained” (Bigland-Ritchie & Woods, 1984). In this sense, performance and fatigue are intrinsically related. Arguments for the aetiology of fatigue during exercise have been largely divided into two camps: peripheral and central fatigue. The dichotomy of peripheral and central fatigue during exercise was considered as early as 1891 by Augustus Waller, who suggested that the central component may act as a protective mechanism over peripheral fatigue. Peripheral fatigue is predominantly characterised by changes in muscle contractility as a result of changes to the contractile properties in the muscle itself. For example, this can be caused by changes in substrate availability, accumulation of metabolites or changes in muscle temperature. Central fatigue refers to mechanisms within the brain which control muscle contractility via descending corticospinal motor pathways. Alessandro Mosso (1903) later concluded that “there exists only one kind of fatigue, namely, nervous fatigue; this is the preponderating phenomenon, and muscular fatigue is also at bottom an exhaustion of the nervous system” (p.243), while Francis Bainbridge elaborated by suggesting that muscular fatigue was superadded to nervous fatigue (1919). However, following the famous work of Archibald Hill (Hill & Lupton, 1923), a greater emphasis was subsequently placed on peripheral mechanisms. Research in this area developed significantly with the advent of analytical techniques such as the muscle biopsy during the 1960s. Bergström and co-workers (1967), demonstrated the importance of muscle glycogen stores for performance during prolonged exercise, further emphasising the importance of muscle metabolism in fatigue. However, in the early 1980s, the central component of fatigue was re-established with the application of twitch interpolation (Grimby *et al.*, 1981; Belanger & McComas, 1981) and transcranial magnetic stimulation (TMS)(Barker *et al.*, 1985). Experiment using these techniques revealed that a difference of force produced in maximal voluntary contractions compared to the force elicited by a superimposed contraction gradually increased with fatigue. This suggested that a central component was limiting the voluntary contractions. In addition to contributing to peripheral fatigue, the

mechanisms for fatigue within the muscle are detected by afferent sensory neurons, which sense a broad range of factors including pH, temperature and accumulation of metabolites. These sensory neurons provide feedback to the CNS, which can modulate motor neurons and inhibitory interneurons, suggesting peripheral and central mechanisms interact to affect performance (Gandevia, 2001). The primary mechanisms in the onset of fatigue depend on the type of exercise and the environment in which it is being performed. Complex movements such as running or cycling are more difficult to analyse, particularly using imaging techniques or TMS, because of the constant movement and ergometers required. Recently, the first study using TMS during prolonged cycling found that cortical excitability does not increase, in contrast to isolated single-muscle exercise. The authors suggest that the greater number of challenges to homeostasis during prolonged cycling may contribute to intracortical inhibitory mechanisms (Sidhu *et al.*, 2012). Similarly, there is evidence that prolonged exercise is modulated by a teleoanticipatory system, which influences pacing relative to feedback (St Clair Gibson *et al.*, 2004, 2006; Noakes *et al.*, 2005). Nonetheless, the degree to which central and peripheral mechanisms determine fatigue in prolonged exercise is still hotly debated (Shephard, 2009; Noakes, 2011a, 2011b). However, in warm conditions, this central component appears to be even especially pronounced (Nybo & Nielsen, 2001a; Nybo, 2008), as discussed below.

1.2- Prolonged Exercise in Warm Conditions

Endurance exercise capacity is reduced in warm conditions. This effect has been demonstrated to be dependent on the rate of heat-gain, which becomes impaired as ambient temperature increases (Galloway & Maughan, 1997). The combination of basal metabolic rate, external work, environmental factors and the body's ability to dissipate heat contribute to this process. The 5 main routes through which heat is lost from the body are conduction, convection, radiation, evaporation and respiration. As the skin to ambient temperature gradient narrows, sweating becomes the only effective mechanism for heat loss. Maximal heat loss via sweating occurs when sweat evaporates from the skin. Large amounts of energy are required to drive the phase change from liquid water to vapour. As a result, the transition of sweat into

water vapour removes considerable thermal energy from the surface of the skin. For the same reason, sweat that drips off the body or is wiped away is considerably less effective for heat loss (Havenith, 2005). Consequently, prolonged exercise performance in warm conditions is further impaired by increasing humidity (Maughan *et al.*, 2012). This is due to increased water vapour saturation of the air which increases the competition between evaporation of water molecules from the skin and condensation of water molecules from the air onto the skin. However, because air can contain larger concentrations of moisture with increased temperature, the same percentage relative humidity of air at higher temperatures will contain a greater concentration of moisture.

These significant challenges to thermoregulation and fluid balance are considered to be the primary causes of fatigue during prolonged exercise in warm conditions (Hargreaves, 2008; Nybo, 2008; Maughan, 2010). During exercise in warm conditions the narrow core-to-skin temperature gradient results in an increased demand of blood flow to the skin to facilitate heat loss. The water and electrolytes which form the majority of sweat are mobilised from intracellular fluid compartments to maintain blood volume (Nose *et al.*, 1988). As exercise continues there is a progressive reduction in blood volume, due to the loss of fluid as sweat, resulting in a fall in cardiac output and a compromised muscle and skin oxygen and nutrient supply via blood flow (Gonzalez-Alonso *et al.*, 2007). Reduced blood flow also diminishes the capacity for convective heat loss from muscle and body core to the surrounding environment (Crandall & González-Alonso, 2010). Muscle glycogenolysis (Febbraio *et al.*, 1996) and glucose oxidation appear to increase with ambient temperature, but fatigue during prolonged sub-maximal exercise in warm conditions occurs long before muscle glycogen depletion (Parkin *et al.*, 1999; Febbraio, 2000). A critical core temperature ($\sim 39.5^{\circ}\text{C}$) was proposed as the determining mechanism for fatigue (Nielsen *et al.*, 1993). Similarly, initial core temperature and rate of increase of core temperature appear to be determining factors for the onset of fatigue and volitional exhaustion during prolonged exercise (González-Alonso *et al.*, 1999). Core temperature was also demonstrated to correlate more strongly to the reduction force of maximal voluntary contraction than muscle temperature (Thomas *et al.*, 2006). Skin temperature has recently been

shown to be a determining factor as changes in cutaneous blood flow contribute to cardiovascular strain by requiring a higher cardiac output to circulate blood through the skin (Cheuvront *et al.*, 2010). This drive to dissipate heat through the skin, paired with fluid loss, has been considered to be more important to the onset of fatigue than an elevated core temperature alone (Sawka *et al.*, 2012). This results in increased relative exercise intensity at a given workload as VO_{2max} decreases and cardiovascular strain increases.

Hyperthermia has been found to impair maximal muscle activation, alter brain activity and increase perceived exertion (Nybo & Nielsen, 2001*b*). This is accompanied by reduced middle cerebral artery blood velocity (Nybo & Nielsen, 2001*c*), though this was later demonstrated not to be the cause of the changes in brain activity or fatigue (Rasmussen *et al.*, 2004). These physiological responses have been implicated in the fatigue process during exercise in the heat via mechanisms residing within the CNS (Nybo, 2008). There is some evidence that central fatigue may be due to dysfunction in metabolic and structural function, which could ultimately lead to catastrophic failure. A general decrease of glucose and oxygen along with increased heat storage in the brain may result in general disruption of normative function (Nybo & Secher, 2004). Nybo and co-workers (2005) also found an increased uptake and retention of ammonia in the brain during prolonged exercise. Ammonia is neurotoxic, and can only be detoxified by conversion to glutamine (Albrecht & Norenberg, 2006). Nybo and co-workers (2005) suggest that this process may affect glutamate metabolism and subsequently both glutamatergic and GABAergic neurotransmission. In addition, temperature determines both the integrity and fluidity of cell membranes in general (Blicher *et al.*, 2009). This is particularly significant to brain cells, which are especially sensitive to changes in these membrane qualities. Depending on the degree of integrity lost detriment to signal transduction, impulse conduction, metabolism and homeostasis in general would occur, which could ultimately lead to significant cell damage, apoptosis and eventually oedema (Kiyatkin & Sharma, 2009).

Prolonged exercise in the heat has been found to increase serum S-100 β concentrations (Watson, Shirreffs, & Maughan, 2005), a small calcium binding neurotrophic protein of 27 kiloDaltons primarily expressed by astroglial and Schwann cells in the CNS. The significance of this is not certain, other than it suggests an increase in blood-brain barrier permeability (Kapural *et al.*, 2002). This could result in serious metabolic disruptions of CNS homeostasis. If a 27 kiloDaltons protein can escape, then a number of ions and molecules of similar or smaller size may escape and/or enter the CNS and affect neurotransmission. In addition to neuroactive substances, other proteins of similar or smaller size, such as calcium binding parvalbumin (12 kiloDaltons), for example, may escape the CNS and have deleterious effects; calcium homeostasis is essential for almost all cell function (Clapham, 2007), but especially so for the CNS (Heizmann, 1993; Clapham, 2007). Interestingly, exercise has been found to increase expression of parvalbumin in the hippocampus in developing male Wistar rats (da Silva *et al.*, 2010). Despite the apparent changes in blood-brain barrier permeability, using magnetic resonance imaging (MRI), Watson and co-workers (Watson *et al.*, 2010) found that although exercised-induced hyperthermia and dehydration resulted in shrinking of cerebral ventricles and cerebrospinal fluid volume, but no significant changes in brain volume was apparent.

An argument against the catastrophic models of fatigue are that even in conditions most conducive to catastrophic failure it is rarely observed, suggesting a protective mechanism terminates exercise before this can happen (Noakes *et al.*, 2005). There is evidence to suggest a teleoanticipatory algorithm within the brain predicts expected outcome of performance relative to sensory and emotional feedback and adjusts power output and pacing with respect to this feedback (St Clair Gibson *et al.*, 2006). It is worth considering that pacing is, in part, determined by expectation based on previous experience. This suggests that the teleoanticipatory pacing mechanism and the changes to performance incurred by this mechanism might reflect a learned limit of expectation for performance rather than an unconscious instinct or survival reflex. However, as expectation and subjective experience are inherently dependent on learned experience, this must be viewed as another facet to central fatigue. Furthermore, except for in the rare cases of catastrophic failure or sudden

unconsciousness, the decision to stop exercising is a conscious one (Kayser, 2003). Nonetheless, subconscious and unconscious processes will contribute to this decision and appear to directly modulate performance as well. For example, the rate of heat storage has been found to modulate the power output in relation to a fixed rating of perceived exertion (RPE)(Tucker *et al.*, 2006) further demonstrating the role of centrally mediated mechanisms of fatigue during prolonged exercise in warm conditions. Changes to the central neurotransmission which underpin the teleoanticipatory system would also alter its function. Though the cerebral mechanisms for the onset of fatigue are not fully understood, there have been many studies attempting to manipulate fatigue by altering CNS function. While those attempting to alter fatigue via manipulation of serotonin have yielded conflicting results (Meeusen *et al.*, 2006b), pharmacological manipulation of central catecholamines has produced more consistent changes in the onset of fatigue and exercise performance in warm conditions (Roelands & Meeusen, 2010).

1.3- The Serotonin Hypothesis of Fatigue

One of the first neurobiological theories for this increased sense of fatigue during exercise was proposed to be related to changes in brain 5-hydroxytryptophan (serotonin). The concept of serotonin-mediated fatigue was founded on the association of 5HT to feelings of drowsiness and in decreasing arousal in the sleep-wake cycle. The basis for serotonin-mediated fatigue during prolonged exercise was largely due to the work of Chaouloff and co-workers which reported increased serotonin levels in rats during prolonged exercise (Chaouloff *et al.*, 1985, 1986b, 1987). They also demonstrated that provision of free tryptophan, the amino acid precursor for serotonin synthesis, increased this response (Chaouloff *et al.*, 1986a). Soon after, Newsholme and co-workers (1987) proposed a widely cited theory linking changes in peripheral substrate availability and mobilisation of free-fatty acids to changes in neurochemistry. This hypothesis suggested that prolonged exercise increases plasma fatty acid concentrations, which liberate tryptophan from albumin, resulting in an increased uptake of tryptophan by the large neutral amino acid (LNAA) transporter across the blood-brain barrier. As tryptophan hydroxylase is not saturated under normal physiological conditions, increased delivery of tryptophan

would result in an elevation in brain serotonin synthesis and release (Ruddick *et al.*, 2006). However, the ratio of free tryptophan to albumin bound tryptophan may be of little importance (Pardridge, 1983), because the exchangeable tryptophan in humans may approximate total plasma tryptophan. Pardridge (1983) explains that this is, in part, due to the fact that the tryptophan-albumin complex dissociates and re-associates many times during transit through brain capillary. As the branched-chain amino acids (BCAAs) compete for binding of the LNAA transporter, Blomstrand and co-workers (1991) suggested that supplementation with BCAA may limit the delivery of tryptophan to the CNS and attenuate serotonin production during exercise. They conducted a study in which BCAAs were administered before two long distance races, reporting an improvement in exercise performance in a group of slower runners.

To substantiate the serotonin hypothesis several strategies have been employed. In rats, administration of serotonin precursors resulted in an enhanced increase of serotonin in response to prolonged exercise (Meeusen *et al.*, 1996). Gomez-Merino and co-workers administered valine in rats and found that it reduced the exercise-induced increase in serotonin levels in the hippocampus by reducing uptake of tryptophan (Gomez-Merino *et al.*, 2001). Yamamoto & Newsholme (2000a) used a LNAA transporter blocker to prevent tryptophan uptake and subsequent serotonin synthesis, which prolonged exercise in rats. Subsequent human studies, however, have failed to support an effect of BCAA administration on central fatigue (Meeusen *et al.*, 2006a). Mittleman and co-workers (1998) administered BCAAs during low-intensity cycling in a warm environment and found a significant increase in time to exhaustion. However, subsequent studies have been unable to produce similar results (Cheuvront *et al.*, 2004; Watson *et al.*, 2004). It is worth noting that the protocol employed by Mittleman and co-workers (1998) did not induce a state of hyperthermia (core temperatures at fatigue of 37.3-37.7°C), due to the very low exercise intensity (40% VO_2max). To this day only two studies have reported an ergogenic effect of BCAAs on prolonged exercise performance (Blomstrand *et al.*, 1991; Mittleman *et al.*, 1998).

In addition to the serotonin hypothesis, Bailey and co-workers observed that dopamine tissue content decreased at fatigue, while serotonin remained high (Bailey *et al.*, 1993) leading to the theory that the ratio of serotonin to dopamine was the determining factor in fatigue (Davis & Bailey, 1997). A recent exercise study in warm conditions by Hobson and co-workers (2012) administered a tryptophan-free amino acid mixture, in order to reduce circulating tryptophan, and consequently serotonin synthesis. Despite a marked decrease in circulating serotonin, no changes in performance were observed. Tyrosine is an amino acid precursor to dopamine and noradrenaline synthesis and is also blocked by BCAA for uptake at the LNAA transporter. Because tryptophan depletion bypasses the problem of concomitantly reduced tyrosine uptake by the LNAA transporter this study provides further evidence that neither circulating tryptophan nor the ratio of serotonin/dopamine are particularly important in the genesis of central fatigue. A similar lack of convincing evidence has been found using pharmacological manipulation of serotonin in human studies. Wilson and Maughan (1992) tested the serotonin hypothesis in humans using the serotonin reuptake inhibitor paroxetine and found that it significantly decreased cycling time to exhaustion. This led to several follow-up studies with serotonin reuptake inhibitors, serotonin agonists and antagonists which failed to support those initial findings (see Meeusen *et al.*, 2006*b* for review). Eventually, Newsholme and Blomstrand (2006) conceded that central fatigue is likely more complex than the serotonin hypothesis.

To account for this variability in results, there are many factors to consider. Firstly, rat exercise studies should be regarded with caution as footshock, a consequence of the electric shock grids used at the rear of the treadmill in many of these studies to encourage the rat to perform for as long as possible, will confound the significance of the changes in monoamine levels throughout the brain (Dishman *et al.*, 1997). Similarly, inescapable/uncontrollable stress activates the brain and alters monoamine levels differently than controllable stress (Weiss *et al.*, 1981). The increased level of serotonin in certain parts in the brain could be affected by several things, including acute stress, which increases circulating glucocorticoids, increases tryptophan hydroxylase (TPH) activity in a dose related manner (Clark & Russo, 1997). Furthermore, the raphe nuclei, the source of most serotonergic projections in

the brain, are located in the brainstem. These neurons have been implicated with regulation of thermogenesis (Cao & Morrison, 2005) and stress induced cutaneous vasomotor tone (Blessing, 2005). serotonergic activity in the anterior hypothalamus/preoptic area has been implicated with stress responses including tachycardia and increased blood pressure (Szabó, Butz, & Alper, 1998). The increase of serotonin found at fatigue in the rat studies (Chaouloff *et al.*, 1986b; Blomstrand *et al.*, 1989; Bailey *et al.*, 1993) could therefore be a result of fatiguing processes, rather than being the cause of fatigue. Furthermore, serotonin is considered to play an important role in stimulating locomotion, which would likely further contribute to observed changes in serotonin concentrations (Takahashi *et al.*, 2000; Vanderwolf *et al.*, 1997). Finally, many of these results were obtained from brain tissue homogenate, rather than *in-vivo* microdialysis, and dynamic changes over time were not observed (Meeusen *et al.*, 2001).

While manipulation of central serotonin in humans has had conflicting results, pharmacological manipulation of catecholamines, particularly with reuptake inhibitors, has seen more consistent success (Roelands & Meeusen, 2010). Chaouloff and co-workers (1987) observed a decrease in serotonin synthesis during treadmill exercise after administration of amphetamine in trained rats, which acts via primarily catecholaminergic effects. Bailey and co-workers observed that a serotonin agonist decreased brain dopamine during prolonged exercise (Bailey *et al.*, 1993) and led to the consideration that the ratio of serotonin to dopamine was the determining factor (Davis & Bailey, 1997). However, for the reasons stated above and the lack of consistent results in studies using nutritional or pharmacological manipulation of central serotonin neurotransmission (2010), the role of serotonin in fatigue appears to be less important than central catecholamines and will no longer be considered in great detail .

1.4- Synthesis and Metabolism of Catecholamines

Dopamine was originally thought only to be the precursor to noradrenaline until it was demonstrated to be a neurotransmitter in its own right by Nobel Prize laureate Arvid Carlsson in 1957. This quickly led Carlsson to establish the role of dopamine in Parkinson's disease and subsequently in motor function. This became the focus of dopamine related research and the understanding of dopamine's function in the brain became defined by this research (Schallert *et al.*, 2009). Dopamine was originally associated with movement and then became associated with reward (Tobler, 2011), but it is now recognised that dopamine is also highly involved with learning, motivation, emotion, affect (Salamone *et al.*, 2007; Wise, 2004) and the attribution of value or salience to sensory stimuli (Tobler, 2011). The role of dopamine in motor function thus became a fundamental theme for research in cerebral control of exercise. This, combined with the observations by the exercise studies in rats mentioned in the previous section (Gerald, 1978; Chaouloff *et al.*, 1987; Bailey *et al.*, 1993) are perhaps why noradrenaline has received comparatively little attention, despite being closely tied with dopamine function, as described below.

Dopamine is synthesised from the amino acid L-tyrosine, which is converted by the enzyme tyrosine hydroxylase (TH) and the necessary cofactor tetrahydrobiopterin, to L-3,4-dihydroxyphenylalanine (L-DOPA) by the addition of a hydroxyl group. The carboxyl group is removed from L-DOPA by the enzyme aromatic L-amino acid decarboxylase (AAAD) to form dopamine (see figure 1.1). L-tyrosine can be acquired in the diet or synthesised from the essential amino acid phenylalanine, which cannot be synthesised by the body. Under normal physiologic conditions, dopamine synthesis is considered to be rate limited by the availability of tyrosine and tetrahydrobiopterin for hydroxylation by TH. Recent evidence suggests that the activity of AAAD is more important than originally thought (Duchemin *et al.*, 2000; Duchemin *et al.*, 2010), specifically during rapid short-term synthesis.

After decarboxylation of L-DOPA to dopamine, noradrenaline is formed from dopamine by dopamine- β -hydroxylase with the addition of a hydroxyl group at the second carbon (beta) from the amino group. Catecholamines are inactivated and metabolised by monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT). MAO uses oxygen, while COMT transfers a methyl group from the cofactor S-adenosylmethionine (SAM). Through other metabolic pathways SAM is also tied to monoamine synthesis, as described in section 1.8. Both enzymes are expressed within neurons and astroglia as well. This allows for degradation of catecholamines within neurons, in the synapse and extrasynaptic space as well, but occurs primarily within the neurons that synthesize them (Eisenhofer *et al.*, 2004). Interestingly, human prefrontal cortex (PFC) has a high concentration of COMT mRNA, where membrane bound COMT can be expressed on the exterior surface of the cell membrane postsynaptically (Matsumoto *et al.*, 2003).

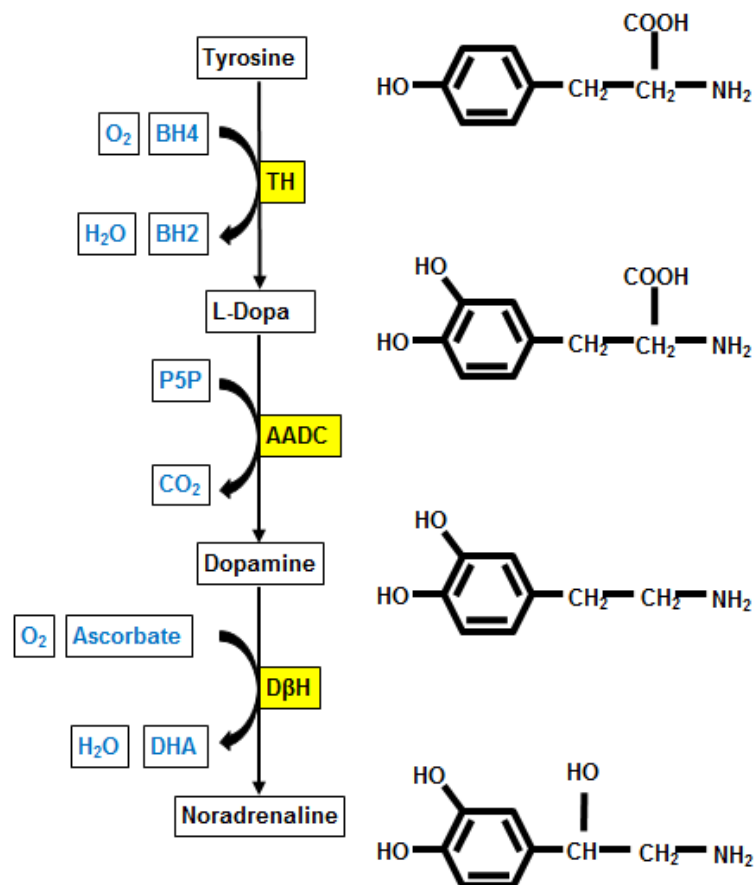


Figure 1.1 Cofactors and by-products (Blue): BH4 = Tetrahydrobiopterin; BH2 = Dihydrobiopterin; P5P=Pyridoxal-5-phosphate; DHA = Dehydroascorbate; Enzymes (Yellow) TH = Tyrosine hydroxylase; AADC = Aromatic L-amino acid Decarboxylase; D β H = dopamine- β -hydroxylase.

1.5- Dopamine Receptors, Adrenoceptors and Signalling

Dopamine receptors and adrenoceptors belong to a large family of transmembrane intracellular signalling proteins called G protein-coupled receptors. G protein-coupled receptors stimulate signal transduction through metabotropic instead of ionotropic mechanisms. This means that instead of directly opening an ion-channel, which rapidly changes the polarity of the neuron membrane, metabotropic receptors affect intracellular signalling via complex cascades (See figure 1.2). This in turn influences cell metabolism, but generally has a less immediate effect on extracellular signalling, including neurotransmitter release into the synapse. However, D1-like receptors have also been found to couple with glutamic NMDA receptors, in which case can directly stimulate calcium flux into the cell, resulting in a more rapid signalling process (Scott & Aperia, 2009). Dopamine receptors have been categorised into two separate classes based on their excitatory or inhibitory effects via alteration adenylyl cyclase activity and cyclic adenosine monophosphate (cAMP) production. The D1-like receptors, which consist of D₁ and D₅, are stimulating G protein (G_s and G_{olf}) - coupled receptors and stimulate cAMP production by increasing adenylyl cyclase activity (Herv *et al.*, 1993). D2-like receptor family, which include D₂, D₃ and D₄, are inhibitory G protein (G_i and G_o)-coupled receptors and decrease cAMP production by inhibiting adenylyl cyclase. While D1-like receptors are relatively well understood, D2-like receptors are not. This is due to the existence of various isoforms that differ at one of the intracellular functional groups (Jaber *et al.*, 1997; Missale *et al.*, 1998).

1.6- Receptor Subtype and Distribution

Table 1.1 below shows the relative distribution of the 5 subtypes of dopamine receptors in the cerebral cortex, hippocampus, amygdala, striatum, nucleus accumbens (NAc), ventral tegmental area (VTA), substantia nigra and the hypothalamus. These are several key areas of the brain for controlling exercise related behaviours.

Table 1.1 Dopamine receptor and transporter (DAT) expression in 6 areas of the human brain (Ciliax *et al.*, 1999; Rankin *et al.*, 2009). Indicated levels of expression are relative expression within receptor and transporter groups. +++ indicates very high, ++ indicates high, + indicates significant and – indicates not detected.

	D1	D2	D3	D4	D5	DAT
Cortex	++	+	+	+++	+	+
Hippocampus	+	+	+++	++	+	+
Amygdala	+	+	+++	++	-	++
Striatum	+++	+++	+	+	++	+++
Nucleus Accumbens	+++	+++	+++	+	+	++
Ventral Tegmental Area	+	++	+	+	-	+
Substantia Nigra	++	++	++	+	+++	++
Hypothalamus	+	+	+	++	++	+

The cerebral cortex is involved in relating to the outside world, via primary sensory areas, the primary motor area, association areas and limbic areas. The amygdala and hippocampus are part of the limbic system, which is involved in memory, emotion and motivation. The striatum and NAc are involved in reward, motor control, motivation and initiation of behaviour. These are key brain areas within the mesocorticolimbic system which is involved in motivation, reward, and learning, dysfunction of which is primarily implicated with impulse control disorders and drug addiction (Wise, 2004). The VTA and the substantia nigra are the main sources of projecting dopaminergic neurons in the brain. The VTA is the source of dopamine for the mesocorticolimbic system. The substantia nigra is the source of dopamine for the

dorsal striatum, is primarily involved in controlling motor behaviour, dysfunction of which is considered the main cause of Parkinson's disease. Finally, the hypothalamus is primarily associated with homeostatic control and neuroendocrine secretory function, however, it is also involved in drives and emotional behaviours (Nolte, 2009). The anterior and preoptic area of the hypothalamus in particular appear to be important for thermoregulation during exercise (Hasegawa *et al.*, 2005), with increases in both dopamine and noradrenaline release in the preoptic area positively correlated with core temperature (Hasegawa *et al.*, 2011). The VTA innervated areas are in turn modulated by noradrenaline (see figure 1.2), particularly in the PFC, regulating effort-related function controlling motivation outcomes and possibly linking the intensity of input saliency to effort intensity (Puglisi-Allegra & Ventura, 2012).

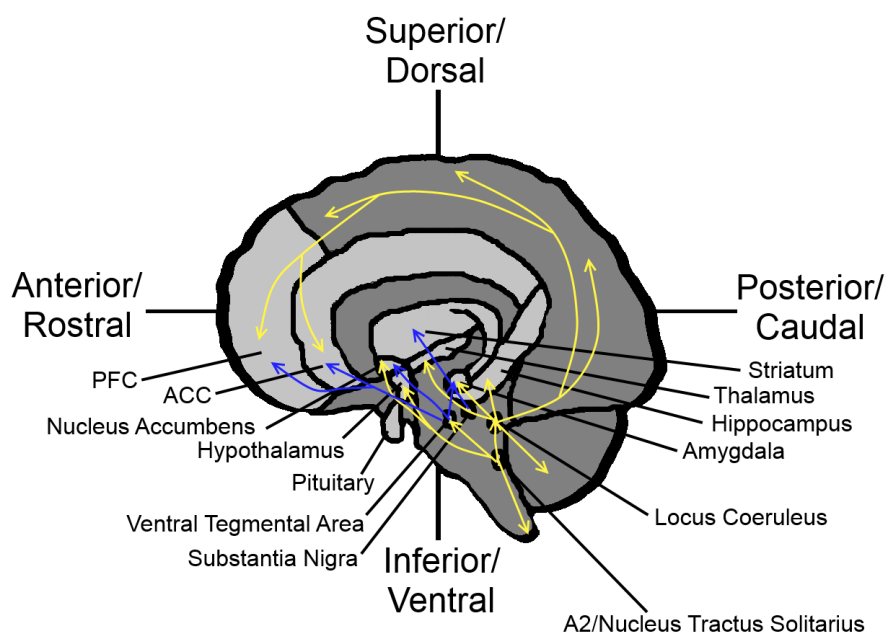


Figure 1.2 Neuroanatomical map, with axes of orientation. Light grey demarks regions of interest. Blue arrows represent dopaminergic projections, yellow arrows represent noradrenergic projections.

In total 9 different adrenoceptor subtypes have been identified, though their categorisation and distribution has not been as well defined as with dopamine receptors. The α_2 and β adrenoceptors are G_i and G_s , respectively. The α_1 adrenoceptors are G_q , which increases phospholipase C activity, which increases cytosolic calcium release. Table 1.2 shows the relative distribution of the 7 subtypes of noradrenaline receptors in the cerebral cortex, hippocampus, striatum, hypothalamus, thalamus, locus coeruleus and nucleus tractus solitarius (NTS). The thalamus is important for information transfer to and from peripheral afferents to the cortex via the spinal cord and is modulated by the basal ganglia and noradrenergic nuclei to control motor pattern generation and arousal, respectively. The locus coeruleus and NTS are particularly important noradrenergic nuclei located in the brain stem and modulate the stress response, arousal and interface with the mesocorticolimbic system to modulate behaviour and motor control.

Table 1.2 Noradrenaline receptor and transporter (NAT) distribution in 7 areas of the rat brain (Nicholas *et al.*, 1996; Schroeter *et al.*, 2000). Indicated levels of expression are relative within receptor and transporter groups. +++ indicates very high, ++ indicates high, + indicates significant and – indicates not detected. *Unfortunately, the understanding of human adrenoceptor distribution within human brain is not as well categorised as dopamine receptors. While interspecies variation of receptor and transporter expression is noteworthy, the afferent targets are very similar and the comparison is suitable as a general reference.

	α_{1b}	α_{1d}	α_{2a}	α_{2b}	α_{2c}	β_1	β_2	NAT
Cortex	+++	++	++	-	++	++	++	+
Hippocampus	-	+++	+	-	+++	+	+	++
Striatum	+	-	-	-	+++	-	-	+
Hypothalamus	+	-	++	-	+	-	+	++
Thalamus	+++	+	+	+	+	+	+	++
Locus Coeruleus	-	-	+++	-	-	-	-	+++
Nucleus Tractus Solitarius	-	-	++	-	-	-	-	++

The control of synthesis and release of catecholamines are determined by cell signalling mechanisms that can also be triggered by the catecholamines themselves as inhibitory feedback. TH and AAD activity is regulated by the number of units expressed in a cell and the activity of each unit. TH activity is increased through the transfer of phosphate groups (phosphorylation) by protein kinase A, protein kinase C and calmodulin-dependent protein kinase II. Protein kinase A and protein kinase C are in turn regulated by cAMP and cytosolic calcium levels (Nestler *et al.*, 2008). AAD is also activated by cAMP (Duchemin *et al.*, 2000). Removal of phosphate groups (dephosphorylation) of TH by protein phosphatase 2A decreases activity. Neurotransmitter synthesis and release in dopaminergic and noradrenergic neurons can thus be regulated via D2-like and α_2 receptors, respectively (see figure 1.3).

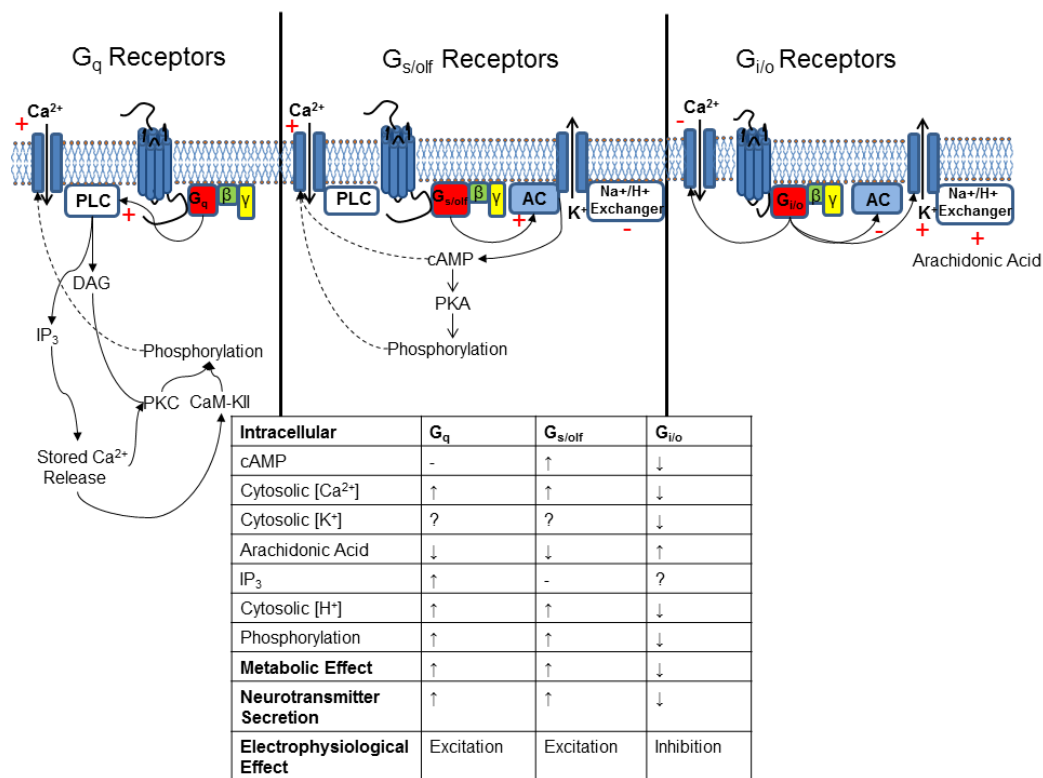


Figure 1.3 Simplified overview of a few key differences between G-protein receptor signalling. G_q receptors represent α adrenergic receptors while G_{s/olf} receptors represent D1 dopamine receptors and β adrenoceptors. G_{i/o} receptors represent D2 and α_2 adrenoceptors. Red pluses and minuses indicate activation and deactivation respectively. Pathways shown where substantial literature supports; Lack of pathways and “?” indicate lack of uniformity in the research. PLC = Phospholipase C; DAG = Diacylglycerol; IP₃ = inositol triphosphate; AC = Adenylyl Cyclase; cAMP = Cyclic adenosine monophosphate; PKA = Protein kinase A; PKC = Protein Kinase C; CaM-KII = Ca²⁺/calmodulin-dependent protein kinases II. DARPP-32 = dopamine and cAMP-related phosphoprotein of 32 kiloDaltons; PP1 = Protein phosphatase 1.

D1- and D2-like receptors increase and decrease cytosolic calcium levels, respectively, which contributes to the modulation of signalling cascades and cell metabolism, but also directly affects membrane excitability. Increased cytosolic calcium concentrations increases the rapidity with which the membrane may repolarise and decreases the refractory period before another action potential may be triggered (see figure 1.4).

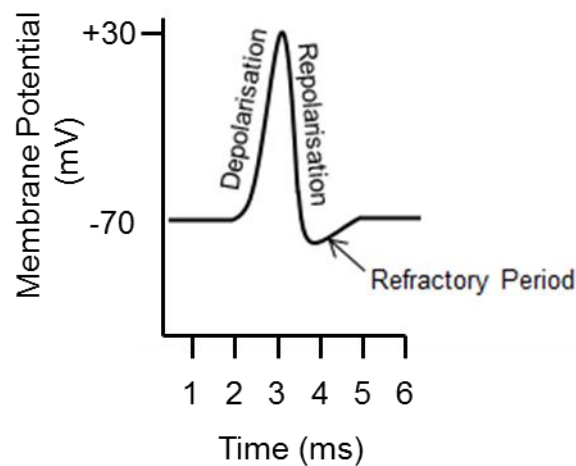


Figure 1.4 Schematic of an action potential.

In a given neural circuit, catecholamines may increase or decrease activity. As an example, excitatory neurotransmission could be increased via D1-like receptors in a glutamatergic neuron or D2-like receptors in a GABAergic neuron to decrease inhibitory neurotransmission. Conversely, dopamine can decrease excitatory neurotransmission in a given circuit by acting at D2-like receptors in glutamatergic neurons, or at D1-like receptors at GABAergic neurons (See figure 1.5). Of course, this is a gross simplification, but demonstrates the way in which catecholamines can modulate neurotransmission differently, dependent on the target receptors and the neurons on which they are expressed. An example of this can be found in the striatum, the target of the most dopaminergic afferents in the brain. In the striatum D1 receptors increase medium-spiny neuron reactivity to glutamatergic signalling while D2 decrease medium-spiny neuron reactivity to glutamate (Surmeier et al., 2007).

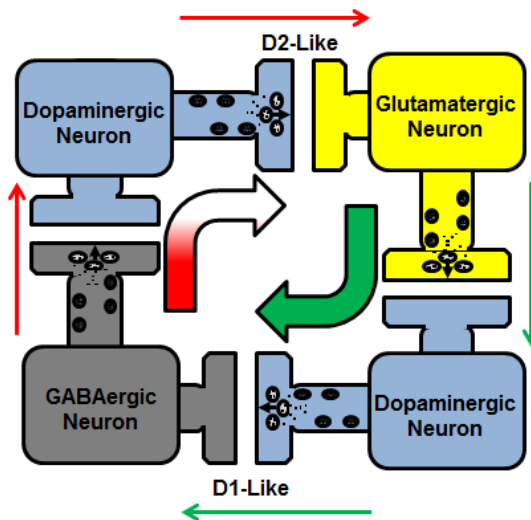


Figure 1.5 A simplified circuit to depict different ways dopamine signalling can influence a circuit. Red represents inhibitory signalling and green represents excitatory signalling. The circuit on the left depicts how inhibition of a GABAergic neuron via stimulation of D2-like receptors leads to the disinhibition of the top left dopaminergic neuron and excitation of the glutamatergic neuron. The circuit on the right depicts how excitation of a GABAergic neuron via D1-like receptors inhibits the top left dopaminergic neuron and leads to disinhibition of the glutamatergic neuron.

Catecholamines can also elicit biphasic responses via concentration-dependent activation of a combination of inhibitory and stimulatory receptors, which are differentially activated due to their expression and their varying affinities for their respective neurotransmitter. For example, Tuberoinfundibular dopaminergic (TIDA) neurons in the hypothalamus exert a biphasic control of prolactin secretion from the pituitary via changes in tonic vs. phasic firing modes. Prolactin feedback at the TIDA neurons shifts firing to phasic to increase dopaminergic inhibition of prolactin secretion (Lyons *et al.*, 2012), while thyrotropin-stimulating hormone shifts TIDA neurons to tonic firing, and potentiates the prolactin response (Lyons *et al.*, 2010). In the PFC both dopamine and noradrenaline exhibit an inverted-U effect on cognitive function (Arnsten, 2007). Concentration-dependent effects are sometimes dependent on the distance from the site of release at which receptors are expressed, which in some cases can be relatively large. In these circumstances monoamines act in a paracrine or extrasynaptic manner to enable volume transmission, a process regulated by release and controlled by reuptake by their respective transporters (Fuxe *et al.*, 2010a). Dopamine receptors and transporters are primarily expressed extrasynaptically, (Missale *et al.*, 1998), as are noradrenaline receptors and

transporters (Schroeter *et al.*, 2000). Because diffusion occurs much faster than reuptake, release largely overwhelms uptake and the combination of tonic and phasic release determine baseline extracellular neurotransmitter levels (Rice & Cragg, 2008).

1.7- Tonic and Phasic Signalling

Tonic and phasic signalling are differentiated by the number of action potentials within a given period of time. Tonic signalling is characterised by sustained frequent action potentials, which maintain background levels of neurotransmitter, while phasic signalling is a transient bursting of action potentials in quick succession (see figure 1.6), which are usually coupled with salient stimuli such as aversive or rewarding cues.

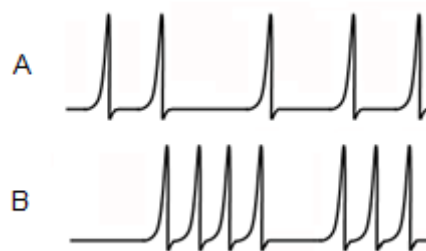


Figure 1.6 Train of action potentials in tonic (A) and phasic (B) firing.

These yield comparatively small and large neurotransmitter quantal release, respectively. In the striatum, ventral and dorsal baseline dopamine levels are modulated by tonic activity from the VTA and substantia nigra pars compacta, respectively, and the ventral striatum is particularly sensitive to changes in tonic firing from the VTA due to a greater range in extracellular dopamine concentrations (Zhang *et al.*, 2009). Supporting this, recent evidence suggests that in the NAc, located in the ventral striatum, dopamine reuptake inhibition increased tonic stimulation of low-affinity post-synaptic receptors and results in desensitisation to phasic dopamine signals (Dreyer & Hounsgaard, 2013). Low levels of baseline dopamine levels in the NAc shell are associated with decreased motivation and exertion of effort for food, whilst reward-seeking remains intact in rats (Salamone *et*

al., 2003). Tonic dopamine signals in the NAc modulate baseline extracellular dopamine and set an ‘average reward’ of current behaviour, which then determines the likelihood to exert effort and vigorous responding to reward cues (Niv *et al.*, 2007). As a result, this relationship between tonic and phasic dopamine signals in the NAc affects behaviour (see figure 1.7). In the cerebral cortex, tonic noradrenaline acts to modulate the level of arousal, while phasic noradrenaline acts to entrain cortical cross-talk toward a specific stimulus (Aston-Jones & Cohen, 2005). DAT/NAT blockade mimics enhanced tonic signalling because the tonic stimulation of receptors is determined by baseline concentrations, which become enhanced by reuptake inhibition, increasing the accumulation of extrasynaptic catecholamines from both phasic and tonic release.



Figure 1.7 An example of the relationship between tonic and phasic firing in the NAc. A represents a normal balance between tonic and phasic dopamine, whereas B represents a decreased tonic dopamine resulting in exaggerated phasic dopamine signalling, which is characterised by impulsive behaviour. C represents excessive tonic dopamine, which masks phasic dopamine spikes and is observed in pain and stress syndromes (Leknes & Tracey, 2008).

DAT is most heavily expressed in the midbrain and basal ganglia (Hall *et al.*, 1999). Here it acts to modulate dopaminergic volume transmission and extrasynaptic signalling, which the results from extracellular concentrations of dopamine spill-over to extrasynaptic receptors when concentrations are elevated beyond the capacity for DAT during phasic signalling (Fuxe *et al.*, 2010). In the midbrain and basal ganglia, tonic signalling delivers smaller, but more constant, dopamine release than phasic signalling and preferentially activates D2 receptors, which have a higher affinity for dopamine than D1 receptors, which only become activated by large phasic dopamine releases. This appears to be particularly important in the ventral striatum, where a broad range of firing frequencies is present, unlike the dorsal striatum where dopamine release is not linearly dependent on firing activity (Rice & Cragg, 2008). The dorsolateral striatum, which receives dopaminergic projections from the substantia nigra pars compacta, has a lower phasic-to-tonic ratio than VTA

innervated areas. This includes the ventral striatum, PFC and amygdala where the contrast between the tonic and phasic dopamine signals modulates attention and behavioural selection (Zhang *et al.*, 2009). Human NAT is most heavily expressed in the brainstem, (particularly at the locus coeruleus), the thalamus and cortex (Schou *et al.*, 2005; Takano *et al.*, 2008). NAT in the locus coeruleus has been considered a key target for the treatment of depression (Klimek *et al.*, 1997), while in the cortex it is implicated in attention deficit hyperactivity disorder (ADHD)(Pliszka, 2005). Attention and behavioural control are also modulated by tonic and phasic noradrenaline signals from the locus coeruleus to the cerebral cortex (Aston-Jones & Cohen, 2005). Together, the dopaminergic signals from the VTA and the noradrenergic signals from the locus coeruleus coordinate to control all motivated behaviour and learning (Puglisi-Allegra & Ventura, 2012). These systems are directly modulated by bupropion and methylphenidate, which have had relatively consistent results in improving exercise performance in warm conditions as described in section 1.9. Furthermore, these systems directly control the behavioural response to stress. Therefore, the performance enhancing effects of these drugs may be due to alterations to the stress response within the CNS.

1.8- Central Catecholamine Precursors and Prolonged Exercise

Attempts to manipulate central fatigue using precursors for catecholamine synthesis have been conflicting. Several studies during prolonged military operational drills found that supplementation with tyrosine reduced fatigue and stress while improving cognitive and motor performance in soldiers (Salter, 1989; Owasoyo *et al.*, 1992; Smith *et al.*, 2003). However, several laboratory exercise studies in humans have found no effect of tyrosine supplementation (Strüder *et al.*, 1998; Chinevere *et al.*, 2002). The apparent disparity in effectiveness might be explained by the type of stress endured by soldiers was more prolonged and is perhaps incomparable to that experienced in laboratory studies due to differences in motivation and psychological stress. L-DOPA is one metabolic step closer to dopamine than tyrosine (see figure 1.1). L-DOPA has been used to treat motor control disorders in Parkinson's disease for over 40 years and is considered the "gold standard" treatment today (Nagatsu & Sawada, 2009). Clinically, L-DOPA is administered with an amino acid

decarboxylase (AADC) inhibitor that cannot readily cross the blood-brain barrier; this prevents decarboxylation of L-DOPA in the periphery, thereby reducing associated gastrointestinal distress and increasing the available L-DOPA to the brain. Tyrosine is not an effective treatment for Parkinson's disease because a symptom of the disease is the significantly decreased expression of tyrosine hydroxylase in dopaminergic neurons (Javoy-Agid *et al.*, 1990). Only one study to date has investigated the effects of L-DOPA administration in healthy participants on exercise performance and found no difference in time to exhaustion compared to placebo (Meeusen *et al.*, 1997a). The lack of effect observed in the exercise studies above may also have been due to having been conducted in normal ambient temperatures. A recent study was conducted to examine whether warm conditions might provide a better environment to test this relationship and found that acute tyrosine administration before exercise did enhance performance (Tumilty *et al.*, 2011). However, a follow-up study using the same conditions found no effect on performance (Watson *et al.*, 2012).

Catecholamine metabolism is also dependent on methyl-group donors and the one-carbon cycle. As the primary methyl donor in human physiology, SAM plays a vast number of important roles in the body. SAM is directly involved in homocysteine metabolism, the synthesis of creatine, metabolism of several neurotransmitters and the regulation of DNA, RNA. SAM is synthesised by the addition of ATP to methionine by SAM synthetase. The transfer of the methyl group yields S-adenosylhomocysteine. S-adenosylhomocysteine hydrolase then removes adenosine from homocysteine (see figure 1.8 below). Chronically elevated homocysteine levels are associated with folate and B12 deficiencies as well as a number of disorders, including depression (Bottiglieri, 2005). SAM has been used to treat depression for over 60 years (Papakostas *et al.*, 2003), osteoarthritis (Soeken *et al.*, 2002) and is considered potentially useful for liver disorders (Purohit *et al.*, 2007). Via the transulfuration pathway SAM is involved in the synthesis of glutathione (Lu & Mato, 2008) and may effect on synthesis of glutamate and GABA as well. SAM is tightly co-dependent with folate and choline metabolism (Bottiglieri, 2002; Zeisel & Blusztajn, 1994). These are necessary for synthesis of acetylcholine, betaine and cell membranes (Zeisel & Blusztajn, 1994). Folate contributes to methyl-

group metabolism via methyltetrahydrofolate (MTHF), which can regenerate methionine via methylation of homocysteine and can therefore contribute to SAM synthesis. MTHF is also necessary for tetrahydrobiopterin synthesis, a cofactor for synthesis of nitric oxide, serotonin, dopamine and noradrenaline (Bottiglieri et al., 1992; Stahl, 2007). It is thought to be through this pathway that SAM has been shown to improve mood (Fernstrom, 2000). Supporting this, folate deficiency is common in depression and has been associated with decreased cerebrospinal fluid SAM concentrations (Bottiglieri, 2002). The relationship of SAM to central catecholamine metabolism has also been characterised by the effects of L-DOPA treatment on SAM concentrations. In rats, brain SAM concentration decreases in response to L-DOPA infusions (Chalmers *et al.*, 1971). In humans, L-DOPA treatment decreases cerebrospinal fluid SAM concentrations (Surtees & Hyland, 1990). Similarly, patients receiving L-DOPA treatment for Parkinson's disease, showed a decrease in plasma methionine and SAM, while homocysteine was elevated (Müller *et al.*, 2001).

While the effect of SAM supplementation on exercise has not been investigated, there have been a few exercise studies investigating related compounds such as homocysteine, choline, choline-containing phospholipids and betaine. In recreational athletes prolonged exercise has been found to increase blood homocysteine and decrease folate and B12 (Herrmann *et al.*, 2003). In addition to increased plasma homocysteine, prolonged exercise decreases plasma choline levels. Following the Boston marathon have been found to be significantly lower than before the race in two studies by the same group (Conlay *et al.*, 1986, 1992). Buchman, Jenden, & Roch (1999) investigated plasma free and phospholipid bound choline before, immediately after, and 2 days after a marathon in 23 experienced male and female marathon runners and found a significant decrease in both free and phospholipid bound choline immediately after the race and persisting decreased plasma phospholipid bound choline levels 2 days following the race.

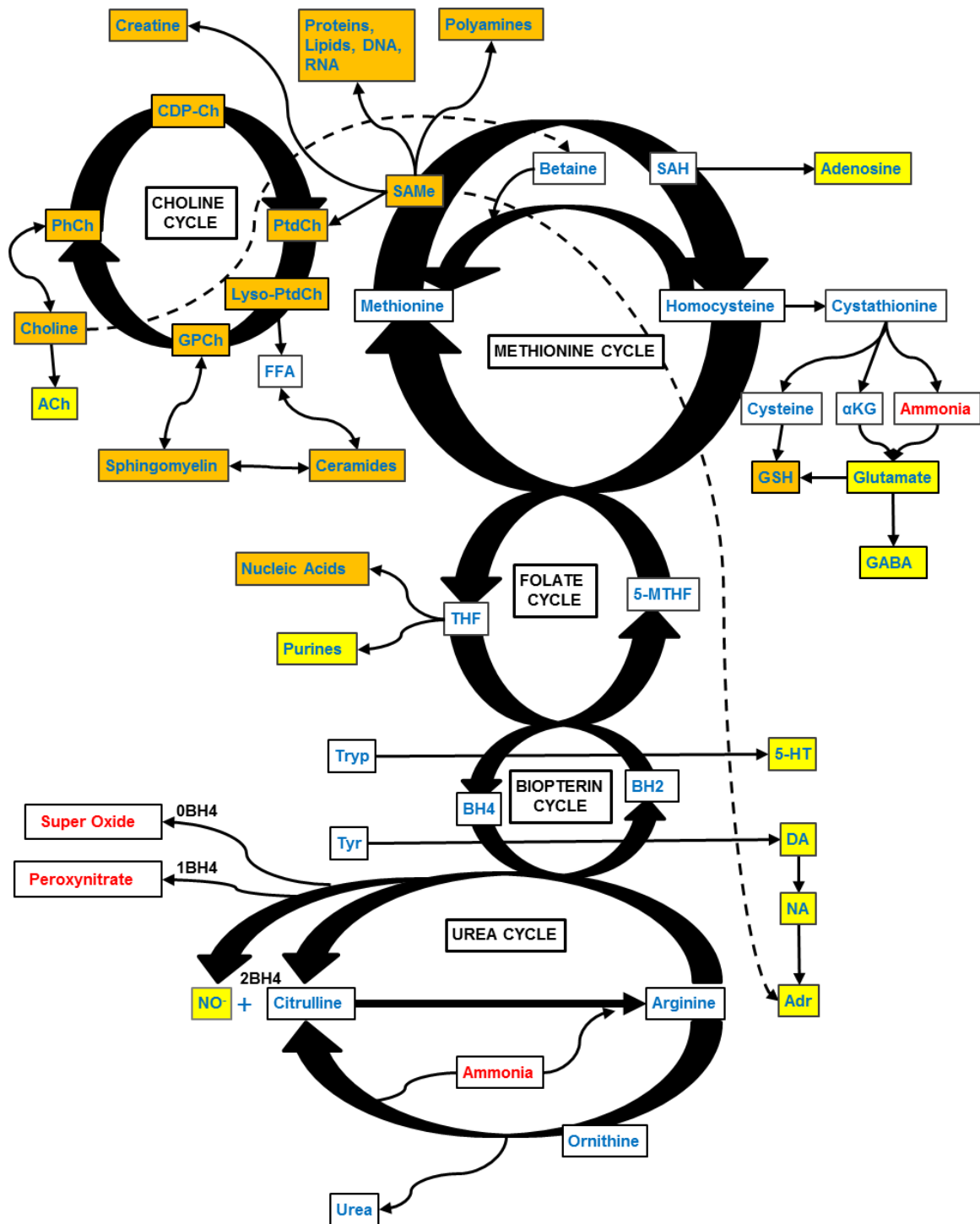


Figure 1.8 A simplified metabolic map of various roles of SAM important to CNS function. NO=Nitric Oxide; BH4 = tetrahydrobiopterin; BH2 = Dihydrobiopterin; Tryp = Tryptophan; Tyr = Tyrosine; Adr= Adrenaline; THF = Tetrahydrofolate; 5-MTHF = 5-methyltetrahydrofolate; GSH = Glutathione; α KG = alpha-ketoglutarate; SAH = S-adenosylhomocysteine; Ach = Acetylcholine; GABA = gamma-aminobutyric acid; PtdEth = Phosphatidylethanolamine; PtdCh = Phosphatidylcholine; FFA = Free fatty acids; LysoPtdCh = Lysophosphatidylcholine; GPCCh = Glycerophosphocholine; PhCh = Phosphocholine; CDP-Ch = Citydine diphosphate-choline

Phosphatidylethanolamine N-methyltransferase accepts the methyl- group from SAM and converts phosphatidylethanolamine to phosphatidylcholine. Membrane phosphatidylcholine is important for membrane physical properties and provides the bulk of cell choline stores (Li & Vance, 2008). Cell membrane phosphatidylcholine synthesis appears to also be regulated by mechanical strain in cell membranes. Cytidine 5'-triphosphate: phosphocholine cytidyltransferase (CCT) activity is modulated by membrane curvature elastic strain (Attard *et al.*, 2000). This occurs because CCT is translocated into the membrane to alleviate the elastic curvature strain, and this simultaneously activates CCT and thus increases its activity and production of phosphatidylcholine. This suggests that physical membrane strain during exercise may result in methylation of phosphatidylethanolamine to form phosphatidylcholine. Mechanical changes in cell membranes during exercise due to during muscle contraction and changes in osmotic pressure may induce increased muscle turnover of phosphatidylcholine. Two studies found that supplementation of lecithin, which is a mixture of phospholipids including phosphatidylcholine, was able to prevent the decline in plasma choline concentrations during prolonged exercise (von Allworden *et al.*, 2000; Buchman *et al.*, 2000). However, Jäger and co-workers (Jäger *et al.*, 2007) point out in their review of phospholipid supplementation that these results should be regarded with caution as there can be great variability in marathon times due to variations in courses and environmental conditions. It should also be considered that these studies used acute dosing protocols, and because plasma choline is extensively metabolised for a number of different processes in almost every type of tissue, it is difficult to say what physiological effects it may produce, especially over such a small period of time.

The ratio of membrane phospholipid methylation is also important to structural function and other methyl-group dependent reactions. This is demonstrated clearly in the liver, where deficiencies of phosphatidylcholine to phosphatidylethanolamine negatively affect folate and homocysteine metabolism (Zeisel & Blusztajn, 1994), supporting the notion that the increased plasma homocysteine and decreased plasma choline after prolonged exercise are correlated. Insufficient membrane phospholipid methylation disrupts lipid metabolism and lipoprotein export, as can be seen in both alcoholic and non-alcoholic steatohepatitis (Li & Vance, 2008).

Membrane integrity and fluidity are especially important for neuronal function and determine the function of membrane-bound proteins such as receptors and ion channels (Lenaz, 1987). This modulation of neuronal membrane fluidity by SAM has been observed in old rats, returning membrane fluidity and β -adrenoceptor expression in the striatum to juvenile values (Cimino *et al.*, 1984). In human brains afflicted by Alzheimer's, phosphatidylethanolamine N-methyltransferase activity is depressed as well as total phosphatidylcholine in the frontal cortex (Guan *et al.*, 1999) and cerebrospinal fluid SAM concentrations are low (Bottiglieri *et al.*, 1990; Linnebank *et al.*, 2010). Membrane phosphatidylcholine is an important pool for acetylcholine synthesis. This is supported by evidence by Párducz, Kiss, and Joó (1976) who found that cholinergic sympathetic ganglion neurons lost significant membrane phosphatidylcholine in the presence of the choline uptake inhibitor hemicholinium during stimulation. Further supporting the connection between folate, choline, lipid metabolism, central acetylcholine, SAM and Alzheimer's, two studies in mice have demonstrated the ability of SAM to restore acetylcholine function in folate deficient mice (Serra *et al.*, 2008; Chan *et al.*, 2008*b*). Due to this multifaceted relationship to cellular function and acetylcholine, SAM is being investigated as a treatment for Alzheimer's (Chan *et al.*, 2008*a*).

In addition to roles of methyl group donors in neurotransmission, cell signalling and metabolism during exercise, they also have a volemic role. Betaine is formed by dehydrogenation of choline, and acts as a methyl-donor and osmolyte. Craig and co-workers (2010) found that the betaine content of sweat in adolescent girls was almost 7 fold greater than in plasma, while choline was 7 fold greater in plasma than in sweat. This may explain the volemic, oxygen consumption and thermal strain differences observed in a study testing the effects of betaine supplementation on exercise performance during running in warm conditions (Armstrong *et al.*, 2008). Even at doses of 100mg/kg body weight used by Schwahn and co-workers (2003), betaine was still found to be eliminated by metabolism rather than excretion, again indicating the importance of consideration for the metabolic versatility of these associated compounds when testing their effects on physiology. Central cholinergic function modulates motor learning and motor control, reward and motivation (Woolf & Butcher, 2011). Central cholinergic tone also influences proopiomelanocortin

derived hormones (Meister *et al.*, 2006), and growth hormone (De Marinis *et al.*, 1997; de Vries *et al.*, 2002). However, the efficacy of SAM in the treatment of depression and schizophrenia are attributed to the role SAM plays in catecholamine metabolism, as the connection between catecholamines and these disorders is well established. The decrease in plasma choline, loss of betaine through sweat and increase in homocysteine observed during prolonged exercise, may reflect a decreased pool of methyl-group donors. Depending on the magnitude of the effect, nutritional status and genetics of an individual, this detriment to methyl group metabolism could have significant ramifications for exercise performance. While choline and betaine represent a significant pool of methyl-group donors, only SAM is recognised for clinical effects on various central nervous disorders. The most significant of these effects appear to be mediated by catecholamine metabolism. This provides a possible link between decreases in methyl-group donors during prolonged exercise, which may subsequently affect catecholamine metabolism via SAM-dependent reactions. SAM supplementation may therefore attenuate these changes, or increase catecholamine synthesis and influence prolonged exercise performance.

1.9- Catecholamine Reuptake Inhibitors

Pharmacological manipulations of central catecholamines with reuptake inhibitors have provided the most consistent changes to exercise performance, particularly in warm conditions. Piacentini and co-workers (2004) found no effect of bupropion, a combined dopamine/noradrenaline reuptake inhibitor on exercise performance in temperate conditions. In the studies by Watson or Roeland and co-workers (2005a; 2008, respectively) neither bupropion nor methylphenidate (also a combined dopamine/noradrenaline reuptake inhibitor) enhanced time trial performance in temperate conditions, respectively, but did in warm conditions. However, in the study by Swart and co-workers (2009), an even smaller dose of methylphenidate (20mg vs. 8mg) improved duration and power-output during cycling exercise to exhaustion at a fixed RPE. Amphetamine has also been found to increase time to fatigue in humans in temperate conditions (Chandler & Blair, 1980). The apparent difference between these studies on exercise performance may be due to the influence of

spacing strategies in the time trial studies (Baron *et al.*, 2011). A recent study found no effect for methylphenidate, but found a detrimental effect of reboxetine, a potent and selective noradrenaline reuptake inhibitor, on time trial, motor evoked potential and a psychomotor vigilance task (Klass *et al.*, 2012). An earlier study using reboxetine in temperate conditions found no difference in time trial performance (Piacentini *et al.*, 2002), while a study in both warm and temperate conditions found a negative effect in both conditions (Roelands *et al.*, 2008a). The relative potencies and effects of these drugs will be described and discussed below. Thus far, three selective catecholamine reuptake inhibitors have been used in studies investigating prolonged exercise in warm conditions: bupropion, methylphenidate and reboxetine (Roelands & Meeusen, 2010). Many CNS stimulants originally thought to impart their effects primarily via dopamine have been found to have significant effects on noradrenaline. This includes those that have been found to benefit exercise performance in humans, including amphetamine (Brauer & De Wit, 1997; Rothman *et al.*, 2001; Wachtel *et al.*, 2002), bupropion (Dwoskin *et al.*, 2006) and methylphenidate (Challman, T.D., and Lipsky, 2000). A recent PET imaging study in humans showed the effective dose to occupy 50% (ED 50) of NAT in the thalamus by methylphenidate (0.14mg/kg) is less than the ED 50 of methylphenidate for DAT in the striatum (0.25mg/kg)(Hannestad *et al.*, 2010). The ED 50 value for DAT in this study was similar to that in previous research (Volkow, Wang, Fowler, & Ding, 2005). Unfortunately, there is a disparate lack of *in-vivo* verification for occupancy of the NAT in humans. This has been due to difficulties synthesising appropriately selective radioligands, however, recent developments with reboxetine analogues, such as (S,S)-[¹¹C]methylreboxetine, promise to further elucidate the role of NAT in neuropharmacology (Kiyono *et al.*, 2008).

While the bupropion and methylphenidate have been demonstrated to improve exercise performance, reboxetine administration has produced negative effects, leading the authors to conclude that noradrenaline negatively influences exercise (Roelands *et al.*, 2008a; Klass *et al.*, 2012). While in those studies this effect is attributed to central effects of the drug, it deserves some mention that reboxetine has significant peripheral activity, which complicates the matter of qualifying the central effects. Boschmann and co-workers (2002) conducted a study of the effects of

reboxetine in combination with isoproterenol, a β -adrenergic agonist with negligible ability to cross the blood-brain barrier, to investigate the involvement of NAT in autonomic control of metabolism. This study found that reboxetine augmented responses to isoproterenol for heart rate, systolic blood pressure, serum glucose (in male participants), glycerol, and free fatty acids. Reboxetine also increased the respiratory quotient in favour of glucose over fat oxidation and interfered with adipocyte metabolism clearly demonstrating a peripheral effect of the potent NAT blockade by reboxetine. Another study found in healthy normal volunteers an acute dose of reboxetine induced a physiological state comparable to orthostatic intolerance which is characterised by symptoms of light-headedness, fatigue, nausea, orthostatic tachycardia and exercise intolerance (Schroeder *et al.*, 2002). While in both papers it is acknowledged that reboxetine's physiological effects are likely to arise from both the peripheral and central nervous systems, it is of considerable importance to recognise that effects in either of these systems have tremendous potential for impact on exercise performance. Indeed, the resting tachycardia observed by Piacentini *et al.*, (2002) (cited as not significant, but ~14% higher in reboxetine than placebo), Boschmann *et al.*, (2002), Schroeder *et al.*, (2002) and Roelands *et al.*, (2008) may be explained by decreased noradrenaline clearance by NAT in the heart, which is more dependent on NAT than any other tissue for noradrenaline clearance (Esler *et al.* 1990; Goldstein, *et al.* 1988). The peripheral effects of reboxetine may also result in sympathetic feedback inhibition. Further to this, a recent review and meta-analysis of reboxetine for use in acute treatment of major depression found no greater therapeutic effect compared to a placebo and higher rates of both adverse events and patient withdrawals due to adverse events (Eyding *et al.*, 2010). Therefore, the results of studies using reboxetine should be considered carefully before drawing conclusions about the role of noradrenaline.

The primary mode of action for bupropion has been considered to be via DAT blockade due to findings from *in-vitro* studies and studies in rats. However, *in-vitro* studies remove the element of bupropion metabolism, which occurs in the liver, and rats do not metabolise bupropion the same way that humans (mice, guinea pigs and dogs) do. Humans rapidly and extensively metabolise bupropion, predominantly to

the metabolites hydroxybupropion and threohydrobupropion (Bondarev et al., 2003). In humans, bupropion metabolites accumulate at levels 10-100 times greater in plasma (Damaj et al., 2004) and 6 times greater concentration and cerebrospinal fluid respectively than bupropion (Cooper et al., 1994). Furthermore, it has been found in guinea pigs, which have the most similar pharmacokinetic profile for bupropion to humans in rodent models (Suckow et al., 1986), that bupropion metabolites accumulate in brain tissue more than bupropion (DeVane, Laizure, & Cameron, 1986) in a linear relationship to the observed plasma ratios. Additionally, a study using rats found that bupropion only increased extracellular dopamine concentrations in the NAc using doses that were significantly higher than are used in clinical doses (25mg/kg)(Nomikos et al., 1992). There is considerable evidence that the differential effects of bupropion metabolites contribute significantly to its action.

Several human studies using acute doses of bupropion have found the area under the curve (AUC) for hydroxybupropion and threohydrobupropion in ranges roughly 4-36 times greater than bupropion, with hydroxy-metabolites obtaining the maximum plasma concentration (C_{max}) and AUC values, while hydro-metabolites had the longest elimination half-life values (Stewart *et al.*, 2001; Turpeinen *et al.*, 2007; Kharasch *et al.*, 2008). Similarly, *in-vitro* binding tests have found that bupropion and hydroxybupropion are equally bound by plasma proteins, while threohydrobupropion is bound 5 times less. hydroxybupropion can exist as two pairs of enantiomers (4 diastereomers) but in human plasma, only the enantiomers (2S,3S)- and (2R,3R)-hydroxybupropion are found, respectively identified as (+) and (-) by their optical rotation (Suckow, Zhang, & Cooper, 1997). The (+)-hydroxybupropion isomer more potently inhibits NAT than DAT *in vitro* (Damaj et al., 2004), while (-)-hydroxybupropion had no significant effect on either. Kharasch et al., (2008) found roughly 95% C_{max} of hydroxybupropion in humans as (-)-hydroxybupropion leaving the more active (+)-hydroxybupropion in quantities over 10 times less than bupropion. In the same study (+)-hydroxybupropion contributed only 2% to the AUC for overall hydroxybupropion due to the significantly shorter elimination half-life. Because (-)-hydroxybupropion and (+)-threohydrobupropion are found in the highest concentrations in humans, it seems reasonable to conclude that the majority of the effects are contributed by these metabolites. This may explain the comparatively

weak reuptake inhibition observed in human imaging studies. Recently, Egerton and co-workers(2010) found that after a single clinical dose (150mg sustained release formula) there was no observable change in striatal extracellular dopamine concentrations in normal healthy volunteers as measured by [¹¹C]raclopride positron emission tomography. Other studies have similarly found that DAT occupancy by bupropion does not correlate with therapeutic efficacy (Argyelán *et al.*, 2005). Therefore, the contribution of noradrenaline reuptake inhibition to bupropion's therapeutic effect at clinical doses appears to more significant than originally thought. Because amphetamine, bupropion and methylphenidate all inhibit noradrenaline and dopamine reuptake, the function and roles of both catecholamines should be considered with respect to exercise and stress.

1.10 - Measuring Central Catecholaminergic Activation via Pituitary Hormones

While the application of microdialysis techniques has enabled measurement of neurotransmitter release during exercise in rodents, the measurement of changes in central neurotransmission is fairly limited in human exercise studies. Modern imaging techniques are often not suitable due to expense, access to experienced operators and logistical problems with performing exercise in or close to the equipment. However, it is possible to measure changes in central catecholaminergic activity indirectly via changes in circulating pituitary hormones. Although a large number of neurotransmitters and hormones affect pituitary hormone secretion, the effects of monoamines on the secretion of adrenocorticotrophic hormone (ACTH), growth hormone (GH) and prolactin have been well characterised. Prolactin secretion is inhibited by high concentrations of dopamine, which is released by tuberoinfundibular neurons at the pituitary as an inhibitory feedback mechanism in response to circulating prolactin. Noradrenaline weakly increases prolactin secretion by inhibiting tuberoinfundibular dopamine release (Freeman *et al.*, 2000). While It is well established that dopamine stimulates GH secretion, noradrenaline has opposing actions at α and β adrenoceptors on GH secretion, but it is generally accepted that endogenous noradrenaline increases GH secretion (Müller *et al.*, 1999). An important mediator of the hypothalamic-pituitary-adrenal (HPA) stress response is

corticotropin-releasing factor (CRF). Noradrenaline stimulates CRF release from projections of the paraventricular nucleus of the hypothalamus (PVN) to the hypothalamo-hypophyseal portal system, which stimulates the secretion of ACTH into the blood stream. Circulating ACTH then acts at the adrenal glands to increase circulating cortisol (Tsigos & Chrousos, 2002).

Monitoring the hormonal response of individuals to nutritional and pharmacological interventions or stressors has become an important tool in both a clinical setting and in exercise physiology to provide information regarding alterations in central neurotransmission, or the individual variability and sensitivity to such changes. Although the regulation of pituitary hormone secretion is complex and modulated by a number of other neurotransmitters and hormones, changes in circulating concentrations can be a useful guide. Nonetheless, caution must be exercised when interpreting the results as complex interactions between factors which increase and factors which decrease secretion of these hormones may conflate the significance of a given hormonal response. This is particularly true during stress, when peripheral and central physiological changes, as well as psychological changes can all contribute to the regulation of their secretion (described below).

1.11 - Central Stress Signalling During Prolonged Exercise

A great deal of emphasis has been given to central dopamine in central fatigue and the role of dopaminergic neurons in motor control and motivation, while comparatively little attention has been given to the role of noradrenaline. This is in spite of being directly involved in many of the same processes as well as being a critical component of the stress response. Of the noradrenergic nuclei, the locus coeruleus has received the most attention due to its aberrant activity in depression (Nestler *et al.*, 1999) and possibly attention deficit hyperactive disorder (Pliszka, 2005; Del Campo *et al.*, 2011). Many drug treatments for these disorders, such as anti-depressants, modulate locus coeruleus activity (West *et al.*, 2009) including bupropion (Cooper *et al.*, 1994) and methylphenidate (Devilbiss & Berridge, 2006; Ishimatsu *et al.*, 2011). The locus coeruleus is well known for its role in the

attention/arousal system, involved in waking from sleep and promoting alertness or mediating the fight/flight stress response. Recent evidence has demonstrated a more complex role for the locus coeruleus in behavioural flexibility and implicate its dysfunction in a broad range of behavioural disorders (Devilbiss & Berridge, 2006). The evidence suggests that both insufficient and excessive noradrenaline activity may result in psychomotor, cognitive, attention-arousal dysfunction, as well as depression, anxiety disorders and ADHD (Aston-Jones *et al.*, 1999; Nieuwenhuis *et al.*, 2005; Aston-Jones & Cohen, 2005; Aston-jones *et al.*, 2007).

Whether the predominant stressor during prolonged exercise in a warm environment is core temperature, cardiovascular strain or both, the stress systems recruited in the CNS which dictate the behavioural response are the same. These systems are heavily dependent upon central catecholamine function in both their bottom-up signalling of the stress event and the top-down control of the behavioural response. The signalling of peripheral stress within the CNS is determined by two key areas of the brain: the hypothalamus and the brain stem (Joëls & Baram, 2009). The hypothalamus and noradrenergic nuclei of the brainstem are interconnected and coordinate the activation of the stress system. Two of these nuclei are especially suited to coordinate the stress response. The A2 nucleus of the nucleus tractus solitarius receives ascending afferents via the spinal cord, while the locus coeruleus does not; however, both are heavily interconnected throughout the brain and spinal cord. They are instrumental in providing the sympathetic activation required for exercise, both directly via spinal pathways and indirectly via the HPA axis. As described in section 1.10, noradrenaline stimulates CRF release from the PVN, resulting in activation of the HPA axis and increases in circulating ACTH and cortisol. Circulating cortisol acts in a negative feedback loop to decrease CRF and ACTH secretion from the PVN and hypothalamo-hypophyseal portal, respectively (Tsigos & Chrousos, 2002). However, the HPA feedback loop can be overridden by noradrenergic afferents from the locus coeruleus and A2 at the PVN, increasing CRF secretion and HPA activity during acute stress (Ziegler *et al.*, 1999).

In both the A2 (Glass *et al.*, 2001) and the locus coeruleus (Nestler *et al.*, 1999) α_2 autoreceptors provide negative feedback to noradrenaline release, however, this regulatory mechanism may become overridden during acute stress. The afferents from the A2 also increase CRF signalling within the CNS via projections from the hypothalamus and amygdala (Johnson *et al.*, 2011). Both the hypothalamus and amygdala send CRF afferents to the locus coeruleus, which forms a feedforward system for the activation of the stress response (Koob, 1999; Gold & Chrousos, 2002). Psychological stress has been found to increase CRF mRNA in the central nucleus of the amygdala, but not in the PVN (Makino *et al.*, 1999). This suggests that psychological stress can act independently or concert with physiological strain to activate or enhance the stress response via these amygdalar afferents (see figure 1.9).

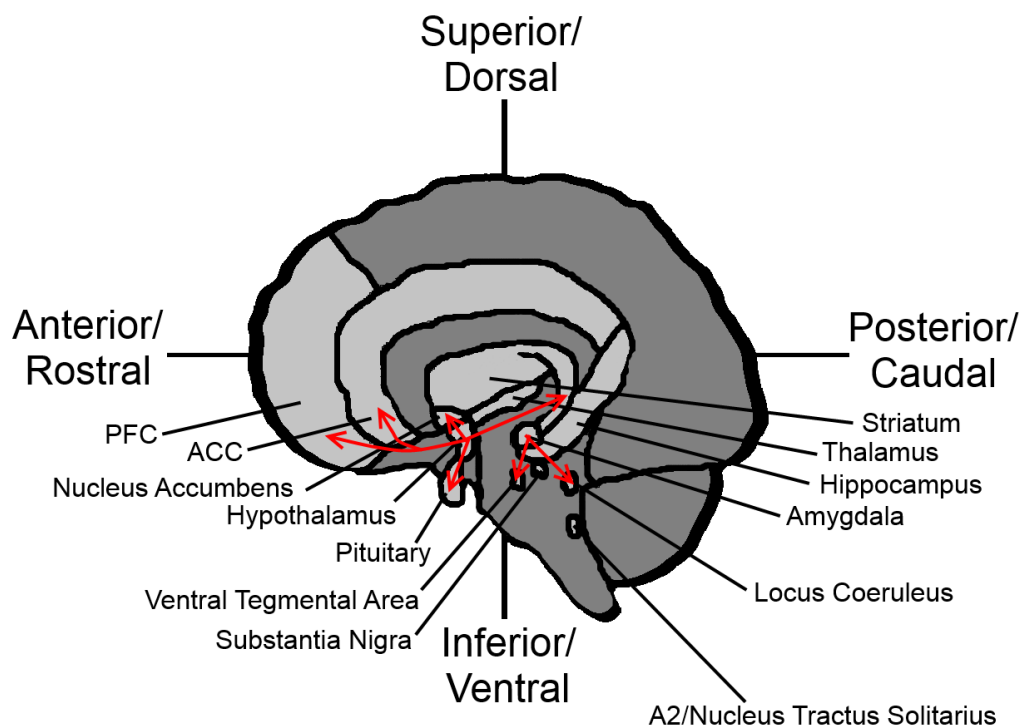


Figure 1.9 CRF projections in the brain displayed as red arrows.

During prolonged exercise CRF, ACTH and arginine vasopressin have been found to increase in a linear time-dependent fashion in male athletes (Inder *et al.*, 2012). Both ACTH and arginine vasopressin also increases locus coeruleus neuronal activity in a dose-dependent manner (Olpe *et al.*, 1987). Thermal strain also increases locus coeruleus neuronal activity (Morilak *et al.*, 1987) and activation of locus coeruleus by hemodynamic strain appears to be due to local release of CRF (Valentino *et al.*, 1991). Arginine vasopressin secretion is also enhanced by hyperosmolality, heat/hyperthermia and hypovolemia (Takamata *et al.*, 1995). Consequently, there may be an additive stimulatory effect on locus coeruleus activity during prolonged exercise in the heat, which increases as core temperature and blood osmolality increase while blood pressure decreases. Similarly, circulating adrenaline is increased during prolonged exercise in warm environments (Febbraio, 2001), which can stimulate the A2 via ascending sympathetic afferent feedback (Rinaman, 2011). Collectively, this provides a link between the thermoregulatory strain, the resulting demands on the cardiovascular system described by Cheuvront and co-workers (Cheuvront *et al.*, 2010) and the catecholaminergic neural circuits within the CNS, which modulate the behavioural response.

The mesocorticolimbic circuit is where dopamine and noradrenaline interact to coordinate the behavioural stress response. The locus coeruleus projects to the cerebellum, thalamus, hippocampus, and cerebral cortex, while the A2 projects to the locus coeruleus, amygdala and NAc and both nuclei project to the hypothalamus (Rinaman, 2011). Therefore, the A2 more directly controls emotion and affect during stress, while also modulating the locus coeruleus, which has more direct control over sensory informational processing and cognitive function. The mesocorticolimbic circuit consists of projections arising from the mesencephalon (or midbrain), the cortex and the limbic system. The midbrain projections are primarily from the VTA, the dopaminergic nucleus of the brain with the most diverse afferent projections. The cortical projections come from the frontal cortex and the limbic system projects from the anterior cingulate cortex (ACC) and ventral striatum. This circuit therefore integrates sensory, memory, emotional, affective, behavioural, motor and executive thought centres, encodes the salience of the stimuli and elicits a response in

accordance with the learned expectation of the outcome (Schultz, 1997; Wise, 2004).

Central to the mesocorticolimbic motivation system is the projection of dopaminergic VTA neurons to the NAc (Wise, 2004). This system was originally considered the pleasure centre of the brain, with dopamine release being responsible for the 'hedonic impact' of drugs and rewarding stimuli. Interestingly, evidence is accumulating to support the notion that both aversive unpleasant stimuli activate the motivational system the in the same ways that rewards do, as confirmed by microdialysis, electrophysiological and voltammetric studies (Salamone *et al.*, 2007). Further supporting this evidence, it has been found that amphetamine enhanced aversive responses in animals, and NAc lesions interfere with aversive responses (Kelley and Berridge 2002). Dysfunction of the mesocorticolimbic circuit is also implicated in pathological pain disorders (Borsook *et al.*, 2007). Aversive responses can be triggered by noxious stimuli, such as noxious heat, by coupling the corticolimbic circuit with the "classic pain circuitry" via nociceptive signals (Becerra *et al.*, 2001). The amygdala, which was originally considered the fear centre, is also active in these processes. The central nucleus of the amygdala also projects to the major brainstem monoamine nuclei, which includes the SN, the VTA, the serotonergic Raphé nucleus, and locus coeruleus as well as the hypothalamus driving appropriate autonomic and motor responsivity to emotionally salient input (Cardinal *et al.*, 2002). The amygdala also has direct interaction with the shell of the NAc, PFC and ACC, providing a direct pathway for emotionally salient stimuli to influence motivation and behaviour.

Recent discoveries on the significance of locus coeruleus activity have demonstrated an important distinction in electrophysiological modes of neuronal firing, which modulate behaviour and determine task performance. Increased phasic firing of locus coeruleus neurons is associated with improved attention and focus, while increased tonic firing of locus coeruleus neurons disengages animals from task performance and promotes behavioural adaptation (Aston-Jones & Cohen, 2005). This is relevant because CRF has been found to increase tonic but not sensory-

evoked phasic activity of noradrenergic locus coeruleus neurons in unanesthetized rats (Valentinol & Foote, 1987). Similarly, CRF was found to modulate locus coeruleus activity in a dose-dependent inverted U-shaped response, initially improving task performance, but as the dose increased, promoted behavioural flexibility and a shift to searching of alternative activities (Snyder *et al.*, 2012). This inverted-U response to locus coeruleus activity is reflected in the control of noradrenaline concentrations over PFC function. Because of this concentration-dependent modulation, this stress/arousal system promotes different behaviours depending on the level of activation. Higher levels of noradrenaline activate $\alpha 1$ and $\beta 1$ receptors and take the PFC “off-line” whereas in other regions they provide a more optimal neurochemical environment and shifts to more reflexive, instinctive behaviour driven more by subcortical structures (Ramos & Arnsten, 2007; Arnsten, 2009).

The PFC and ACC are interconnected and both send and receive projections to catecholaminergic nuclei, collectively contributing to the integration of emotional and cognitive processes (Bush *et al.*, 2000) and directing motor behaviour, allowing the transformation of intention into action (Paus, 2001). Their activity is also integrated in the generation of the error-related negativity (ERN) component of event-related potentials measured by electroencephalogram (EEG)(Herrmann *et al.*, 2004). The ERN is thought to reflect internal monitoring errors and has been shown to occur even as a result of unperceived, or subconscious errors and are proportionate to the degree of mismatch between expectation and outcome (Nieuwenhuis *et al.*, 2001). Interoceptive information provided from the insular cortex allows for autonomic management of behavioural economics, linking intent, action and consequence (Sanfey *et al.*, 2006; Salamone *et al.*, 2009; Medford & Critchley, 2010). Although all motivated behaviour is dependent on NAc dopamine (Salamone *et al.*, 2009), the appropriate selection and maintenance of behaviour in response to these signals appears to be dependent on the ACC, particularly in extended or prolonged behaviour (Holroyd & Yeung, 2012). noradrenaline facilitates processing by the ACC, while phasic dopamine bursts transmit information based on reinforcement history for decision making, particularly in difficult or demanding tasks (Warren & Holroyd, 2012).

The teleoanticipatory centre proposed by St Clair Gibson and co-workers (2006) closely mirrors the role of the ACC and ERN. As power output is modulated a prediction of performance feedback will be computed. If afferent stress signals do not match those predicted by the algorithm, a change in power output may be made to compensate and the experience of this change of demand will be reflected in perceived exertion. Indeed, the perception of errors acts as aversive, or unpleasant stimuli (Hajcak & Foti, 2008). This suggests that when unexpected negative teleoanticipatory feedback is received it would have a negative emotional and cognitive impact. This may explain the findings by Baden and co-workers (2005) in which participants were surprised by a small extension in the amount of exercise asked of them, resulting in an increase of negative affect which corresponded with an increase in RPE, despite being otherwise well within their means to perform. Supporting this notion, negative affect increases the amplitude of ERN in response to errors (Wiswede *et al.*, 2009), suggesting a potential feed-forward effect of an increasingly negative impact on affect and stress by error detection and awareness. There is also evidence supporting the contribution of unconscious stress signals to the same teleoanticipatory algorithm for pacing. A study comparing the cycling power output at a fixed RPE until power reached below 70% max in different temperatures showed that power output is decreased when rate of heat storage is increased (Tucker *et al.*, 2006). The relationship between PFC, insular cortex and ACC represent a possible neuroanatomical substrate for the subjective experience of perceived exertion. This neural construct serves as a bridge between conscious and unconscious regulation of effort, in which psychological and physiological strain interact and modulate the performance of motivated behaviours.

Because of the difficulty of measuring brain function during exercise, there is little direct evidence of the processes underlying central fatigue. However, fatigue during exercise in the heat has been associated with decreased EEG cortical β power without significant changes to α power, which was positively correlated with ratings of perceived exertion (Nybo & Nielsen, 2001*b*; Rasmussen *et al.*, 2004). β power has also been investigated with respect to movement disorders, as relatively high β power over the motor cortex is associated with advanced stages of Parkinson's disease and dopamine depletion (Jenkinson & Brown, 2011). Recently, it has been

proposed that total cortical β power represents a cross-network communication, which maintains the status quo, facilitating and reinforcing the networks promoting appropriate behaviour, while decreasing β power is associated with increasing bottom-up stimuli, distractibility and promoting a shift in behaviour (Engel & Fries, 2010). It is worth noting that individuals with ADHD display decreased frontal β power compared to controls (Snyder & Hall, 2006) and bupropion, amphetamine and methylphenidate, which have been successful in improving performance during prolonged exercise in the heat are treatments for ADHD (Wilens, 2006). Similarly, clinically relevant doses of psychostimulants preferentially increase extracellular catecholamines in the PFC (Berridge & Arnsten, 2012) while also slightly decreasing locus coeruleus cell firing by inhibiting reuptake (Stahl *et al.*, 2004; Devilbiss & Berridge, 2006). This includes methylphenidate (Berridge *et al.*, 2006) and bupropion (Bares *et al.*, 2010) and amphetamine (Berridge & Arnsten, 2012).

1.12 - Summary and Aims

Studies using central catecholamine reuptake inhibitors have thus far been the most successful pharmacological manipulation of central fatigue during prolonged exercise, particularly in a warm environment. These drugs share a common therapeutic efficacy in treating ADHD and appear to preferentially affect the PFC and brain stem nuclei. However, the effects of these drugs during prolonged exercise in warm conditions warrants further characterisation. Similarly, few studies have been conducted to examine the effects of changes to catecholamine metabolism on prolonged exercise performance in warm conditions. Therefore the aim of this series of studies is to contribute to both elements of the role of central catecholamines during prolonged exercise in a warm environment. The studies described in Chapters 3 and 4 are designed to further explore the effects of central catecholamine reuptake inhibition with bupropion. Chapter 3 was the first study to examine the gender differences in the role of central catecholamines during prolonged exercise. The aim of this chapter was to determine whether the same effects observed by Watson and co-workers (2005) are the same for women as they were in men. The aim of Chapter 4 was to determine the effects of different doses of bupropion on exercise performance. Both Chapters 3 and 4 were conducted with funding from the

World Anti-Doping Agency, and were designed to help determine the necessary guidelines and regulations for bupropion. Chapter 5 was designed as a follow up to the study conducted by Meeusen and co-workers (1997). In this study the pharmaceutical Sinemet, which is the catecholamine precursor L-DOPA combined with an AADC inhibitor (carbidopa), was administered before prolonged exercise and had no effect on performance. The study described in Chapter 5 was designed to examine the effect of the same pharmaceutical on prolonged exercise in warm conditions, with the consideration that warm conditions provide an environment more sensitive to central catecholamine function. Furthermore, Chapter 5 included a dosing schedule better suited to the pharmacokinetics of this particular drug. The study described in Chapter 6 was an experimental probe into alternative nutritional manipulation of central catecholamines. SAM is readily available as a nutritional supplement in the UK and USA, but is the most heavily researched and supported alternative antidepressant treatment (Bottiglieri, 2002). It is hypothesised that these manipulations of central catecholamines will improve performance during prolonged exercise in warm conditions.

Chapter 2

General Methods

2.1 - Ethical Approval

All work described in Chapters 3, 5 and 6 received approval from the Loughborough University Ethical Advisory Committee. The study in Chapter 4 was approved by the Research Council of the Vrije Universiteit Brussels, Belgium. Prior to the start of each investigation all potential participants were first approached either in person, or contacted via email or poster. Those expressing an interest in taking part received written details approved by the local Ethics Committee outlining the background to the study, information regarding the protocol and any possible discomfort or adverse effects that could arise during the investigation. Following an opportunity to ask questions, those interested in participating completed a health screen questionnaire and signed a written statement of consent. In the studies using prescription drugs, the health screen questionnaire was viewed by the prescribing doctor to ensure no contraindications prior to enrolling in the study. All participants were fully aware from the outset that they were free to withdraw from the study at any time without providing any reason for doing so.

2.2 - Participants

Participants were recruited from local university staff and student populations as well as local sports clubs. Female participants were recruited in Chapter 3 and male in Chapters 4, 5 and 6. Due to the physically demanding nature of the investigations, participants were familiar with the sensation of strenuous and prolonged exercise. Participants were aged between 18 and 35 for all investigations. Participants recruited to take part in the studies investigating responses to exercise in a warm environment were unaccustomed to exercise in the heat at the time of the investigation. Due to the nature of these series of studies, those with a history of psychiatric illness and/or metabolic disease were excluded. For the investigations described in Chapters 3, 4, and 5, participants were provided with manufacturer information on contraindications and adverse effects, which served as additional exclusion criteria. For the investigation described in Chapter 3, only female participants who were not using hormonal contraceptives were selected due to a potential interaction with bupropion metabolism (described in Chapter 3). Those individuals that did not fit the inclusion criteria were thanked for their interest and

politely told that their help would not be required. In addition, participants were asked to sign an informed consent form after completing a health screen questionnaire approved by the prescribing doctor.

2.3 - Experimental Design and Standardisation of Experimental Conditions

All studies were placebo-controlled trials. All trials were randomised using a Latin-square design to minimise any order effect and were administered in a double-blind manner. All studies employed a cross-over design. The study in Chapter 4 employed a randomised 4-block design to control for order effects that may have occurred over the 4 experimental trials. Trials were performed at the same time of day to minimise the influence of circadian variation. Prior to the start of the experimental trials, a familiarisation trial was undertaken to ensure the participants were accustomed to the procedures employed during the investigations and to minimise any potential learning or anxiety effects. This followed the exact protocol used in the experimental trials. In Chapters 3 and 5, an additional single-blind placebo treatment was administered to serve as a second familiarisation and provide an extra comparison of reliability for any observed effects.

To help ensure metabolic conditions were similar before each experimental trial, participants were instructed to record all food and fluid intake (household measures technique), as well as any exercise performed, in a diary over the 2 days prior to the first trial. Participants were asked to replicate this dietary intake and physical activity as closely as possible during the 2 days before subsequent trials. Participants were also asked not to perform any strenuous exercise or consume alcoholic beverages in the 24 hours prior to all trials. Trials in Chapters 3 and 6 were performed following an overnight fast. In Chapter 4, participants consumed a standardised breakfast 90 minutes before arriving for testing. In Chapter 5, participants were instructed to consume a standardised breakfast (provided) 4 hours and 30 minutes before entering the lab. These differences in protocol were due to timing of food around drug dosing. Participants in all investigations were asked to consume 500mL of plain water during the 90 minutes before entering the lab. The environmental conditions

for exercise trials were controlled using a climatic chamber with integrated thermostat and hygostat (Weiss Technik UK Ltd, Loughborough, UK). When participants were required to remain seated at rest, the ambient temperature was maintained within a comfortable range (20-25°C). All trials were separated by at least 7 days to limit the development of heat acclimation (Barnett and Maughan, 1993).

2.4 - Measurement of Peak Oxygen Uptake

To determine the workloads required during experimental protocols, participant peak oxygen uptake (VO_2peak) was determined in advance. This was achieved with a discontinuous protocol (Chapters 3, 5, and 6) or continuous (Chapter 4) incremental graded exercise test on an electrically braked cycle. The discontinuous protocol required participants to complete between 4 and 6 discrete 4 minute increments, beginning at an initial workload of 100 watts (W) for male participants (Chapters 5 and 6) or 50W for female participants (Chapter 3). Depending on the participant's performance in the previous stage (e.g. verbal feedback, ratings of perceived exertion, heart rate), the workload was increased by 50 or 25W. Between each bout, a supervised rest period of 3 to 5 minutes was observed, during which the participant was able to walk around and drink plain water. This was repeated until the participant retired through volitional exhaustion. The continuous protocol required participants to begin exercise at an initial workload of 80W, with the intensity increased by 40W every 3 minutes until volitional exhaustion. Maximum workload (W_{max}) was determined using the following equation: $W_{\text{max}} = W_{\text{out}} + (t/180) \times 40$ where ' W_{out} ' is the workload of the last completed stage and ' t ' is the time in seconds of the final stage (Jeukendrup et al., 1996). The experimenters provided verbal encouragement during both protocols to help ensure a maximal effort.

Expired gas was collected during the last 60 seconds of each stage in the discontinuous protocol, using the Douglas bag method. Throughout the continuous test, expired gas was analysed using an automated spirometry system (Metamax, Cortex, Biophysik GmbH, Germany). At the end of each increment in both protocols, heart rate was recorded using telemetry (Polar Favor, Kempele, Finland).The

expired gas collected using Douglas bags was analysed for oxygen (O_2) and carbon dioxide (CO_2) composition by drawing gas through a paramagnetic O_2 transducer and infra-red carbon dioxide analyser (Servomex 1440c, Crowborough, UK). The gas analysers were calibrated with gases of known concentration (British Oxygen Company, London, UK). The volume of gas expired was measured through a dry gas meter (Harvard dry gas meter, Harvard Apparatus Ltd, Kent, UK) and the temperature of the expired gas was recorded using an electronic temperature sensor at the dry gas meter inlet (Edale Instruments Ltd, Cambridge, UK). All expired gas volumes were corrected to standard temperature and pressure for dry gas (STPD). Barometric pressure was measured using a standard mercury barometer. O_2 consumption, CO_2 production and the respiratory exchange ratio were calculated using the equations detailed by Frayn (1983). These data were used to calculate the workloads corresponding to the desired percentage of participant's VO_{2peak} .

2.5 - Exercise Trials

All the exercise was completed using cycle exercise on a stationary, electrically braked cycle ergometer (Lode Corival or Lode Excalibur Sport, Groningen, Holland). During the VO_{2peak} test, participants were asked to find a comfortable saddle height, which was recorded and used for the remaining trials. In Chapters 3 and 5, expired gas was collected every 15 minutes of the steady-state work period to verify the workload was correct. In Chapter 6 expired gas was collected every 15 minutes for the first hour of exercise to verify the workload and observe any potential changes in substrate utilisation. Participants were supervised by the same experimenters for all experimental trials to help ensure standardised conditions. To assess exercise performance in Chapters 3 and 5, participants completed preloaded workload challenge. The preload consisted of cycle exercise at a steady state (60% VO_{2peak}), followed by completion of as much work as possible in 30 minutes. During this 30 minute workload challenge participants were free to manipulate the workload, which was initially set corresponding to 75% VO_{2peak} . No feedback was provided regarding power output or heart rate. In Chapter 4 a preloaded time trial (TT) protocol was employed. The TT required participants to complete an amount of work equal to 30 minutes at 75% W_{max} as quickly as possible (Jeukendrup et al., 1996).

Participants began the TT at a workload corresponding to 75% W_{max} and were free to increase or decrease their power output as desired. During the TT a computer program displayed a bar indicating the percentage of total work completed to give the participant an indication of their progress. No feedback was provided regarding time, power output, pedal cadence or heart rate. In Chapter 6, time to exhaustion, defined as volitional cessation of exercise or inability to maintain cadence above 50-60 rpm after 3 warnings (below which the Lode Corival can no longer apply constant load), was used as a performance measure. This method was selected to promote fatigue-associated physiological and psychological changes that could be affected by SAM supplementation.

In all studies, post-void nude body mass was measured before and after exercise to quantify sweat loss. This difference was corrected for any urine output and fluid ingestion during the trial. These data were not corrected for respiratory water or losses due to substrate oxidation. To monitor core body temperature whilst at rest and during exercise, participants inserted a flexible rectal thermistor (YSI UK Ltd, Hampshire, UK or Gram Corp. LT-8A, Saitama, Japan) 10cm beyond the anal sphincter. Surface skin thermistors (Grant Instruments Ltd, Cambridge, UK or Gram Corp. LT-8A, Saitama, Japan) were positioned at four sites (chest, upper arm, thigh and calf). Thermistors were held securely in contact with the skin using transpire medical tape (3M, Loughborough, UK). Weighted mean skin temperature was calculated using the methods described by Ramanathan (1964). Ratings of perceived exertion (RPE) were assessed at regular intervals during exercise using the 15-point Borg scale (Borg, 1982). Ratings of perceived thermal strain were assessed at the same intervals using a 21-point thermal sensation scale ranging from unbearably cold (-10) to unbearably hot (+10) adapted from Hardy (1970)(see appendix). Heart rate was measured at rest and during exercise using short-range telemetry (Polar Favor, Kempele, Finland).

2.6 - Blood Collection, Handling and Analysis

In chapters 3, 5 and 6, participants submerged their forearm into warm water (40-42°C) for approximately 10 minutes to arterialise venous blood and improve visibility of superficial veins. In chapter 5 and 6, an indwelling 21 gauge butterfly cannula (Surflo winged infusion set, Terumo, Tokyo, Japan) was inserted into a superficial forearm vein and a three way tap (Luer-Loc 360, BD Connecta, Heidelberg, Germany) was attached to the end to allow repeated blood sampling. The indwelling cannula was kept patent by flushing with 2-3mL of heparinized saline after each sample. In Chapter 3, blood sampling was done via venepuncture in the antecubital region due to the difficulty of finding superficial forearm veins in female participants. In Chapter 4, blood samples were collected from the antecubital region using the vacutainer system. Assays used widely throughout this thesis are described below, with assays common to a single study described in the methods section of the appropriate chapter. All biochemical analyses performed throughout this thesis were performed in duplicate, unless otherwise stated.

In Chapters 3, 5 and 6, collected blood was immediately dispensed into plain tubes or tubes containing K₂EDTA. Duplicate 100µL aliquots of blood were rapidly deproteinised in 1000µL of ice-cold 0.3N perchloric acid. These were centrifuged and the resulting supernatant was used for spectrophotometric determination of blood-glucose using Randox GOD-PAP kit (Randox Laboratories Ltd, Crumlin, UK). EDTA-treated whole blood was used for the spectrophotometric determination of haemoglobin (Hb) by the cyanmethaemoglobin method as well as packed cell volume (PCV), which was measured in triplicate using microcentrifugation (Hawksley, Sussex, UK). Both Hb and PCV were determined within 2 hours after each experimental trial. These data were used to estimate percentage changes in blood, plasma and red cell volumes relative to the first sample using the methods proposed by Dill and Costill (1974). Untreated and EDTA-treated whole blood was centrifuged at 1500g for 15 minutes at 4°C to obtain serum and plasma, respectively. The supernatants were transferred into eppendorf tubes and stored at -20°C until analysis. In all investigations serum was used to measure cortisol, and in Chapters 4, 5 and 6, prolactin with enzyme-linked immunosorbent assay (ELISA) kits (DRG,

International Inc. New Jersey, USA). For all ELISAs standard curves and a 3 level control sera were measured in duplicate, while participant samples were measured in singlicate. Additional hormone analysis undertaken in Chapter 3 is described therein.

2.7 - Statistical Analysis

Data are presented as means \pm standard deviation (SD). The Shapiro-Wilk test was used to examine whether the outcome variables had a normal distribution. Homoscedasticity was checked using Levene's test. Data sphericity was determined using the Mauchly's test, and, where appropriate, further analysis was corrected as described by Atkinson and Nevill (2001). Exercise performance data were examined using one-way repeated measures of analysis of variance (ANOVA). To identify differences in data collected throughout each trial, two-way (time-by-trial) ANOVA was employed. Where a significant interaction was apparent pair-wise differences were evaluated using the Bonferroni post hoc procedure. Hormone AUC was compared using paired-samples t-tests. Statistical significance was accepted at $P < 0.05$. Non-parametric data were analysed with Friedman's test with Wilcoxon-rank sum for pairwise comparisons.

2.8 – Coefficients of Variation of Methods

CV averaged from duplicate and triplicate samples

Measure	Method	Mean	CV (%)
Haemoglobin	Cyanmethaemoglobin	153.3g/L	0.8
Packed Cell Volume	Microcentrifugation	43.90%	0.7
Blood Glucose	GOD-PAP (Randox)	4.45mmol/L	1.5
Serum Prolactin	Microplate ELISA	21.29ng/mL	7.4
Serum Cortisol	Microplate ELISA	228.7ng/mL	8.0
Plasma ACTH	Microplate ELISA	108.8pg/mL	10.5
Serum FSH	Evidence Investigator (Randox)	3.63mIU/mL	4.2
Serum LH	Evidence Investigator (Randox)	4.63mIU/mL	9.0
Serum Prolactin	Evidence Investigator (Randox)	904.7mIU/L	5.0
Serum Testosterone	Evidence Investigator (Randox)	2.63nmol/L	3.8
Serum Progesterone	Evidence Investigator (Randox)	12.74nmol/L	17.4
Serum Oestrogen	Evidence Investigator (Randox)	222.6pmol/L	3.2

Chapter 3

The Role of Central Catecholamines in the Development of Fatigue in Women during Prolonged Exercise in Warm Conditions

3.1 – Abstract

Bupropion, a dual noradrenaline/dopamine reuptake inhibitor, improves time trial performance in male participants in the heat. Gender differences in neuropsychopharmacology have been shown in animal and human studies, but it is not known whether these differences may alter the effect of bupropion on exercise performance in women. With local ethics committee approval, 9 physically active women (Mean \pm SD age 21 ± 2 y; height 1.68 ± 0.08 m; body mass 64.1 ± 6.0 kg; VO_{2peak} 50.9 ± 7.2 mL/kg/min) were recruited to examine the effect of pre-exercise administration of bupropion (4 x 150 mg) on prolonged exercise performance in a warm environment ($30.2 \pm 0.2^{\circ}\text{C}$, $50\% \pm 1\%$ rh). Participants completed a VO_{2peak} test, a familiarisation trial, and a single-blinded placebo control trial before a randomised, double-blind, placebo-controlled crossover design was employed. Experimental trials took place during the first 10 days of the follicular phase of the menstrual cycle. Participants cycled for 1 h at 60% VO_{2peak} followed by a 30 min workload challenge, during which they were instructed to complete as much work as possible. Heart rate, skin and core temperature, and ratings of perceived exertion and thermal sensation were recorded throughout exercise. Total work done was higher on the bupropion trial (291 ± 48 kJ) than on the single-blind (267 ± 48 kJ, $P=0.021$) and double-blind trials (269 ± 46 kJ, $P=0.042$). No differences were found between all trials for core temperature throughout rest, the first hour or the workload challenge. However, at the end of the workload challenge core temperature was higher on the bupropion trial (39.5 ± 0.4 $^{\circ}\text{C}$) than the single-blind (39.2 ± 0.6 $^{\circ}\text{C}$, $P=0.028$) and double-blind trials (39.2 ± 0.6 $^{\circ}\text{C}$, $P=0.021$). Heart rate was also higher at the end of the workload challenge on the bupropion trial (185 ± 9 beats/min) than the single-blind (180 ± 13 beats/min, $P=0.048$) and double-blind trials (179 ± 13 beats/min, $P=0.043$). The results indicate that during the follicular phase of the menstrual cycle an acute dosing protocol of bupropion can improve self-regulated work rate in warm conditions.

3.2 – Introduction

Endurance exercise capacity is reduced in warm conditions. This effect has been demonstrated to be ambient temperature-dependent (Galloway & Maughan, 1997) and exacerbated by increasing humidity (Maughan *et al.*, 2012). This is primarily due to the significant challenges to thermoregulation and, consequently, fluid balance as well (Hargreaves, 2008; Nybo, 2008; Maughan, 2010). This cumulative strain results in the eventual onset of fatigue and subsequent impairment in performance. This appears to be regulated largely by mechanisms within the CNS (as described in section 1.2). Though the cerebral mechanisms for the onset of fatigue are currently not understood, there have been many studies attempting to manipulate fatigue by altering CNS function. While those attempting to alter fatigue via manipulation of serotonin have yielded conflicting results (Meeusen *et al.*, 2006b), manipulation of central catecholamines has produced more consistent changes in the onset of fatigue and exercise performance in warm conditions (Roelands & Meeusen, 2010).

The physiological strain during prolonged exercise in the heat is similar for men and women, but sex hormone fluctuations throughout the menstrual cycle cause important physiological changes, which can affect performance. De Jonge (2003) provided a comprehensive review on studies conducted to determine the effects of menstrual cycle phases on exercise physiology and performance. The review found no differences or conflicting results for haemoglobin, haematocrit, muscle contractility and lactate response during the menstrual cycle. However, differences in VO_2 were observed during higher intensity exercise, which the author attributed to cardiovascular strain due to the increased resting body temperature during the luteal phase. This effect was exacerbated in warm conditions during light intensity intermittent exercise, but not at relatively low intensities (20%, 30% and 60% VO_2 max). A follow up study corroborated this decrease in performance during prolonged, high intensity exercise in warm conditions (de Jonge *et al.*, 2012). This was characterised by increased resting core temperature, increase rate in core temperature rise during exercise as well as increased RPE and perceived thermal strain. In summary, it appears that the main performance-affecting factors to

consider throughout the menstrual cycle are the fluctuating thermoregulatory processes and consequent resting body temperature, which is increased during the luteal phase. This negatively affects high intensity prolonged exercise in normal temperature and even more so in high ambient temperatures.

Ovarian sex hormones appear to modulate thermoregulation by regulating brown adipose tissue thermogenesis by directly acting at the progesterone receptors and sympathetic modulation by estrogen in the CNS (Quarta *et al.*, 2012). In addition to energy balance and thermoregulation, ovarian sex steroids modulate CNS activity in a number of ways, including the stress response (Chrousos *et al.*, 1998) and activity in areas of the brain associated with motor function and motivation (McEwen & Alves, 1999). Estrogen has been shown to modulate dopaminergic neurotransmission (Colzato *et al.*, 2010; Disshon, Boja, & Dluzen, 1998) and increases activity of tryptophan hydroxylase, an enzyme involved in serotonin synthesis, decreases 5HT_{1a} autoreceptor binding, and modulates the serotonin transporter, which leads to increased expression in the hypothalamus (Bethea *et al.*, 2002). In addition to the challenges presented by de Jonge (2003), the interaction between ovarian sex steroids and monoaminergic neurotransmission suggest possible sex-dependent differences in physiological and performance outcomes in response to pharmacological manipulation of CNS activity.

Women are more susceptible to depression than men, though this may be due to sociological factors, rather than differences in physiology (Piccinelli, 2000; Nolen-Hoeksema, 2001). Nonetheless, there appear to be gender differences in response to antidepressants; women tend to respond better to selective-serotonin reuptake inhibitors and worse to tricyclic antidepressants than men (Young *et al.*, 2009). While there are gender differences in neuropharmacology due to sex hormone interactions (Young & Becker, 2009), there are also differences in attitudes and expectations. Women are generally more willing to seek help, whereas this is generally stigmatised amongst men (Addis & Mahalik, 2003). Furthermore, gender differences in placebo and nocebo responses have been observed, with men more affected by expectancy and women to conditioning (Klosterhalfen *et al.*, 2009). Women are more likely to

report adverse side-effects during placebo treatment than men (Mora *et al.*, 2011), while men tend to be more susceptible to placebo analgesia (Aslaksen *et al.*, 2011). All of the studies conducted to investigate the effects of pharmacological manipulation of fatigue during prolonged exercise in warm conditions to date, have used male participants. It is therefore not known whether the same increases in exercise capacity during prolonged exercise in warm conditions will be seen in women. The aim of the present investigation is to determine the effects of bupropion, a dual dopamine /noradrenaline reuptake inhibitor on performance during prolonged exercise in warm conditions in physically active women. It is therefore hypothesised that acute administration of bupropion will improve prolonged exercise performance in women during the same menstrual cycle phase.

3.3 – Methods

Nine habitually active women were recruited (age 21 ± 2 y; height 1.68 ± 0.08 m; body mass 64.1 ± 6.0 kg; VO_{2peak} 51 ± 7 mL/kg/min). All participants actively took part in regular endurance exercise training, but were not accustomed to exercise in a warm environment at the time of the study. Prior to their admission to the study, participants were provided with information regarding the purpose and design of the study, including manufacturer information for bupropion. Thereafter, if participants confirmed their interest and eligibility, a statement of informed consent was signed. The Loughborough Ethics Advisory Committee approval number for this study was R10-P7. As oral contraceptives have been shown to interfere with bupropion metabolism via cytochrome P450 2B (Palovaara *et al.*, 2003), this study investigated participants not taking any form of hormonal contraception. To account for the hormonal fluctuations in the menstrual cycle, visits were coordinated with participants in relation to the self-reported length of cycle and the onset of menses as has been done in other studies (e.g: Minson *et al.*, 2000). Accordingly, those reporting irregular menstrual cycles and pregnancy were excluded from the study. The primary concern was to schedule the max test and experimental trials to fall within the follicular phase to avoid the increased cardiovascular strain and subsequent VO_2 described by de Jonge (2003) during the luteal phase. A recent study found the follicular phase to last between a minimum of 10 days after the onset

of menses and a maximum of 22 days (Fehring *et al.*, 2006). Therefore, participants were asked to visit the laboratory one week after the onset of menses for their max test and the following week for a familiarisation trial in order to minimise any learning or anxiety effects. Within a week of the onset of their next menses, participants completed a single-blinded placebo control trial. This served as both an experimental trial to compare against the double-blinded crossover trials and as a second familiarisation. Following the single-blinded placebo control trial a randomised, double-blind, placebo-controlled crossover design was employed. These trials were scheduled as soon as possible after the onset of the following menses and 7 days later for the cross-over trial (see figure 3.1 below). While this scheduling was subject to the participants' availability, the timing of the visits was coordinated in the same manner with respect to their menstrual cycle. In the 24 hours preceding the experimental trials participants took either placebo (~1g starch/capsule) or bupropion (4 x 150 mg spread over 24 h).

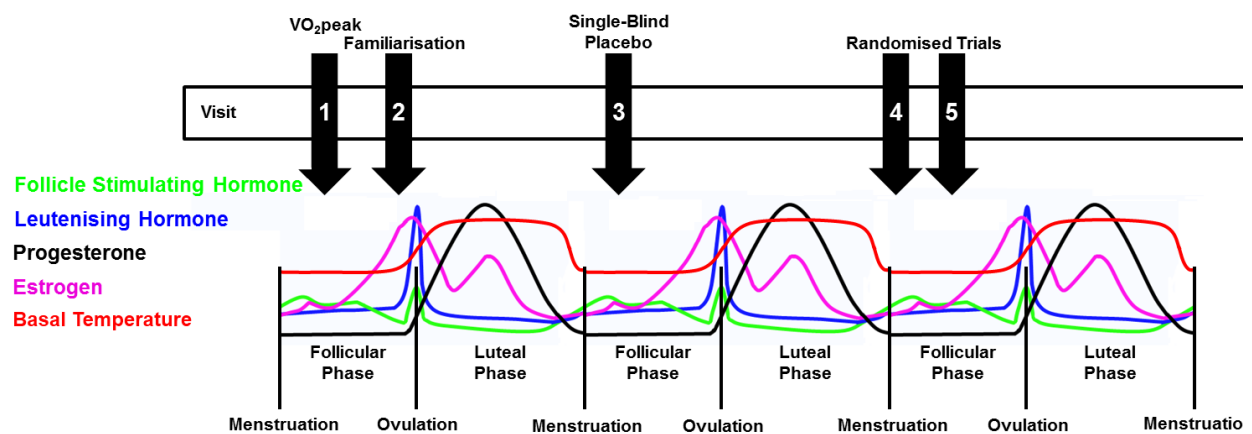


Figure 3.1 This schematic is an example of lab visit scheduling for the control for the hormonal and basal body temperature fluctuations throughout the menstrual cycle.

The experimental trials were designed to be similar to previous studies (Watson *et al.*, 2005a; Roelands *et al.*, 2008d, 2008b). To determine the work rates for the familiarisation and experimental trials, an incremental discontinuous VO₂peak test to volitional exhaustion was performed by participants using a Lode Corival cycle ergometer (Lode B.V., Groningen, Netherlands) in an environmental chamber (Weiss-Gallenkamp, UK) at 20°C and 50% relative humidity as described in Chapter 2. VO₂peak was then used to calculate work rates corresponding to this value using linear regression. The standardisation of pre-trial conditions is outlined in Chapter 2.

The experimental protocol is illustrated in figure 3.2. Participants were asked to cycle at a work rate corresponding to 60% VO_2peak for 60 min, followed by a 30 minute workload challenge, in which participants were asked to complete as much work as possible. Initial work rate during the workload challenge corresponded to 75% VO_2peak ; thereafter participants were free to manipulate the work rate to complete as much as they felt possible.

Participants arrived at the laboratory in the morning (before 9 am), overnight fasted and having consumed 500mL of plain water. After the collection of baseline measurements during the rest period, participants entered the climatic chamber maintained at $30.2 \pm 0.2^\circ\text{C}$, $50\% \pm 1\%$ relative humidity to begin exercise. During exercise, participants were permitted to drink water *ad libitum*. Water consumption was recorded by weight and deducted from post-exercise body mass, to enable the calculation of sweat loss. Throughout the trial heart rate, core and skin temperatures, were measured every 5 minutes. During the 15 minute rest period subjective thermal sensation was measured. During the 1 hour fixed work rate period subjective thermal sensation and rating of perceived exertion were measured every 15 minutes, when expired gas samples were also collected for verification of work rate. During the time trial subjective thermal sensation and rate of perceived exertion were measured every 10 minutes. Standardised verbal encouragement was provided by the experimenter to help ensure a maximal effort. Feedback during the time trial was limited to the time lapsed (power output, cadence, heart rate, etc.) were hidden from the participant. Following completion of the workload challenge and the collection of the final blood sample, participants left the climatic chamber. Skin thermistors were removed and participants removed the heart rate telemetry band and rectal thermistor in privacy before towelling off and nude body mass was recorded behind a screen.

During the experimental trials a maximum of four 5mL blood samples were drawn. Samples were collected at rest, after 30 minutes and at the end of the hour steady state period as well as upon completion of the time trial. Due to the difficulty of blood sampling from some of the female participants, only the resting blood sample

became a priority for hormone measurement to confirm the phase of the menstrual cycle. The 5mL blood samples were drawn into dry syringes and immediately dispensed into 1mL and 2.5mL tubes containing potassium EDTA (1.5mg/mL) and the remaining whole blood into 5mL plain tubes. The 2.5mL EDTA tubes were kept on ice. The 1mL EDTA blood samples were used to analyse haemoglobin and haematocrit, allowing for estimation of percentage changes in blood, plasma and red cell volumes relative to the first resting sample (Dill & Costill, 1974). Two aliquots of 100 μ L were pipetted from the 1mL EDTA tubes into eppendorfs containing 1mL of 0.3M perchloric acid kept on ice for measurement of blood glucose. The 2.5mL EDTA and 5mL plain tubes were centrifuged to obtain plasma and serum, respectively, which was then frozen at -20°C and later -80°C for hormone analysis at a later date. Cortisol was measured using ELISA kits (DRG, International Inc. New Jersey, USA). Follicle-stimulating hormone, luteinising hormone, prolactin, testosterone, progesterone and oestrogen were measured using a Randox Evidence Investigator and Fertility Array Biochips (Randox Laboratories Ltd, Crumlin, UK) in order to confirm the menstrual cycle phase the participants were in during each experimental trial.

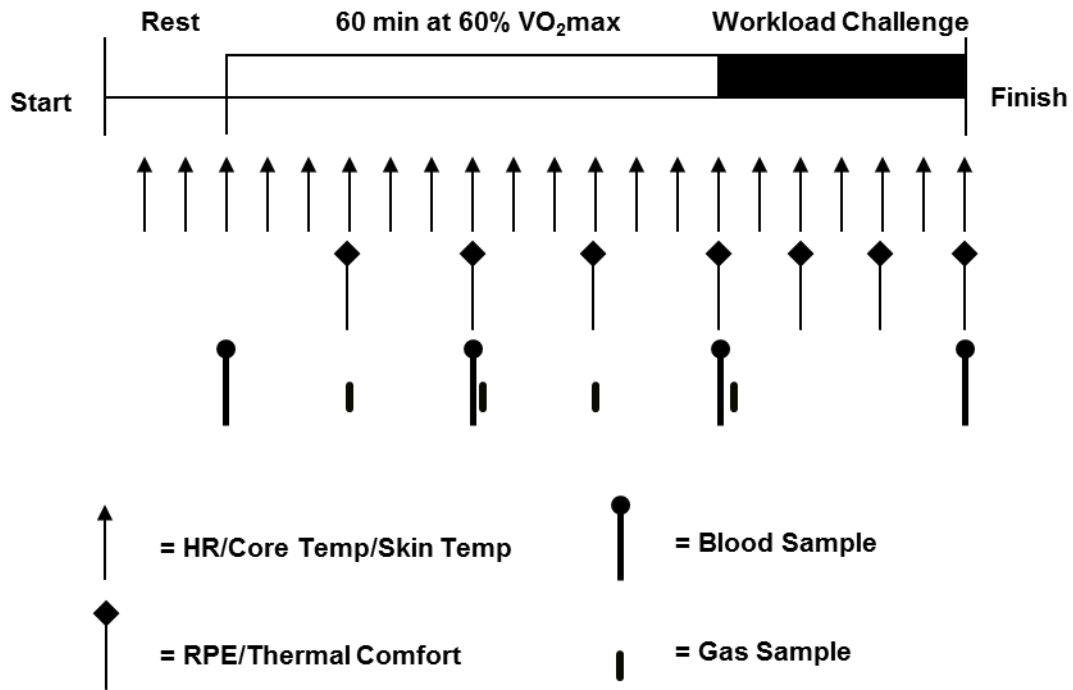


Figure 3.2 Schematic demonstrating the order and frequency of procedures during experimental trials.

3.4 – Results

Total work done was higher during the bupropion trial (291 ± 48 kJ), than during the single-blind (267 ± 48 kJ, $P=0.021$) and double-blind trials (269 ± 46 kJ, $P=0.042$) (figure 3.3A). The difference between the crossover trials represents an increase in performance of $7.5 \pm 9.6\%$ in the bupropion trial (figure 3.4). There was no evidence of an order effect during the experimental trials (visit 1 267.1 ± 48.2 kJ, visit 2 282.8 ± 36.5 kJ, visit 3 277.1 ± 54.3 kJ; $P=0.230$). During the bupropion trial, 7 of 9 participants completed more work than in the double-blind placebo trial; individual work completed during each trial is presented in figure 3.3B.

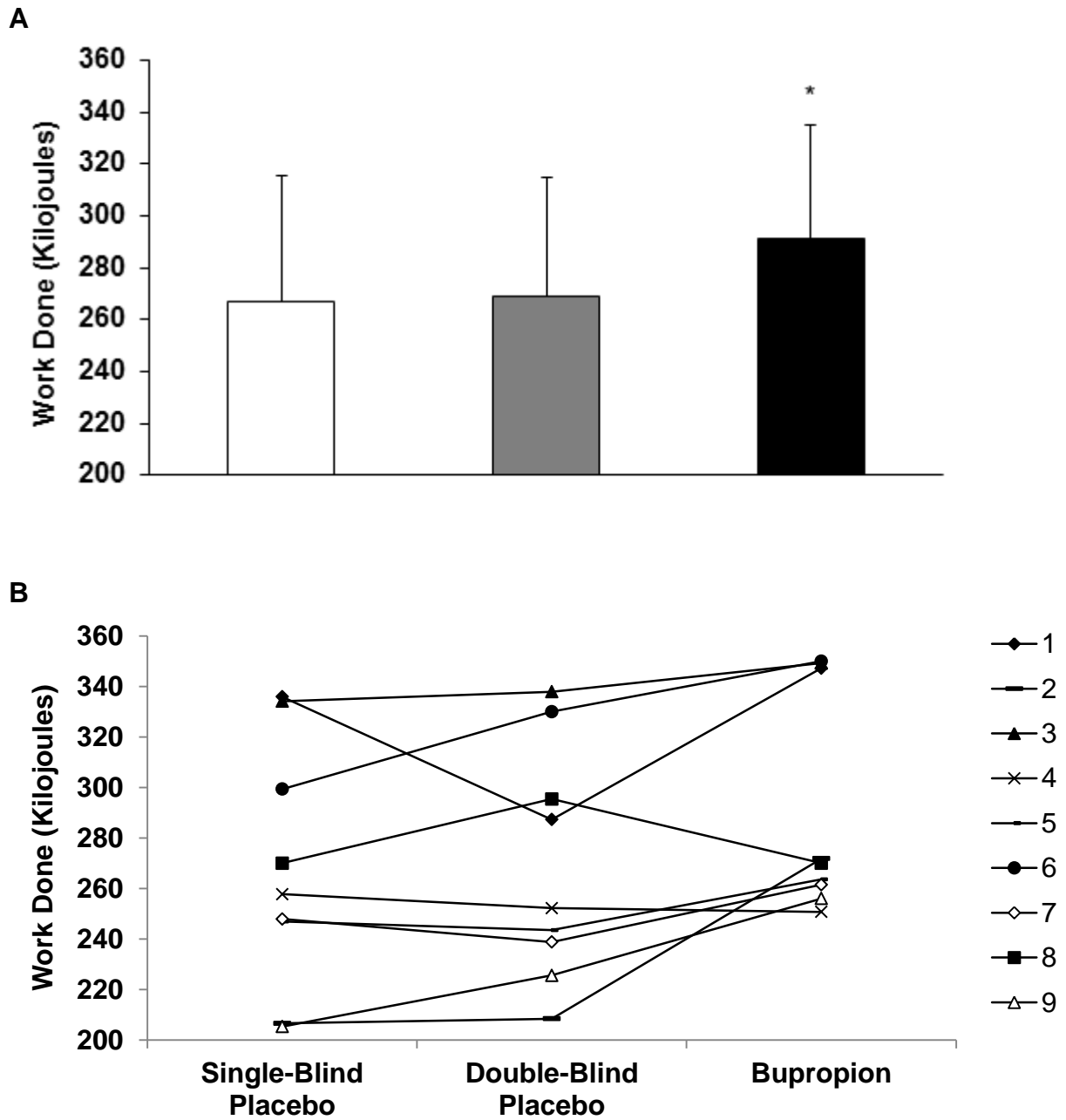


Figure 3.3 Exercise performance by trial (A) and individual performance (B) in each trial. * denotes a significant difference for bupropion vs single-blind and double-blind placebo trials ($P=0.021$ and 0.042 , respectively).

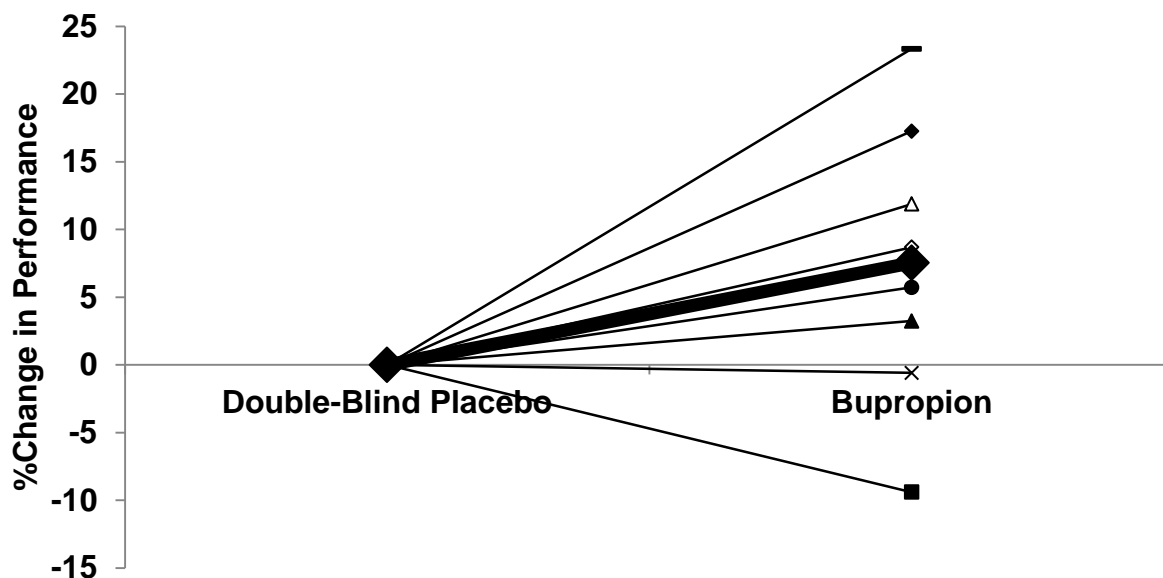


Figure 3.4 Individual percentage change in exercise performance between the double-blind placebo and bupropion trial. The thick black line represents the average change in performance (+7.5%).

Exercise resulted in a significant elevation in core temperature over resting values ($P < 0.05$). Despite the apparent trend for greater core temperature during the bupropion treatment, no differences were found between trials for core temperature at rest or during the first hour or the workload challenge ($P > 0.05$). However, at the end of the workload challenge core temperature was higher during the bupropion trial (39.5 ± 0.4 °C), than during the single-blind (39.2 ± 0.6 °C, $P = 0.028$) and double-blind trials (39.2 ± 0.6 °C, $P = 0.021$) (figure 3.5A). Weighted mean skin temperature rose rapidly with the first 15 minutes of exercise in all trials, becoming relatively stable thereafter (figure 3.5B). There were no significant differences for weighted mean skin temperature between groups ($P > 0.05$).

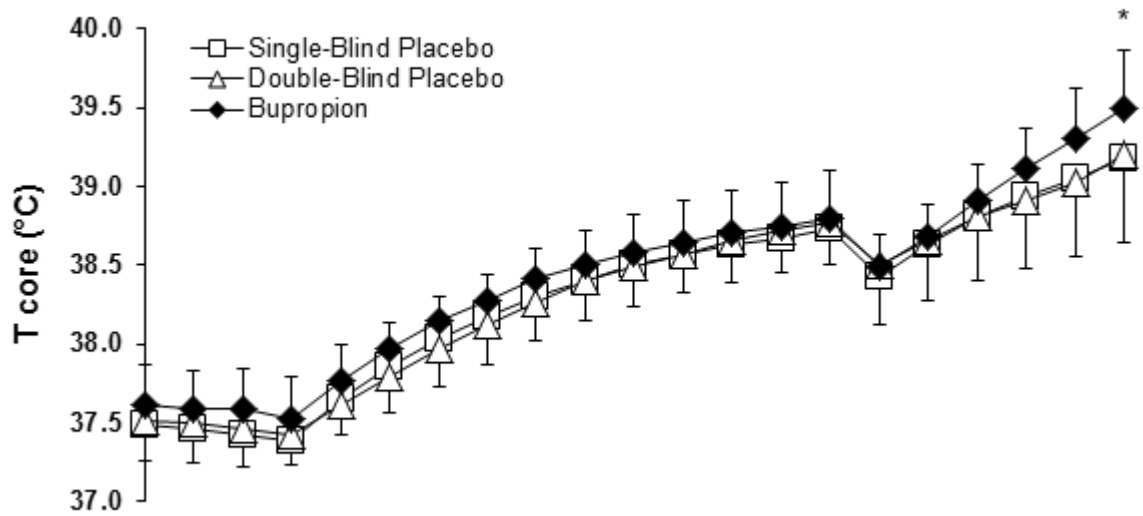
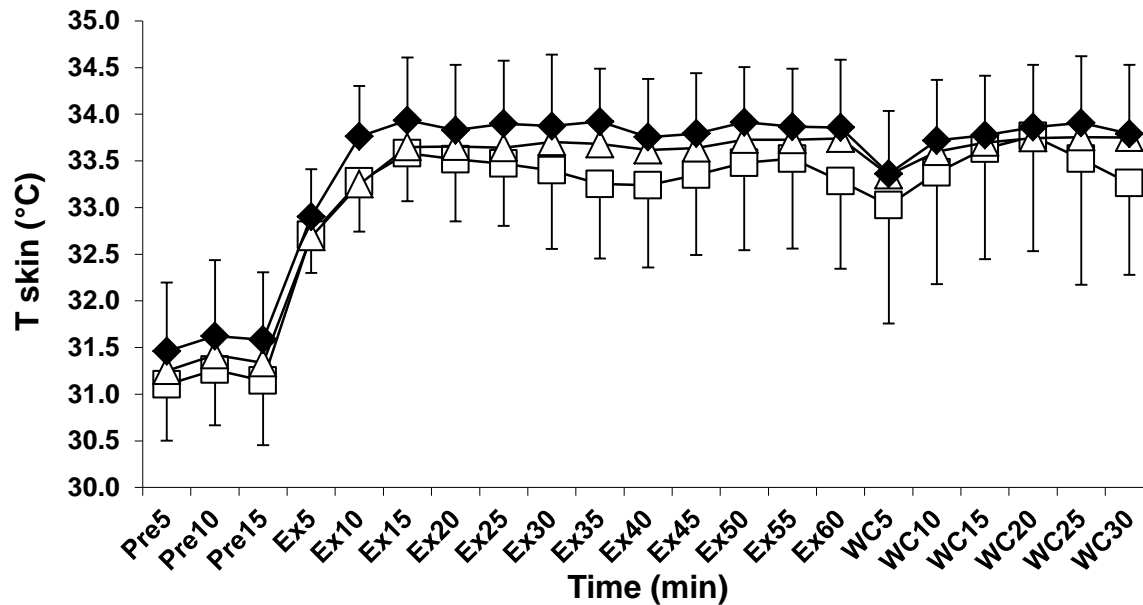
A**B**

Figure 3.5 Core (A) and weighted mean skin (B) temperature at rest and during exercise. * denotes a significant difference for bupropion vs single-blind and double-blind placebo trials ($P=0.028$ and 0.021 , respectively).

Heart rate increased rapidly with the first 10 minutes of exercise in all trials and continued to rise slowly thereafter (figure 3.6). Heart rate was higher at the end of the workload challenge during the bupropion trial (185 ± 9 beats/min) than the single-blind (180 ± 13 beats/min, $P=0.048$) and double-blind trials (179 ± 13 beats/min, $P=0.043$).

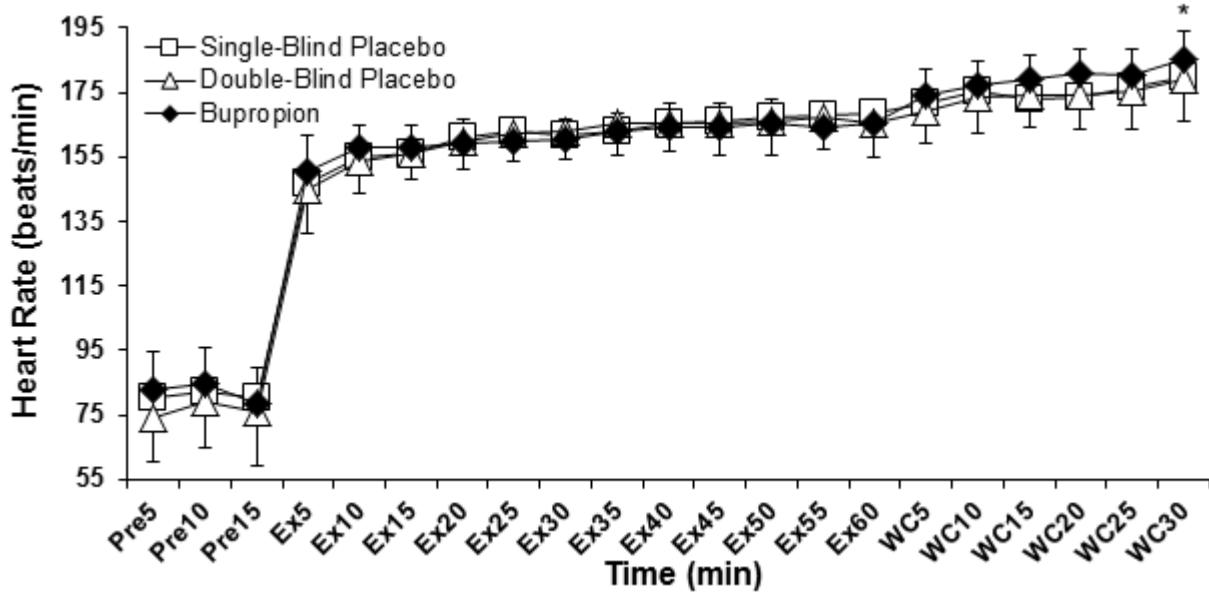


Figure 3.6 Heart rate at rest and during exercise. * denotes a significant difference for bupropion vs single-blind and double-blind placebo trials ($P=0.048$ and 0.043 , respectively).

Ratings of perceived exertion rose slowly through the first hour of exercise and climbed rapidly during the workload challenge in all trials (figure 3.7; $P<0.01$). No significant differences were found between trials ($P>0.05$). Ratings of thermal sensation increased rapidly after the first 15 minutes of exercise and continued to steadily increase until the end of the workload challenge for all trials (figure 3.8; $P<0.05$). No significant differences were found between trials ($P>0.05$).

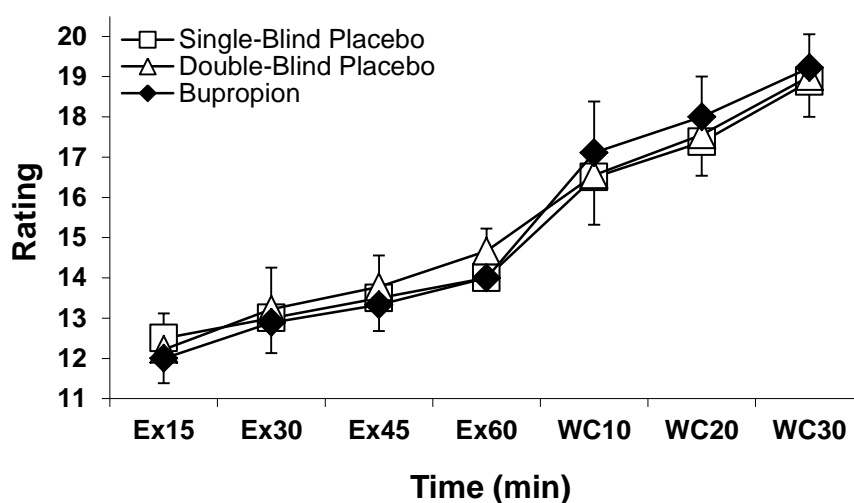


Figure 3.7 RPE during experimental trials.

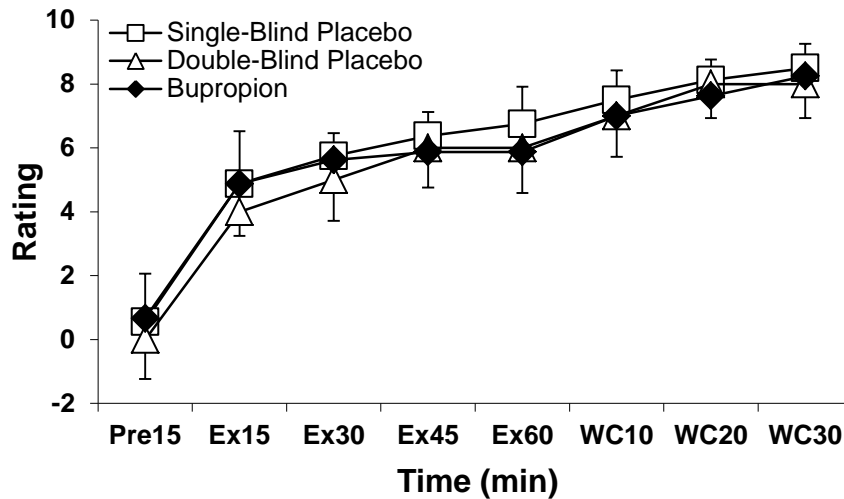


Figure 3.8 Ratings of thermal sensation during experimental trials.

Resting sex hormone levels were not significantly different between trials, nor were there any order effects ($P > 0.05$). There were no differences between trials for exercise-dependent hormone changes (table 3.1). Only five complete data sets were available for these comparisons due to blood collection difficulties. Serum prolactin concentration increased at the end of exercise across all trials, but no significant differences between trials were observed. Serum testosterone, progesterone, oestrogen concentrations were significantly increased at the end of exercise across all trials. Cortisol concentration was increased across all trials, but this was not significant. There were no significant differences for follicle stimulating hormone or luteinising hormone after exercise.

Table 3.1 Serum hormones measured at rest and at the end of the each experimental trial. ^a and ^b denote significant difference (P<0.05) for between rest and end between all trials and within the corresponding trial, respectively. FSH=Follicle Stimulating Hormone; LH=Luteinising Hormone; PRL=Prolactin; TEST=Testosterone; PRO=Progesterone; EST=Estrogen; CORT=Cortisol.

		Single-Blind Placebo	Double-Blind Placebo	Bupropion
FSH (mIU/mL)	Rest	3.73 ± 2.12	4.07 ± 2.23	2.99 ± 1.11
	End	3.59 ± 2.40	4.24 ± 2.29	3.19 ± 1.22
LH (mIU/mL)	Rest	5.64 ± 4.25	4.07 ± 2.78	4.10 ± 2.22
	End	5.07 ± 5.35	4.65 ± 3.38	4.31 ± 2.86
PRL (mIU/mL)	Rest	563.7 ± 46.2	673.2 ± 203.4	668.3 ± 244.0
	End	962.2 ± 386.2 ^a	1377.7 ± 730.7 ^{ab}	1183.3 ± 302.5 ^a
TEST (nmol/L)	Rest	2.30 ± 1.44	1.73 ± 0.58	2.25 ± 0.89
	End	3.14 ± 1.22 ^a	3.21 ± 2.28 ^{ab}	3.18 ± 1.01 ^{ab}
PRO (nmol/L)	Rest	6.52 ± 3.02	12.85 ± 19.69	13.72 ± 20.12
	End	7.53 ± 4.14 ^a	18.53 ± 28.53 ^a	17.33 ± 24.03 ^{ab}
EST (pmol/L)	Rest	177.9 ± 83.7	201.0 ± 206.3	136.4 ± 51.8
	End	196.0 ± 101.6 ^a	435.7 ± 588.5 ^{ab}	188.7 ± 99.4 ^{ab}
CORT (ng/mL)	Rest	172.7 ± 17.2	156.0 ± 66.4	183.6 ± 39.1
	End	187.8 ± 44.7	219.9 ± 86.8	222.4 ± 52.5

No significant differences were found between trials for haematocrit, haemoglobin or glucose (n=6). Haematocrit and haemoglobin increased across all trials after exercise as non-significant trends (P=0.074 and P=0.053, respectively). These trends manifested as a significant increase in percentage change in plasma volume compared to rest and within the single-blind placebo trial (table 3.3). Blood glucose was increased significantly at the end of exercise across and within all trials (table 3.2).

Table 3.2 Haematocrit (Hct), haemoglobin (Hb), and glucose from rest to end of each trial. ^a and ^b denote significant difference (P<0.05) for between rest and end across all trials and within the corresponding trial, respectively.

		Single-Blind	Double-Blind	Bupropion
		Placebo	Placebo	
Hct (%)	Start	40.7 ± 1.4	41.0 ± 1.3	40.6 ± 1.3
	End	42.4 ± 1.5	42.3 ± 1.5	41.7 ± 1.8
Hb (g/L)	Start	139.9 ± 9.0	141.2 ± 6.1	139.6 ± 7.8
	End	145.4 ± 9.3	144.1 ± 7.3	143.6 ± 8.2
Glucose (mmol/L)	Start	4.6 ± 0.3	4.4 ± 0.3	4.2 ± 0.4
	End	6.5 ± 1.6 ^{ab}	6.3 ± 1.4 ^{ab}	6.3 ± 1.1 ^{ab}

Table 3.3 Percentage changes in blood volume (BV), cell volume (CV) and plasma volume (PV) at the end of each trial. Calculated using the method described by Dill and Costill (1974). ^a and ^b denote significant difference (P<0.05) for between rest and end between all trials and within the corresponding trial, respectively.

	Single-Blind	Double-Blind	Bupropion
	Placebo	Placebo	
ΔBV%	-2.8 ± 3.1	-0.8 ± 4.1	-3.1 ± 2.3
ΔCV%	1.0 ± 3.5	1.8 ± 2.5	0.1 ± 2.7
ΔPV%	-5.4 ± 3.4 ^{ab}	-2.4 ± 6.6 ^a	-5.1 ± 4.9 ^a

No differences between were observed for sweat loss (figure 3.9) or steady state expired gas values between trials ($P>0.05$). Expired gas showed a steady increase of $\%VO_{2peak}$ throughout steady state exercise, but this was not significant ($P>0.05$).

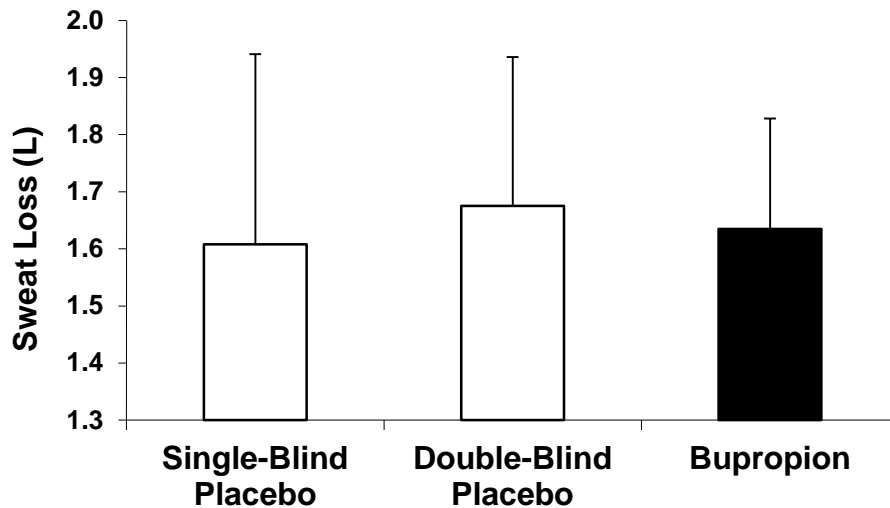


Figure 3.9 Sweat losses as calculated by changes in body mass adjusted for water consumption during exercise.

3.5 – Discussion

The onset of fatigue during prolonged exercise in warm conditions is more rapid and appears to be more heavily affected by factors residing within the CNS compared to exercise of a similar intensity undertaken in thermoneutral environments. Previous studies have found that acutely increasing central catecholaminergic activity, via administration of a dual dopamine/noradrenaline reuptake inhibitor, can improve exercise performance in warm conditions compared to a placebo. Gender differences in the regulation of neurotransmission and response to neuropharmacological interventions, as well as sex hormone-dependent fluctuations in thermoregulation, may alter this performance response to dopamine/noradrenaline reuptake inhibitors. The aim of this investigation was to determine whether or not performance benefits would manifest with the same pharmacological manipulation in women.

The results of the present study demonstrate that an acute administration of bupropion during the early follicular phase can improve performance in women during prolonged exercise in warm conditions. Oestrogen and progesterone levels were not significantly different between trials, suggesting that the method of controlling for menstrual cycle phase was successful. The lack of difference between trials for core temperature, skin temperature, heart rate, RPE, and ratings of thermal sensation during rest and steady state exercise support this. The increase in performance was comparable to those found by Watson and co-workers (2005) and Roelands and co-workers (Roelands *et al.*, 2012) who were also using bupropion (7.5% vs. 9% and 5%, respectively). The dose to body mass ratio for this study was 9.4mg/kg, while it was 7.98mg/kg and 8.15mg/kg, respectively, in the others. As the increase in performance falls between the two, there does not appear to be any obvious dose-dependent differences in performance. However, the inherent variability in response to pharmacological manipulation may obscure this relationship. In addition to genetic variation neurological factors, bupropion pharmacology would be affected by variation in cytochrome PY450B2. In general, there do not appear to be any gender differences for bupropion efficacy for treatment of smoking cessation or depression, or in pharmacokinetics for adult men and women (Dwoskin *et al.*, 2006). In agreement with this, the results of this study appear to demonstrate that bupropion has similar effects for men and women during prolonged exercise in a warm environment.

Hyperthermia and the resulting challenges to cardiovascular function and thermoregulation are considered to be primary contributors to the development of fatigue in warm conditions (Hargreaves, 2008; Nybo, 2008; Maughan, 2010). However, it appears that pharmacological manipulation of central catecholamines can, in part, override processes that mediate hyperthermic fatigue. This is not only evidenced by the increases in performance, but the significantly higher core temperatures and heart rates achieved at the end of the workload challenge in the present study and the time trials in previous studies using similar pharmacological agents (Watson *et al.*, 2005a; Roelands *et al.*, 2008d, 2012). The concept of a critical core temperature has been proposed (González-Alonso *et al.*, 1999) and in the present study core temperatures approached those described by Nielsen and co-

workers (Nielsen *et al.*, 1993). However, the validity of a critical core temperature as the main determining factor of fatigue during prolonged exercise in the heat has recently been considered less important than the impact of increased cardiovascular strain (Cheuvront *et al.*, 2010; Sawka *et al.*, 2012). Because the higher core temperature and heart rate were only significant at the end of the workload challenge, they are likely due to the increased work rate during the bupropion trial, rather than pharmacologically induced changes in thermoregulation or sympathetic activity. This effect was also observed in the study by Watson and co-workers (Watson *et al.*, 2005a). Despite completing more work and achieving higher core temperature and heart rates at the end of the workload challenge, perceived exertion and ratings of thermal sensation were not significantly different.

It has been proposed that pacing strategy relies upon teleoanticipatory algorithm of behavioural economics which utilises peripheral feedback to calibrate power output and this process is interrelated to perceived exertion (St Clair Gibson *et al.*, 2006). In this model, power output is modulated and subsequent period of uncertainty ensues as the new adjustments take time to affect a change detected in the periphery. The error detected by the teleoanticipatory algorithm contributes to perceived exertion. For example, if afferent stress signals do not match those predicted by the algorithm, a change in power output may be made to compensate and the experience of this change of demand will be reflected in perceived exertion. However, peripheral stress signals alone do not explain perceived exertion. There is a psychological and emotional component of stress that is affected by, but not dependent upon, peripheral signals or external stimuli. This has been demonstrated in a study where participants were deceived into thinking the amount of exercise they had to complete was less than was asked during experimental trials (Baden *et al.*, 2005). During the minute after the deception, RPE increased, while ratings of affect decreased significantly, without changes in physiological indices or power output.

There is neurobiological support for the integration of the emotional and cognitive aspects of RPE with the teleoanticipatory algorithm, as proposed by St Clair Gibson and co-workers (2006). ERN in event-related potentials detected via EEG, which are associated with errors during internal-monitoring at the ACC, have been demonstrated to be aversive stimuli, increasing stress and promoting negative affect (Hajcak & Foti, 2008). ERN at the ACC have been shown to occur even as a result of unperceived, or subconscious errors (Nieuwenhuis *et al.*, 2001). This suggests that when unexpected negative teleoanticipatory feedback is received it would have a negative emotional and cognitive impact, supporting the findings by Baden and co-workers (2005). Furthermore, negative affect increases the amplitude of ERN in response to errors (Wiswede *et al.*, 2009), suggesting a potential feed-forward effect of an increasingly negative impact on affect and stress by error detection. The neural networks associated with affect, cognition, and the cognitive/emotional experience of stress are interconnected and catecholamines are key neurotransmitters in their function (Ashby *et al.*, 1999). This will be reviewed in greater detail in the general discussion. By altering the perception and/or impact of stress or exertion, bupropion may allow for greater power output to be maintained relative to peripheral feedback. This is supported by a study in temperate conditions using another dopamine/noradrenaline reuptake inhibitor methylphenidate during which participants were instructed to maintain work rate at an RPE of 16 until they could no longer maintain a work rate of 70% the initial value for two minutes (Swart *et al.*, 2009). Participants maintained a higher power output for longer at the same RPE during the methylphenidate trial, without significant differences in other measurements between trials. A study comparing the rates of fatigue at a fixed RPE in different temperatures showed that power output is decreased at the same RPE with increasing temperature (Tucker *et al.*, 2006). Therefore, heat strain appears to contribute to the same teleoanticipatory algorithm for pacing strategy, which appears to be modulated by central catecholamines. Acute reuptake inhibition of dopamine and noradrenaline may interfere with the negative feedback of stress in the control of power output. The results of the present study indicate that this effect is similar for women during the follicular phase of the menstrual cycle.

There were several limitations with the present study. The lack of blood samples precluded a more robust depiction of hormone and blood volume changes throughout exercise. In addition to the method for controlling menstrual cycle phase, basal body temperature tracking could have been included; however, this is not necessarily reliable (Bauman, 1981). Requesting self-testing with ovulation kits to identify the end of the follicular phase may have improved the reliability of trial coordination. Future studies should investigate whether the effects of bupropion are preserved during the luteal phase and check for possible hormone interactions (described in section 3.2). In summary, the results of the present study suggest that acute administration of bupropion can improve self-regulated work rate for women exercising in warm conditions during the follicular phase of the menstrual cycle. Despite the apparent trend for greater core temperature during the bupropion treatment, this difference was not significant. In conclusion, acute administration of bupropion at therapeutic levels appears to have similar performance effects for both men and women in warm conditions. This suggests a common mechanism for fatigue during prolonged exercise in warm environments that can be manipulated with a combined dopamine/reuptake inhibitor.

Chapter 4

A Dopamine/Noradrenaline Reuptake Inhibitor Improves Performance in the Heat, But Only at the Maximum Therapeutic Dose

4.1 – Abstract

Bupropion, a dopamine/noradrenaline reuptake inhibitor, has previously enabled participants to maintain a higher power output with the same perception of effort and thermal sensation reported during the placebo session. However, it is not known if lower doses exert the same effects during exercise in high ambient temperature. Ten healthy well-trained male cyclists participated in this study. Participants ingested either placebo or a dose of bupropion (Bup50:150mg; Bup75:225mg; Bup100:300mg) the evening before and on the morning of the experimental trial. All trials were conducted in 30°C conditions (humidity 40-60%). Participants cycled for 60min at 55% W_{max} , immediately followed by a time trial to measure exercise performance. The maximal dose of bupropion significantly improved performance ($p=0.035$), while the lower doses did not change performance compared to a placebo condition ($p>0.05$). Bupropion significantly increased core temperature at the end of exercise and during recovery in all trials compared to placebo ($p<0.05$). Heart rate was significantly higher in the Bup100 trial during the recovery period after exercise ($p<0.05$). No changes in ratings of perceived exertion and thermal sensation were found. An ergogenic effect was only present when the highest dose (2 x 300mg) was administered to the participants. Despite an increase in core temperature and improved performance, there was no change in the perception of effort or thermal sensation.

4.2 – Introduction

Exercise capacity during endurance exercise has been demonstrated to be ambient temperature-dependent (Galloway & Maughan, 1997) and exacerbated by increasing humidity (Maughan *et al.*, 2012). These effects appear to be driven by the physiological challenges to thermoregulatory and fluid balance factors (Hargreaves, 2008; Nybo, 2008; Maughan, 2010). This cumulative strain results in the eventual onset of fatigue and subsequent impairment in performance. This appears to be regulated largely by mechanisms within the CNS (as described in section 1.2). These challenges appear to be temporarily overridden by acute administration of drugs that inhibit catecholamine reuptake in the CNS (Roelands & Meeusen, 2010).

Bupropion is a dual dopamine/noradrenaline reuptake inhibitor that was introduced in 1980s as a new and atypical antidepressant (Stahl *et al.*, 2004). In excess of 40 million people use bupropion for various clinical purposes (Jefferson *et al.*, 2005). In 2003 bupropion was removed from the WADA list of prohibited substances, to be placed on the WADA monitoring list. Recently it was shown that use of bupropion and other anti-depressant medications are becoming increasingly prevalent amongst elite athletes (Machnik *et al.*, 2009). Watson and co-workers demonstrated that bupropion can enhance exercise performance in warm conditions (Watson *et al.*, 2005a). The maximum recommended daily dosage for bupropion is 300mg, but the performance effect was found using 600mg within a 24 hour period. Many *in vivo* animal studies have found bupropion to increase dopamine and noradrenaline throughout the brain. However, these studies should be regarded conservatively, as doses used are typically far in excess of the maximum dosage used in human studies relative to body mass. Bupropion metabolites are relatively weaker dopamine reuptake inhibitors and collectively act more potently in inhibiting noradrenaline reuptake at the NAT (Damaj *et al.*, 2004). Human metabolism of bupropion renders the overall effects less potent in directly inhibiting dopamine reuptake than in rats. Dose-dependent pharmacokinetic studies have revealed a relatively linear relationship between metabolites (Suckow *et al.*, 1986). This suggests that drug effects are determined by total transporter occupancy, rather than a change in its direct action, such as is the case with amphetamine. Human positron emission

tomography (PET) imaging studies have found sub-chronic dosing of 150mg/day for 3 days, followed by 300mg/day for 7 days of bupropion produced mean DAT occupancy of 26% (Learned-Coughlin, 2003) and a 4-week 300mg/day study found 20.84% (Argyelán *et al.*, 2005). Recently, a study using the same dosages, achieving the same level of DAT occupancy, did not significantly increase extracellular dopamine in the striatum (Egerton *et al.*, 2010). No appreciable increase in extracellular dopamine in the striatum were found even with 50% DAT blockade and only surprisingly small increases in extracellular dopamine with almost complete blockade (Volkow *et al.*, 2002a). However, using a similar dose this research group showed a small, but significant increase (10%) in extracellular dopamine release in response to food stimulation (Volkow *et al.*, 2002b).

It was originally believed that both methylphenidate and bupropion inhibit DAT more potently than NAT. This was due to estimation of NAT blockade made using radiolabelled Nisoxetine, which has greater affinity for NAT than noradrenaline in the conditions used, resulting in underestimation of binding (Reith *et al.*, 2005). Using radiolabelled noradrenaline instead, methylphenidate and bupropion are found to have 5 and 2 times greater affinity for NAT than DAT, respectively, and methylphenidate has 38 and 15 times greater affinity for NAT and DAT, respectively, than bupropion (Eshleman *et al.*, 1999). Similarly, bupropion has been found to reduce the electrophysiological activity of the locus coeruleus by 50% (IC₅₀) at 13mg/kg compared to the 42mg/kg needed to reduce activity at dopaminergic neurons by the same amount (50%) in rats (Cooper *et al.*, 1994). In the study by Watson and co-workers (Watson *et al.*, 2005a) as well as the previous chapter the dose averaged at 8mg/kg. Together, the dose-dependent effects observed in human imaging, animal and in vitro studies suggest that both bupropion more potently occupies NAT than DAT in humans. The efficacy of such a relatively low dose would imply that striatal increased sensitivity to striatal phasic dopamine and stimulus salience is not necessarily the determining mechanism by which these drugs improve exercise performance. In spite of the large amount of research investigating the effects of these drugs at the striatum, recent evidence suggests that the therapeutic doses used preferentially affect the PFC (Berridge *et al.*, 2006), where DAT expression is minimal (Hall *et al.*, 1999) and NAT expression is high (Logan *et*

al., 2007). Here, extracellular dopamine is actively cleared by NAT (Morón *et al.*, 2002), suggesting that for significant dopaminergic reuptake inhibition to occur in the PFC, the NAT must also be blocked.

The dose-dependent effects of these dual reuptake inhibitors are dependent on linear increases in blockade of both transporters. The resulting neurophysiological effects, however, are dependent on transporter expression at relevant neuroanatomical structures. Drug effects will therefore be determined by unique concentration thresholds at various structures throughout the brain and their interactions. Since often lower doses of bupropion than those used in previous studies are prescribed, it is important to investigate whether there is a dose-response relationship for this pharmacological agent with regard to exercise in high ambient temperature. Previous research on the effects of bupropion during prolonged exercise performance in a warm environment (Watson *et al.*, 2005 and Chapter 3) suggests that the maximal dose will improve exercise performance, so it is hypothesised that there will be a linear, dose-dependent improvement in performance.

4.3 – Methods

Ten healthy males (age 25 ± 4 y; height 1.82 ± 0.05 m; body mass 73.6 ± 9.1 kg; W_{\max} 351 ± 28 W; $VO_{2\text{peak}}$ 64 ± 6 mL/kg/min) participated in this investigation. All participants were well-trained cyclists or triathletes, but were not accustomed to exercise in a warm environment at the time of the study. Prior to the start of the study all volunteers received written information regarding the nature and purpose of the experimental protocol. Following an opportunity to ask any questions, a written statement of consent was signed. The protocol employed was approved by the Research Council of the Vrije Universiteit Brussels, Belgium. The experimental design used in this study is identical to the protocol used by Watson and co-workers (2005) and Roelands and co-workers (Roelands *et al.*, 2008d, 2008a). All participants completed a preliminary maximal exercise test, a familiarisation trial and 4 experimental trials. The preliminary trial consisted of continuous incremental cycle

exercise to volitional exhaustion and was used to determine the power output required to elicit 55% and 75% of maximal workload (W_{max}) and VO_{2max} . A familiarisation trial was undertaken to ensure the participants were accustomed to the procedures employed during the investigation and to minimize any potential learning or anxiety effects. This trial was identical to the experimental trials in all respects. Experimental trials were undertaken in warm (30°C) conditions with relative humidity maintained between 40 – 60%. Experimental trials were separated by 7 days to minimise the development of heat acclimation and to ensure drug washout. Participants were instructed to record dietary intake and physical activity during the two days before the first trial, and to replicate this in the two days prior to the subsequent experimental trials. No exercise, alcohol or caffeine consumption was permitted in the 24 hours before each trial.

Participants ingested a placebo (200mg lactose) or a dose of bupropion (Bup50: 150mg; Bup75: 225mg; Bup100: 300mg) the evening before and on the morning of the experimental trial. A dose of 150mg is typically administered during the first week of the treatment in depression or to assist in the cessation of smoking, while 300mg is equivalent to the maximal daily therapeutic dose (Holm & Spencer, 2000). The treatment was randomized and administered in double-blind crossover manner. All capsules were prepared by an independent pharmacy to appear indistinguishable with regard to dimensions, weight and colour. Experimental trials were designed in accordance with previous studies in this laboratory (Watson *et al.*, 2005a; Roelands *et al.*, 2008d, 2008c). Participants entered the laboratory in the morning approximately 90 minutes after consuming a standard breakfast that included 500mL of plain water. Nude post-void body mass was measured after which participants inserted a rectal thermistor 10cm beyond the anal sphincter for the measurement of core temperature. Surface skin temperature probes were attached to four sites (chest, upper arm, thigh and calf) to enable the determination of weighted mean skin temperature (Ramanathan, 1964) and a heart rate telemetry band was positioned. Participants were dressed in only cycling shorts, socks and shoes for all trials. Participants then entered a climatic chamber maintained at the appropriate environmental condition and rested in a seated position for 15 minutes. During this period temperatures and heart rate were recorded at 5 minute intervals and a resting

venous blood sample was drawn immediately before the start of exercise. The exercise protocol consisted of 60 minutes constant load exercise at a workload corresponding to 55 % W_{max} , followed by a TT to measure exercise performance. There was a 1 to 2 minute delay between the end of the constant load exercise and the beginning of the TT, to program the ergometer. The TT required the participants to complete a predetermined amount of work equal to 30 minutes at 75 % W_{max} as quickly as possible (Jeukendrup *et al.*, 1996). Participants began the TT at a workload corresponding to 75 % W_{max} , but were free to increase or decrease their power output as desired from the outset. During the TT a computer program displayed a bar indicating the percentage of total work completed to give the subject an indication of their progress. Throughout the protocol no feedback was provided regarding time lapsed, power output, pedal cadence or heart rate. During exercise participants had *ad libitum* access to plain water.

Core and skin temperatures and heart rate were recorded at 5 minute intervals during exercise. Ratings of perceived exertion (RPE; Borg 1982) and thermal sensation (assessed using a 21-point scale ranging from unbearable cold to unbearable heat; adapted from Hardy 1970) were assessed every 15 minutes during the initial 60 minute constant load period and at 10 minute intervals during the TT. Venous blood samples were drawn after 60 minutes of constant load exercise and at the end of the TT. Following the completion of the TT participants returned to a seated position where recovery was monitored for 15 minutes (see figure 4.1). The probes and cannula were then removed and nude body mass was re-measured to allow the estimation of sweat losses. Venous blood samples were drawn directly into pre-cooled vacutainer tubes). 10mL samples were collected into plain tubes and left to clot for 1 hour at room temperature before centrifugation. The resulting serum was stored at -20°C for the determination of prolactin and cortisol. Samples for plasma ACTH were collected into 4.5mL tubes containing K_3EDTA . All hormones were measured using ELISA kits. A 0.5mL aliquot of whole blood was extracted and used for the determination of haemoglobin and haematocrit, with these used to estimate percentage changes in plasma volume relative to the pre-exercise sample.

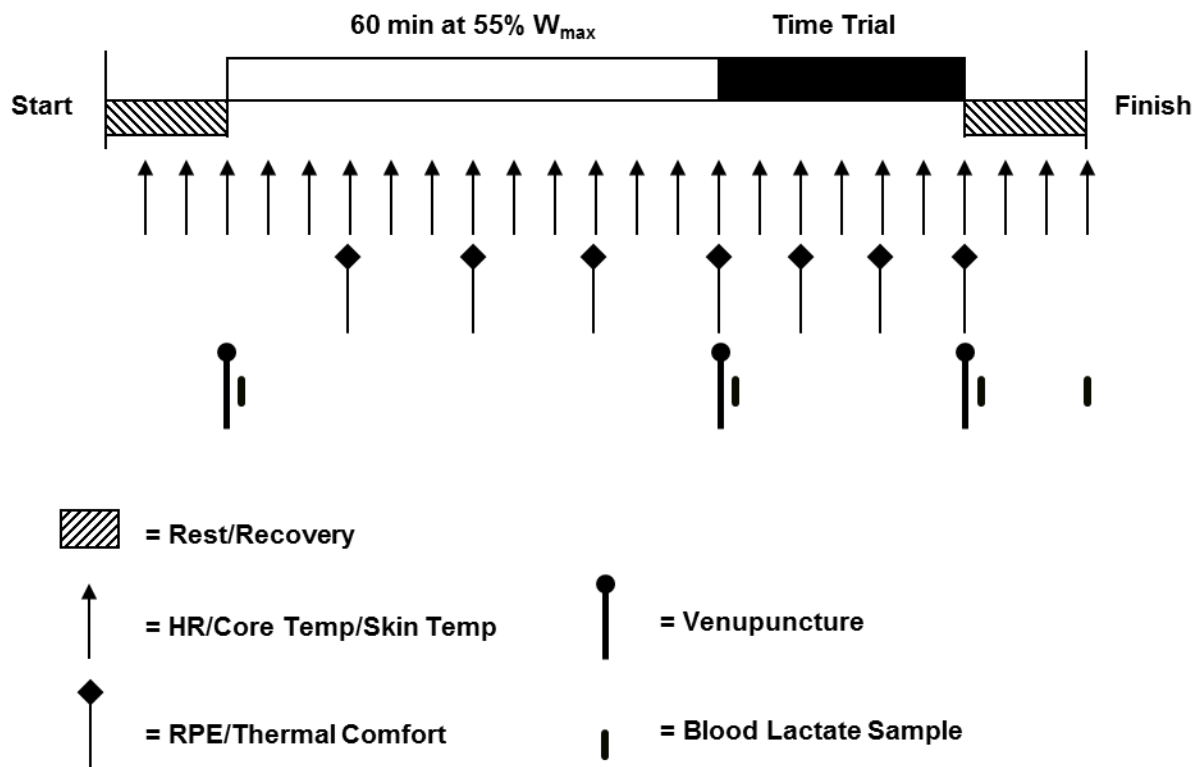


Figure 4.1 Schematic of experimental trials.

4.4 – Results

All participants completed all experimental trials with no reported side effects. Participants finished the TT significantly faster in the Bup100 trial compared to the placebo trial ($p=0.035$; placebo: $33'42'' \pm 2'12''$, Bup100: $32'06'' \pm 1'54''$; Fig. 1) equivalent to a ~5% reduction in time to completion. TT performance during the Bup50 ($p=0.411$) and the Bup75 ($p=0.423$) trials were not significantly different to the placebo trial (figure 4.2). A linear performance response to increasing dose was not apparent for the majority of participants (figure 4.3).

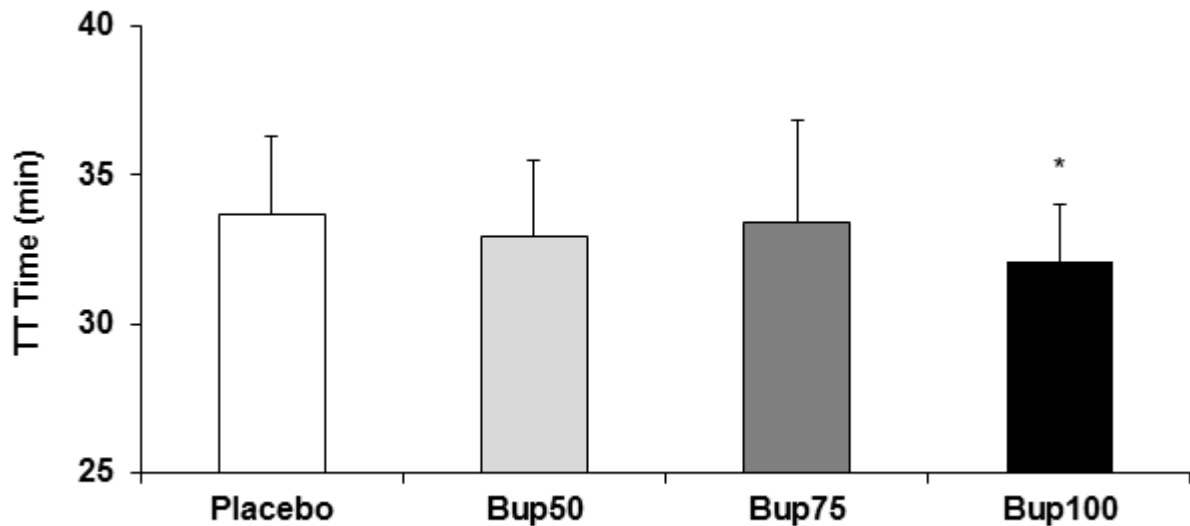


Figure 4.2 Group time trial performance * denotes significant difference compared to placebo (32'06" ± 1'54 vs. 33'42" ± 2'12", respectively; p=0.035).

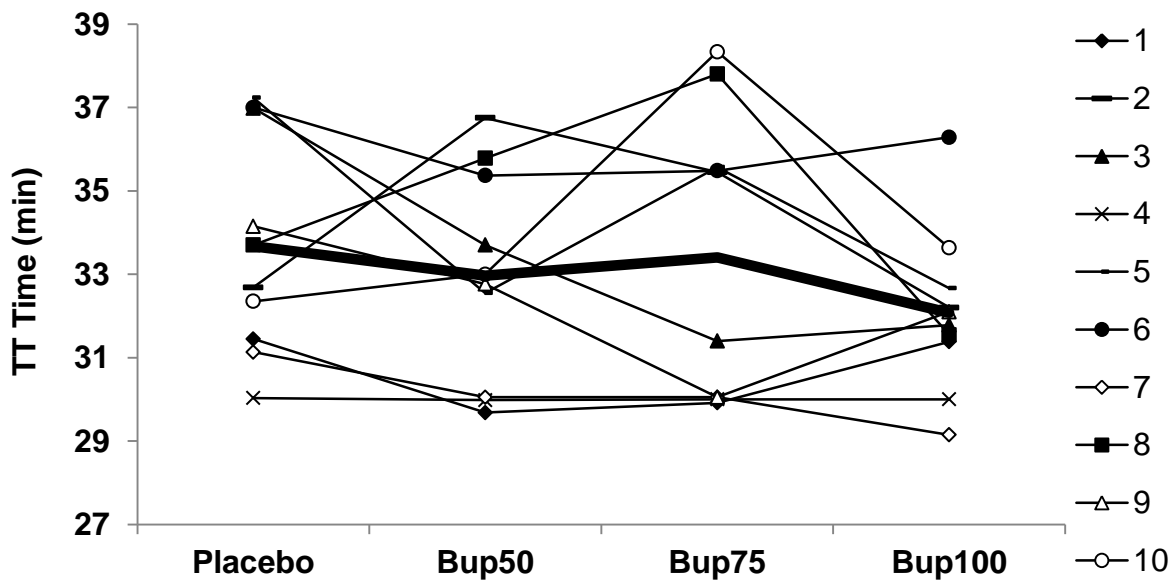
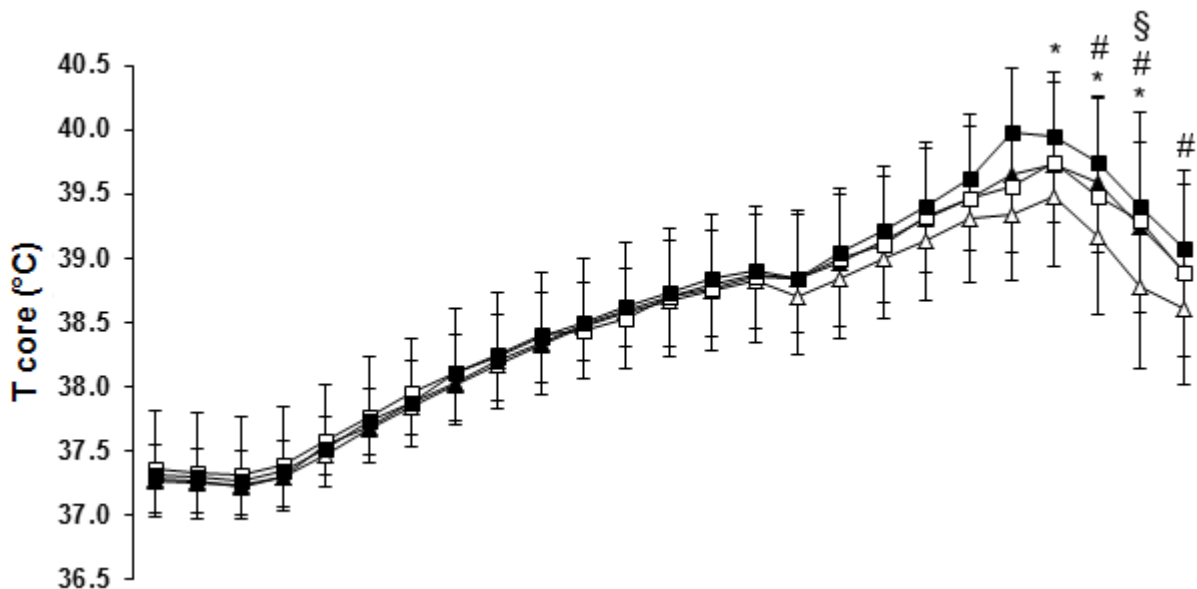


Figure 4.3 Individual time trial performance. Thick black line represents the mean.

Exercise caused a gradual increase in core temperature in all trials (p=0.001; figure 4.4A). In all bupropion trials, there was a tendency for the core temperature to be increased during the time trial compared to the placebo trial. Core temperature rose significantly higher in Bup100 trial ($40.0 \pm 0.6^\circ\text{C}$) than during the placebo trial ($39.5 \pm 0.6^\circ\text{C}$) near the end of the TT and during recovery (p<0.05). During the Bup50 and

Bup75 trial significantly higher core temperatures were reached compared to the placebo trial during recovery phase ($p < 0.05$). No differences in weighted mean skin temperature were apparent between the placebo and bupropion trials (figure 4.4B). Skin temperature increased during exercise in all conditions, reaching a plateau after 10 min of exercise.

A



B

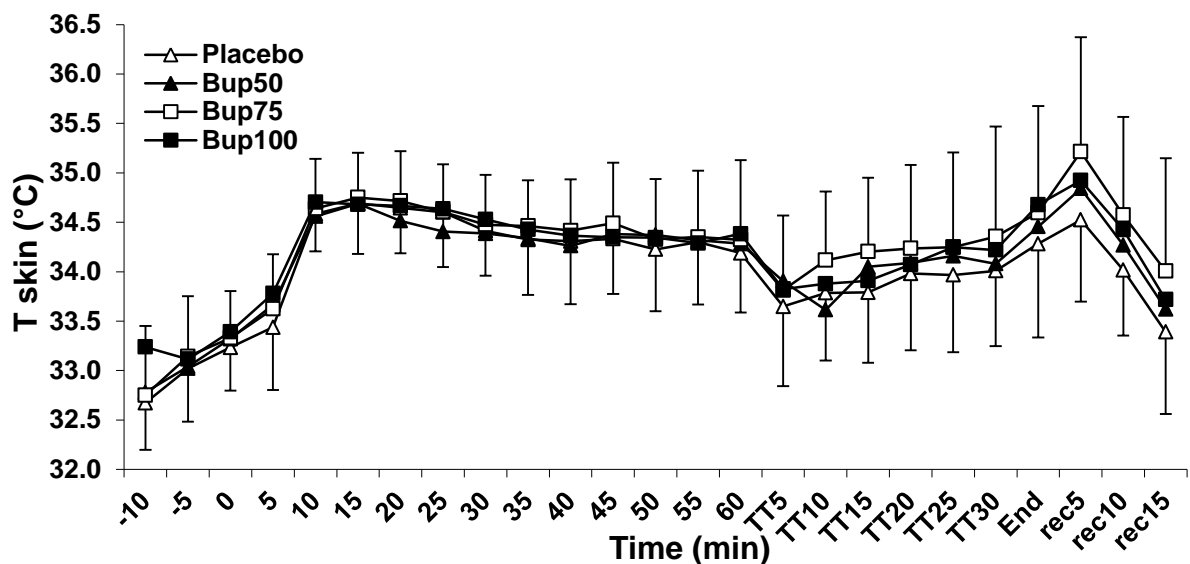


Figure 4.4 Core (A) and weighted mean skin (B) temperature throughout trials. Significant differences ($P < 0.05$) compared to placebo at the corresponding time point are denoted as # for Bup50, § for Bup75, and * for Bup100.

Heart rate increased significantly in all trials ($p < 0.001$). In the Bup100 trial heart rate showed a tendency to be increased near the end of exercise and was significantly higher during the recovery phase compared to the placebo ($p < 0.024$; figure 4.5). No differences in heart rate were found between the lower doses of bupropion and the placebo trial.

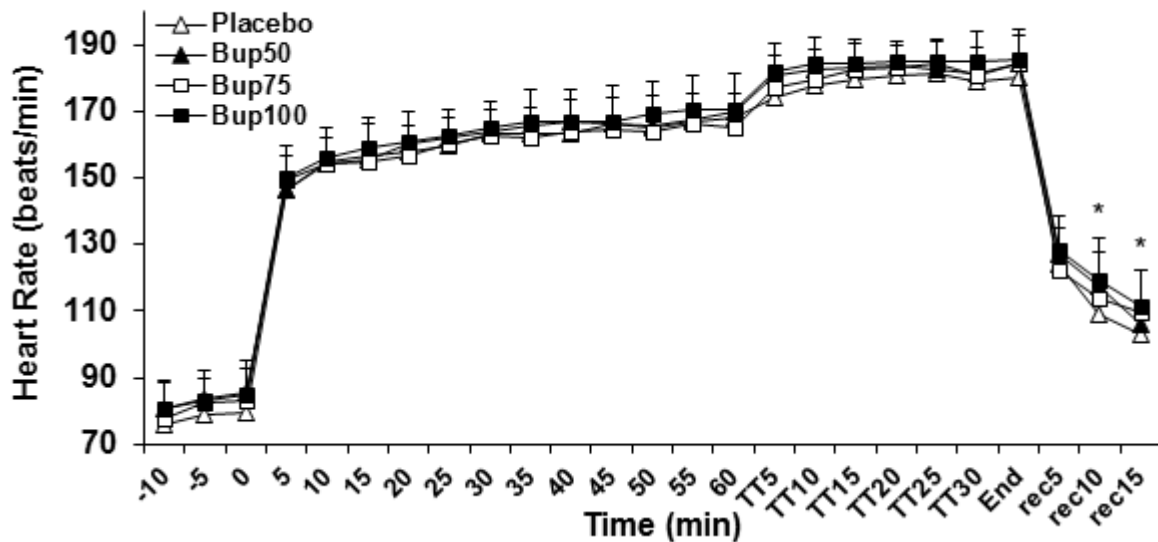
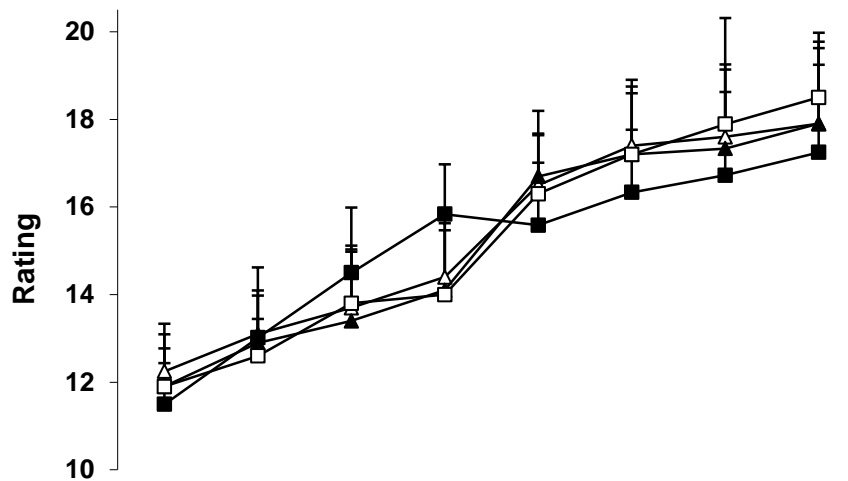


Figure 4.5 Heart rate during experimental trials. Significant difference compared to placebo ($P < 0.05$) denoted by * for Bup100.

Both RPE and TS scores significantly increased during exercise ($p < 0.05$; figure 4.6). Ratings of perceived exertion were similar between the placebo and different bupropion treatments. The participants' ratings of thermal sensation were also not influenced by the drug treatment. The loss of body mass after exercise, corrected for fluid intake, did not show any change due to the bupropion administration.

A



B

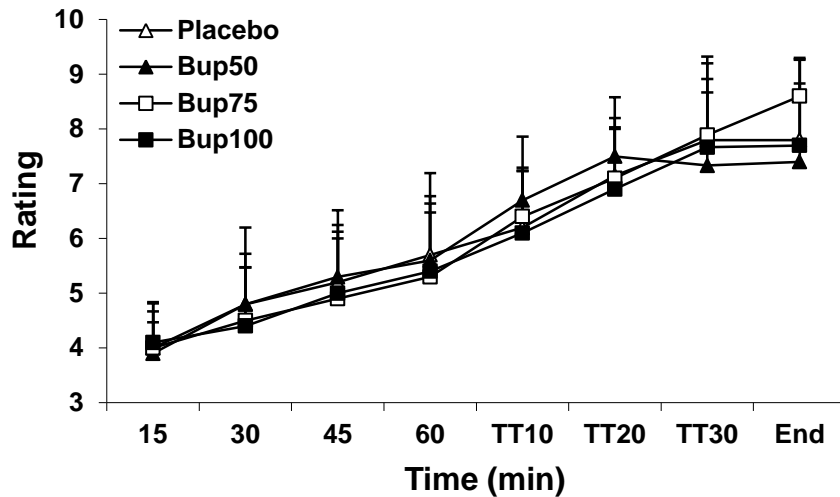


Figure 4.6 RPE (A) and ratings of thermal sensation (B) during experimental trials.

All measured hormone concentrations rose during exercise in all trials (figure 4.7). ACTH similarly increased significantly after constant load exercise ($P < 0.005$) and at the end of TT had increased significantly compared to at rest ($P < 0.001$). Rise in circulating cortisol concentrations were significant only at the end of TT in all trials ($P < 0.05$) compared to at rest and after the constant load exercise. Prolactin concentrations were significantly elevated at the end of TT and had increased significantly compared to both at rest ($P < 0.001$) and after the initial 60 min ($P < 0.001$) for all trials.

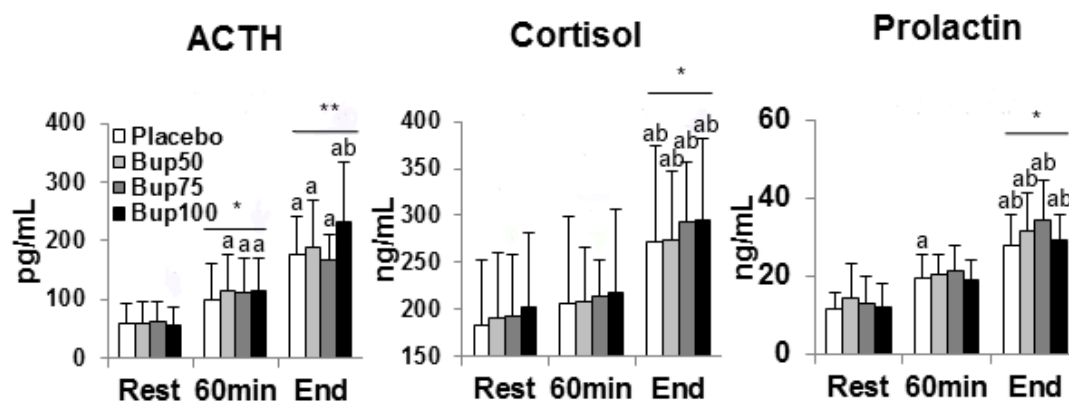


Figure 4.7 Circulating hormone concentrations throughout and within trials. Significant difference ($P < 0.05$) compared to placebo is indicated as * and significant difference compared to both rest and 60min is denoted as **. Within trial significant difference ($P < 0.05$) compared to baseline and 60min is denoted as ^a and ^b, respectively.

There were no differences between trials in haematocrit, haemoglobin or percent changes in blood volume, cell volume or plasma volume. Packed cell volume and haemoglobin significantly increased compared to baseline overall ($P < 0.05$), but only significantly increased between steady state and the end of the time trial in all trials except at 75% dose (table 4.1). This trend was preserved through the calculation for percentage changes in blood volume and plasma volume, while cell volume did not significantly change throughout (table 4.2). There were no differences between trials for sweat loss (figure 4.8).

Table 4.1 Haematocrit (Hct) and haemoglobin (Hb) during experimental trials. ^a and ^b denote significant difference between start and 60min, respectively.

		Placebo	Bup50	Bup75	Bup100
	Start	42.3± 2.2	42.3 ± 2.7	42.6± 2.6	42.5± 2.4
Hct (%)	60min	43.9 ± 2.9 ^a	43.7± 3.3 ^a	44.0 ± 3.2 ^a	44.2 ± 3.3 ^a
	End	44.8± 2.9 ^{ab}	44.6 ± 3.7 ^{ab}	44.6± 3.8 ^a	45.4± 3.2 ^{ab}
	Start	149.9± 9.0	149.5 ± 10.4	150.1 ± 9.9	150.7± 9.1
Hb (g/L)	60min	157.1 ± 11.2 ^a	156.0± 13.4 ^a	156.8± 12.8 ^a	157.0 ± 12.1 ^a
	End	159.3 ± 11.5 ^{ab}	158.5± 13.9 ^{ab}	159.1± 14.5 ^a	160.5± 11.9 ^{ab}

Table 4.2 Percentage changes in blood, cell and plasma volume compared to rest throughout trials as calculated by the method put forth by Dill and Costill (1979). ^a and ^b denote significant difference between start and 60min, respectively.

		Placebo	Bup50	Bup75	Bup100
Δ BV%	60min	-4.5± 1.5 ^a	-4.0± 2.4 ^a	-4.1± 2.2 ^a	-3.9± 2.3 ^a
	End	-5.8 ± 1.9 ^{ab}	-5.5± 2.2 ^{ab}	-5.4 ± 2.7 ^a	-6.0 ± 1.7 ^{ab}
Δ CV%	60min	-0.9± 1.3	-1.0 ± 1.2	-0.9± 1.2	-0.1± 0.5
	End	-0.3 ± 1.4	-0.6± 0.9	-1.1± 1.2	0.3± 1.2
Δ PV%	60min	-7.2± 3.1 ^a	-6.3 ± 3.9 ^a	-6.6 ± 3.7 ^a	-6.8 ± 4.3 ^a
	End	-9.9± 3.8 ^{ab}	-9.3 ± 4.3 ^{ab}	-8.8± 4.8 ^a	-10.7± 3.3 ^{ab}

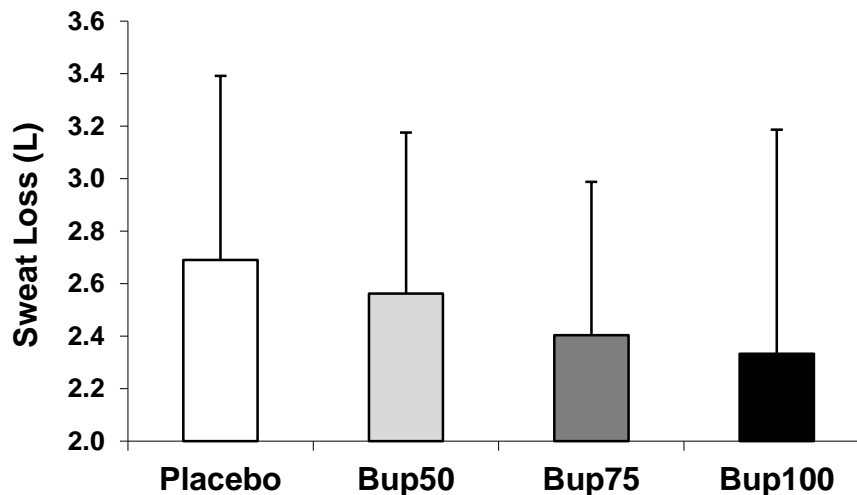


Figure 4.8 Post-exercise corrected body mass losses due to sweat.

4.5 – Discussion

Previous studies have found that acutely increasing central catecholaminergic activity via dual dopamine/noradrenaline reuptake inhibitors can improve exercise performance in warm conditions compared to placebo. The present study is the first to investigate the effects of a range of lower doses. The results of the present study demonstrate that only the maximum acute dose of bupropion improved performance in during prolonged exercise in warm conditions. The increase in performance was comparable to those found by Watson and co-workers (2005) and in those in Chapter 3 (5% vs. 9% and 7.5% respectively). Acute manipulation of central

catecholaminergic neurotransmission can enhance performance in the heat, despite the attainment of core temperatures in excess of those observed during the placebo trial. Bupropion is not currently included in the list of prohibited substances, meaning that athletes are currently free to use this agent in training and competition. A consequence of this improvement in performance was the maintenance of a greater rate of heat production, resulting in the attainment of a higher core temperature towards the end of the TT phase of the trial. In a similar manner to previous investigations (Watson *et al.*, 2005; Roelands *et al.*, 2008a), this was accompanied by a lack of difference in perceived exertion or ratings of thermal sensation, so it seems possible that the use of drugs of this nature has the potential to increase the likelihood of developing heat illness.

This ability to maintain a higher power output and attain higher core temperatures may be due to catecholaminergic modulation of error detection in the teleoanticipatory algorithm described by St Clair Gibson and co-workers (2006). Heat strain appears to contribute to the same teleoanticipatory algorithm for pacing strategy. A study comparing the rates of fatigue at a fixed RPE in different temperatures showed that power output is decreased at the same RPE with increasing temperature (Tucker *et al.*, 2006). As power output is modulated a prediction of performance feedback will be computed. If afferent stress signals do not match those predicted by the algorithm, a change in power output may be made to compensate and the experience of this change of demand will be reflected in perceived exertion. Methylphenidate, another dopamine/noradrenaline reuptake inhibitor, has been demonstrated to alter this relationship in RPE and power-output in a study controlling for RPE. Participants maintained a higher power output for longer at the same RPE during the methylphenidate trial (Swart *et al.*, 2009). The perception of exertion has also been demonstrated to be related to affect (Baden *et al.*, 2005). This is supported by evidence from research focused on the ACC, which has been identified as a key neuroanatomical structure for internal monitoring and autonomic management of behavioural economics (Sanfey *et al.*, 2006; Salamone *et al.*, 2009). The relationship between affect and RPE may be explained by internal-monitoring errors acting as aversive stimuli, increasing stress and promoting negative affect (Hajcak & Foti, 2008). ERN at the ACC has been shown to occur

even as a result of unperceived, or subconscious errors (Nieuwenhuis *et al.*, 2001). This suggests that when unexpected negative teleoanticipatory feedback is received it would have a negative emotional and cognitive impact, supporting the findings by Baden and co-workers (2005). Furthermore, negative affect increases the amplitude of ERN in response to errors (Wiswede *et al.*, 2009), suggesting a potential feed-forward effect of an increasingly negative impact on affect and stress by error detection. The neural networks associated with affect, cognition, and the cognitive/emotional experience of stress are interconnected and catecholamines are key neurotransmitters in their function (Ashby *et al.*, 1999). This includes the PFC, a key target of action for therapeutic doses of methylphenidate (Berridge *et al.*, 2006) and bupropion (Bares *et al.*, 2010). PFC and ACC are interconnected, collectively contributing to the integration of emotional and cognitive processes, and catecholamines modulate their activity (Bush *et al.*, 2000). Their activity is also integrated in the generation of the ERN (Herrmann *et al.*, 2004). By altering the perception and/or impact of stress or exertion, acute bupropion administration may allow for greater power output to be maintained relative to peripheral feedback.

The prevalence of antidepressant and psychostimulant use appears to be increasing among elite athletes (Eichner, 2008; Machnik *et al.*, 2009). While many individuals may have legitimate reasons for seeking this type of treatment, the inappropriate use of these agents by athletes to enhance performance is also a distinct possibility. The combination of manipulating catecholaminergic neurotransmission and strenuous exercise in the heat has been shown to enable the attainment of higher core temperatures, potentially increasing the risk of heat illness (Watson *et al.*, 2005; Roelands *et al.*, 2008a). Because lower doses of bupropion (compared with the recommended maximal daily dose) are often prescribed (150mg/day), the present study investigated the dose–response effects of acute administration of bupropion on performance, thermoregulation and hormonal responses to prolonged exercise in warm conditions. Given that an ergogenic effect was present only when the highest dose (2 x 300mg) of bupropion was administered, this suggests a threshold at which the reuptake inhibition of dopamine and noradrenaline begin to affect performance. In addition, administration of a more potent catecholaminergic agent (such as

methylphenidate) results in greater performance improvements (Roelands et al., 2008a).

The therapeutic use of these drugs entails chronic administration over a period of weeks, months or years. A previous study using a sub-chronic dosing protocol (10-day) reported no benefit to exercise performance (Roelands et al., 2009). Daily administration of bupropion has been found to result in a decrease in the tissue levels of dopamine, lower concentrations of its metabolites, and a reduction in voluntary motor activity (Santamaría & Arias, 2010). The authors attribute this effect of bupropion administration over a period of several days to excessive dopamine/noradrenaline release, which eventually depletes the tissue pool of dopamine. However, it should be noted that this study used very high concentrations of bupropion relative to body mass (~30mg/kg). Furthermore, reuptake inhibitors typically reduce synthesis of the respective neurotransmitters they affect due to negative feedback, as demonstrated by reduction in the presence of synthetic enzyme mRNA, which is also the case for bupropion and tyrosine hydroxylase (Nestler *et al.*, 1990). This effect has been observed in tandem with the changes in electrophysiological adaptations at the locus coeruleus after treatment with antidepressants, including bupropion (Nestler *et al.*, 1999). These findings may be of interest to WADA, because bupropion is currently on the monitoring program, meaning its use by athletes is not restricted in competition, unlike the use of methylphenidate or amphetamine. Unfortunately, determining whether an athlete has been using the treatment in the appropriate manner would be difficult to determine.

Chapter 5

The Effect of a Catecholamine Precursor on the Development of Fatigue during Prolonged Exercise in Warm Conditions

5.1 – Abstract

Acute doses of the catecholamine precursor L-DOPA and peripheral amino acid decarboxylase inhibitor (Sinemet) on prolonged exercise previously failed to change exercise performance in normal ambient temperatures. This may have been due to the short half-life of L-DOPA and sensitivity to food consumption. Therefore, it is not known whether acute doses of L-DOPA timed to reach C_{max} during exercise will improve prolonged cycling performance in warm conditions. 10 physically active men (age 26 ± 4 y; height 1.76 ± 0.08 m; body mass 76.3 ± 10.6 kg; VO_{2peak} 57.4 ± 8 mL/kg/min) were recruited for this study. Participants cycled for 1 h at 60% VO_{2peak} followed by a 30 min workload challenge, during which they were instructed to complete as much work as possible. Heart rate, skin and core temperature, as well as ratings of perceived exertion and thermal sensation were recorded throughout exercise. A finger tap test at the beginning and end of exercise were employed to examine fine motor control. No significant difference in exercise performance was observed between trials. Prolactin concentrations were significantly increased at the end of exercise in all trials ($P < 0.001$) but this response was attenuated at the end of exercise for the L-DOPA trial (11.4 ± 5.5 ng/mL) compared to single-blind (23.6 ± 5.6 ng/mL) and double-blind placebo trials (20.8 ± 3.3 ng/mL; $P = 0.024$). No differences between trials were found for all other measures. The results indicate that augmenting central catecholamine pools inhibit the normal prolactin response to exercise in the heat, but do not alter performance, thermoregulation or sympathetic outflow.

5.2 – Introduction

Endurance exercise capacity is reduced in warm conditions in a temperature- (Galloway & Maughan, 1997) and humidity-dependent manner (Maughan *et al.*, 2012). The main physiological difference to exercise in normal ambient conditions is the challenge to thermoregulation and, consequently, fluid balance as well (Hargreaves, 2008; Nybo, 2008; Maughan, 2010). This cumulative strain results in the eventual onset of fatigue and subsequent impairment in performance. This appears to be regulated largely by mechanisms within the CNS (as described in section 1.2). Pharmacological inhibition of catecholamine reuptake consistently improves performance during prolonged exercise in warm conditions, whereas studies attempting to influence catecholamine metabolism have been conflicting (Roelands & Meeusen, 2010). L-DOPA is the precursor molecule for dopamine synthesis. L-DOPA has been used to treat motor control disorders in Parkinson's disease for over 40 years and is considered the "gold standard" treatment today (Nagatsu & Sawada, 2009). Clinically, L-DOPA is administered with an amino acid decarboxylase (AADC) inhibitor that cannot readily cross the blood-brain barrier; this prevents decarboxylation of L-DOPA in the periphery, thereby reducing associated gastrointestinal distress and increasing the delivery of L-DOPA to the brain. To date, only one study has investigated the effects of L-DOPA on prolonged exercise performance (Meeusen *et al.*, 1997b). This study was conducted in normal ambient temperature and reported no effect on exercise performance. This study used Sinemet, a combination drug containing L-DOPA and carbidopa, an AADC inhibitor, in a ratio of 4:1. Instant Sinemet is designed begin absorption 30 minutes after ingestion. Thereafter C_{max} of L-DOPA occurs roughly 1 hour after ingestion and the half-life is only approximately 2 hours (Contin & Martinelli, 2010). In the study by Meeusen and co-workers (1997), the dosing protocol is described as 4mg/kg Sinemet and is taken 24 hours before exercise and the morning of the trial. It is not clear whether this meant the dose was 4mg/kg Sinemet (4:1 L-DOPA/carbidopa) or 4mg/kg L-DOPA. Furthermore, it isn't clear if the 4mg/kg was divided between the two doses or if it is taken twice. The study imposed a standardised breakfast before exercise. Whether this was before or after the morning dose is not mentioned, but in either case, this would have a drastic impact on L-DOPA pharmacokinetics, severely reducing circulating L-DOPA concentrations (Contin & Martinelli, 2010). Therefore,

the dosing protocol employed was not ideal with respect to L-DOPA pharmacokinetics. Nonetheless, peripheral catecholamines were increased as was circulating growth hormone, suggesting a central effect. While this study found no effect on exercise performance, the effects of central catecholaminergic manipulation appears to be more pronounced during exercise undertaken in warm conditions. Therefore, the aim of the present study is to determine the effects of a dosing protocol designed to provide peak L-DOPA concentrations during a performance test during prolonged exercise in a warm environment. It is hypothesised that an acute dose of Sinemet will improve prolonged exercise performance in warm conditions.

5.3–Methods

10 physically active men (age 26 ± 4 y; height 1.76 ± 0.08 m; body mass 76.3 ± 10.6 kg; VO_{2peak} 57.4 ± 8 mL/kg/min) were recruited to participate in this study. All participants took part in regular endurance exercise, but were not accustomed to exercise in a warm environment at the time of the study. Prior to their admission to the study, participants were provided information regarding the purpose and design of the study, including manufacturer information about Sinemet. Thereafter, if participants confirmed their interest and eligibility, a statement of informed consent was signed. Participants visited the laboratory 5 times in total. The first visit was a VO_{2peak} test to determine work rates for the experimental trials. Subsequently, participants returned for a familiarisation trial to minimise any learning or anxiety effects and to ensure proper work rate configuration. This was followed by a single-blinded placebo control trial, which served both as an additional comparison against experimental trials and as a second familiarisation. The experimental trials which followed were arranged in a randomised double-blind, placebo-controlled crossover design.

VO_{2peak} was determined by a discontinuous test to volitional exhaustion was performed by participants using a Lode Corival cycle ergometer (Lode B.V., Groningen, Netherlands) in an environmental chamber (Weiss-Gallenkamp, UK) at 20° C and 50% relative humidity as described in Chapter 2. VO_{2peak} was then used

to calculate work rates corresponding to this value using linear regression. The standardisation of pre-trial conditions is outlined in Chapter 2. On the day of testing, participants ingested 100/25mg L-DOPA/carbidopa or placebo (glucose) upon waking with water. After 1.5 hr, participants consumed a standardised aprotic breakfast consisting of two small cereal bars and 500mL of orange juice. 1.5 hr later they consumed their second dose of Sinemet. During the following 2.5 hours, participants were asked to steadily consume 500mL of water, after which they arrived at the laboratory for testing. This dosing protocol was intended to augment central catecholamine stores and coordinate peak blood concentrations to occur during exercise, while avoiding nausea which is relatively common with administration of L-DOPA. Experimental trials were designed to be similar to previous studies (Watson *et al.*, 2005a; Roelands *et al.*, 2008d, 2008b; chapters 1 & 2). Upon arrival participants were asked to void their bowels and bladder, before nude body mass was recorded. Participants then changed into cycling clothing, positioned a rectal thermistor 10cm beyond the anal sphincter and a radio telemetric heart rate monitor in privacy. Surface skin thermistors were placed at four sites (triceps, chest, quadriceps and calf) for the measurement of weighted mean skin temperature using the Ramanathan method (1964). Whilst seated for 15 minutes in a thermoneutral environment a 2g butterfly cannula was introduced to a superficial forearm vein to allow repeated blood sampling throughout the experimental protocol. Due to the short C_{max} and half-life of L-DOPA, participants were asked to consume a final dose of Sinemet or placebo immediately before beginning exercise; totalling 300mg L-DOPA and 75mg carbidopa, which is the minimum recommended daily dose to start with treating of Parkinson's disease.

The experimental protocol is illustrated in figure 5.1. After the collection of baseline measurements during the rest period, participants entered the climatic chamber maintained at 30.2 ± 0.2 °C, $50\% \pm 1\%$ relative humidity. Before and after exercise participants performed two finger-tap tests with both hands, to determine any changes in fine motor control. During exercise, participants were given 100mL of water to drink every 10 minutes, amounting to 900mL in total, which was deducted from post-exercise body mass, to calculate sweat loss. Throughout the trial heart rate, core temperature and skin temperature, were recorded every 5 minutes. During

the 15 minute rest period subjective thermal sensation was measured. During the 1 hour fixed work rate period subjective thermal sensation and rate of perceived exertion were measured every 15 minutes. Expired gas samples were also collected for verification of work rate at 30 min and just before steady state exercise was complete. During the time trial perceived thermal sensation and exertion were measured every 10 minutes. Participants were asked to cycle at a work rate corresponding to 60% VO_2peak for 60 minutes, followed by a 30 minute workload challenge, in which participants were asked to complete as much work as possible. Initial work rate during the workload challenge corresponded to 75% VO_2peak , thereafter participants were free to manipulate the work rate to complete as much as they felt possible (see figure 5.1). Standardised verbal encouragement was provided by the experimenter to help ensure a maximal effort. Feedback during the time trial was limited to the time lapsed (power output, cadence, heart rate, etc. were hidden from the participant). Following completion of the workload challenge and the collection of the final blood sample, participants completed a second finger tap test before leaving the climatic chamber. Skin thermistors, heart rate telemetry band and rectal thermistor were removed in privacy before towelling off and nude body mass was recorded behind a screen. The second measurement of nude body mass was used to calculate sweat loss, correcting for the weight of water consumed during exercise.

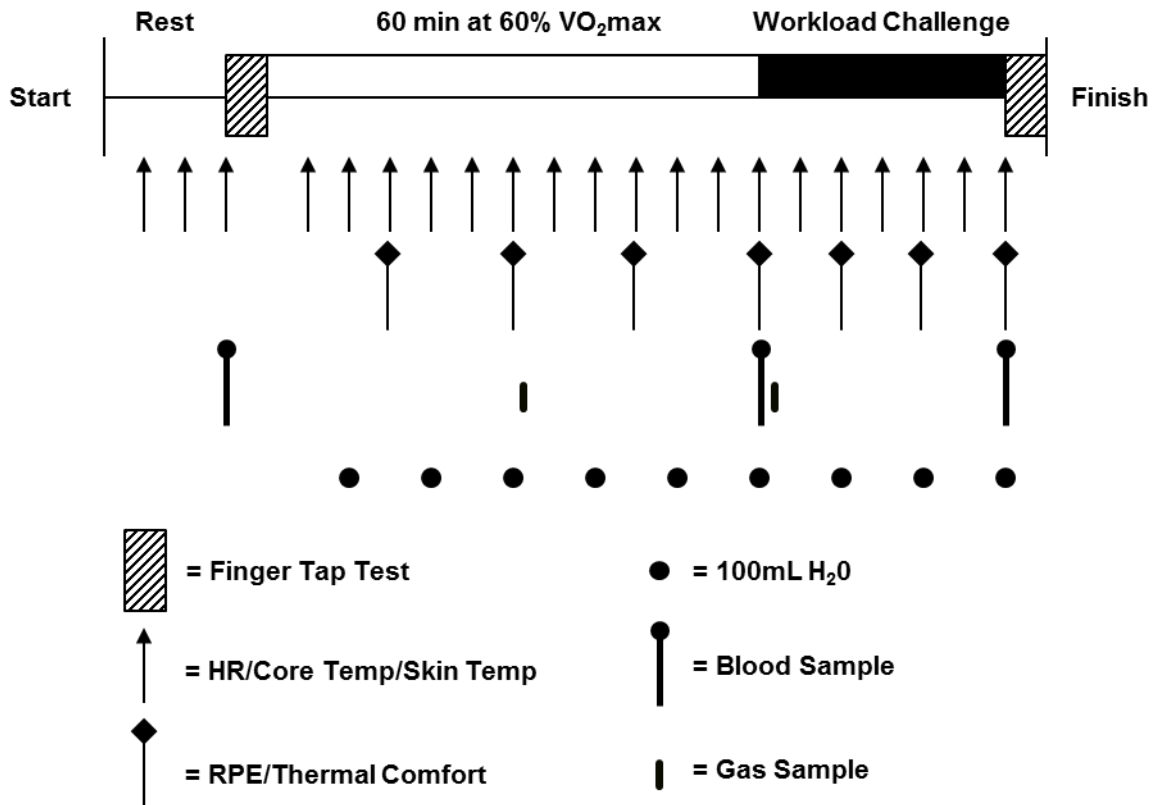
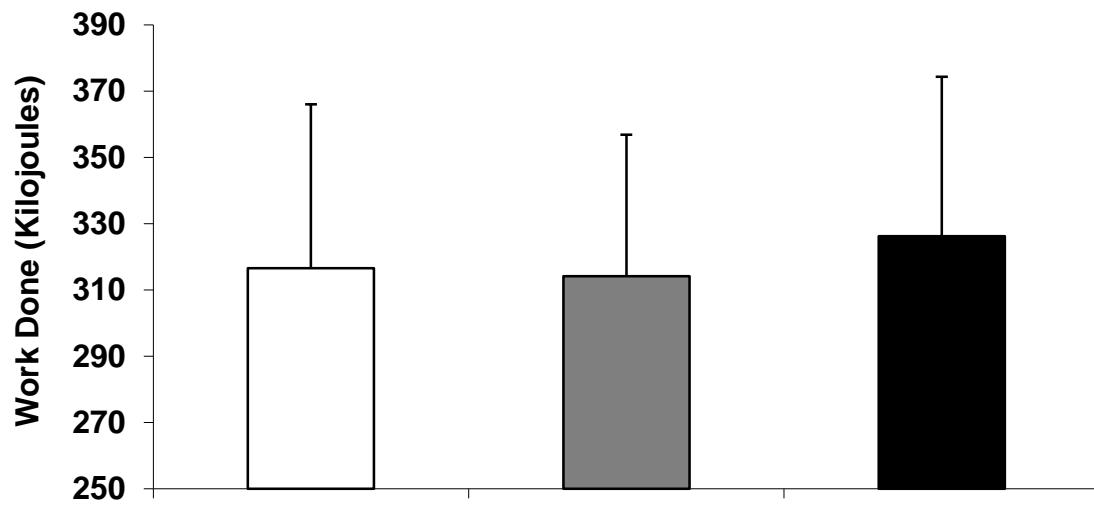


Figure 5.1 Schematic of experimental trials.

5.4 –Results

Multivariate analysis revealed that there were no significant differences between trials for performance ($P=0.08$) (figure 5.2A). Post-hoc paired-sample t-tests were employed to observe between trial differences, which revealed a significant difference between the single-blind placebo (316.6 ± 49.4 kJ) and L-DOPA trial (326.3 ± 48.1 ; $P=0.023$) and no significant difference between single-blind and double-blind (314.1 ± 42.7 ; $P=0.797$), or double-blind placebo and L-DOPA ($P=0.276$). However, because these trials are not conducted under the same conditions, the significance is untenable. No order effect was observed on performance ($P=0.553$). Two participants performed substantially worse on their double-blind placebo compared to their single-blind placebo trial (see figure 5.2B). This resulted in a larger effect size for the L-DOPA trial and should therefore be considered with caution (see figure 5.3).

A



B

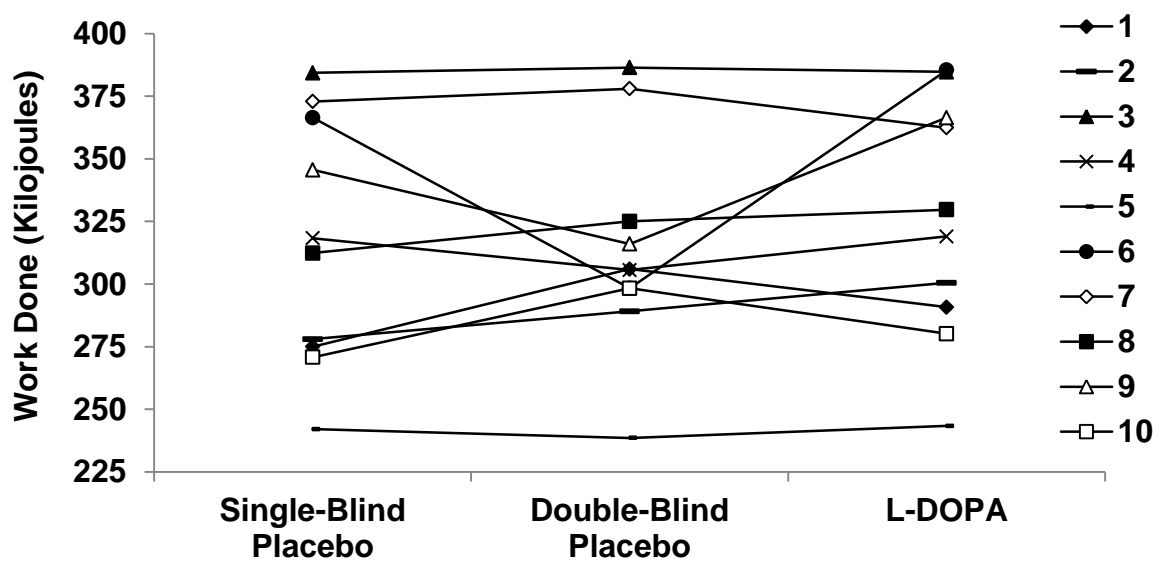


Figure 5.2 Total work done during the time trials expressed by trial (A) and for each individual (B).

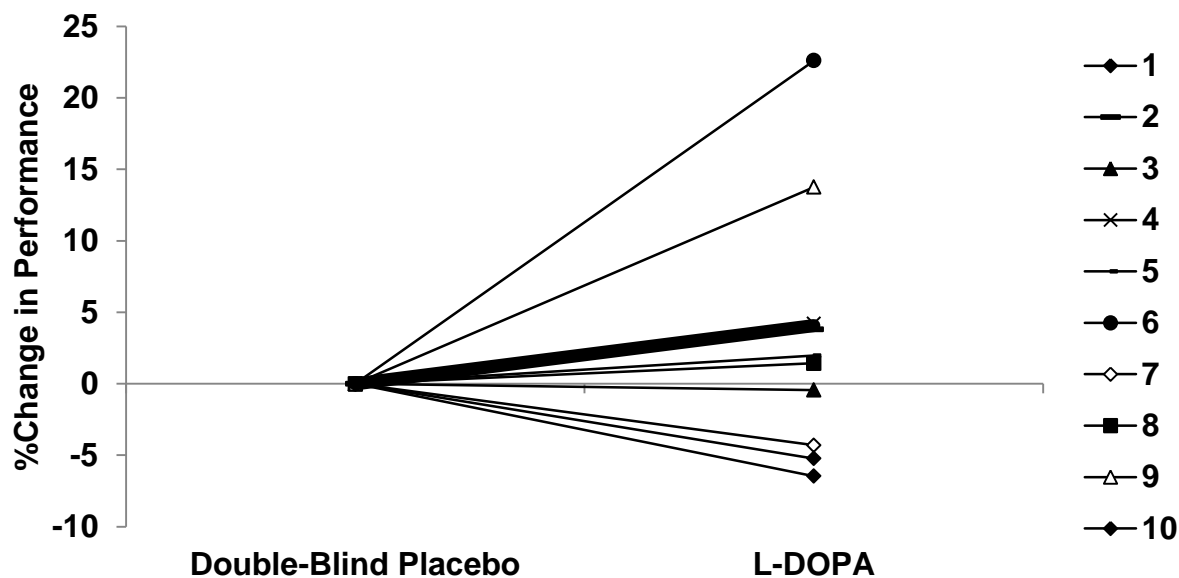


Figure 5.3 Percentage change in performance from double-blind placebo to L-DOPA trials.

Core temperature rose steadily in all trials during exercise, but there was no significant difference between trials (figure 5.4A). Weighted mean skin temperature rose rapidly in the first 15 minutes of exercise after which it plateaued until the end of exercise (figure 5.4B). No significant differences were observed between trials. Similarly, heart rate rose rapidly in the first 15 minutes of exercise, then stabilised and increased gradually throughout the steady state period. Heart rate increased sharply again after the start of the workload challenge and continued to rise slowly until the end (figure 5.5). No significant differences were observed between trials.

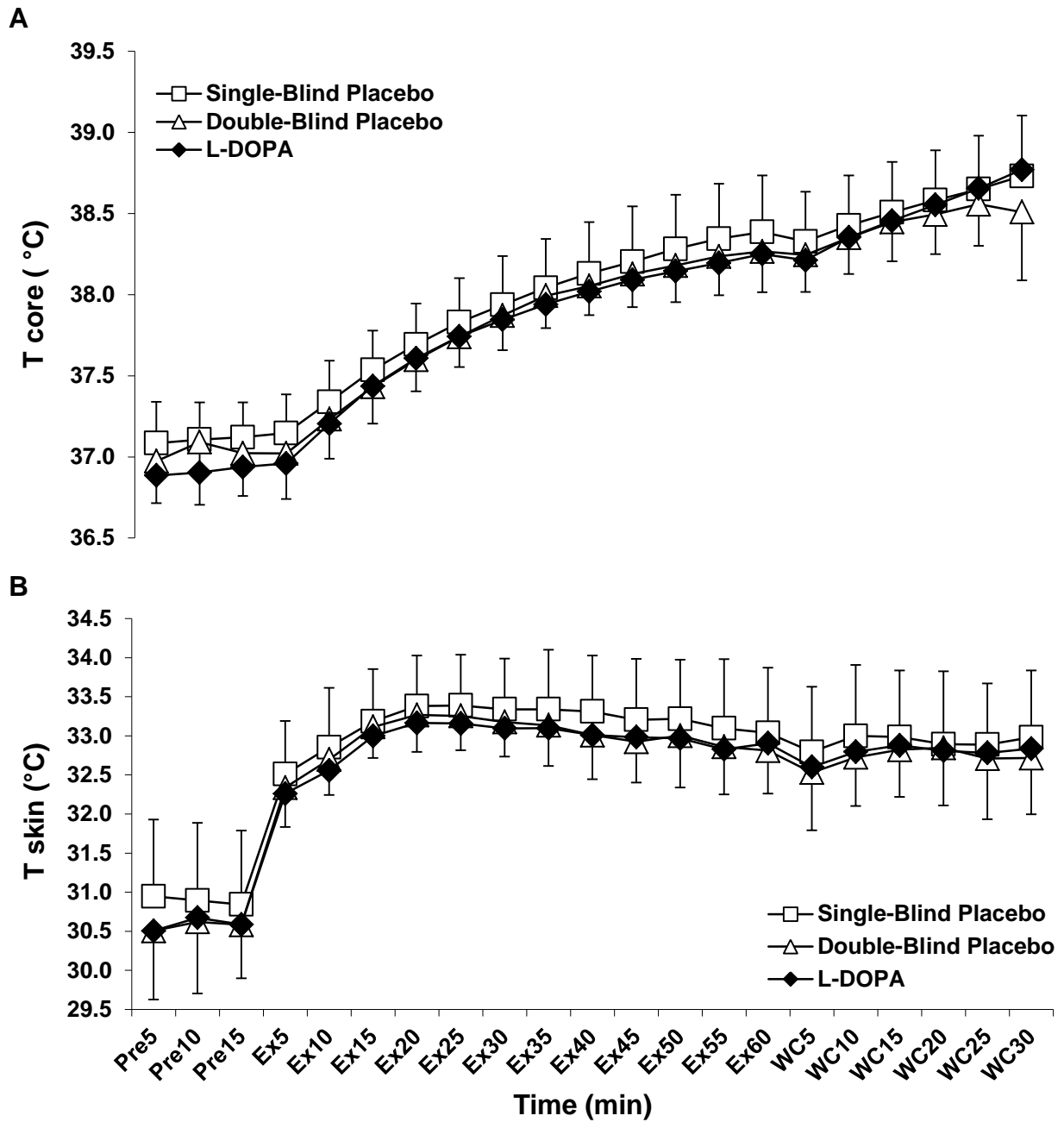


Figure 5.4 Core temperature (A) and weighted mean skin temperature (B) throughout trials.

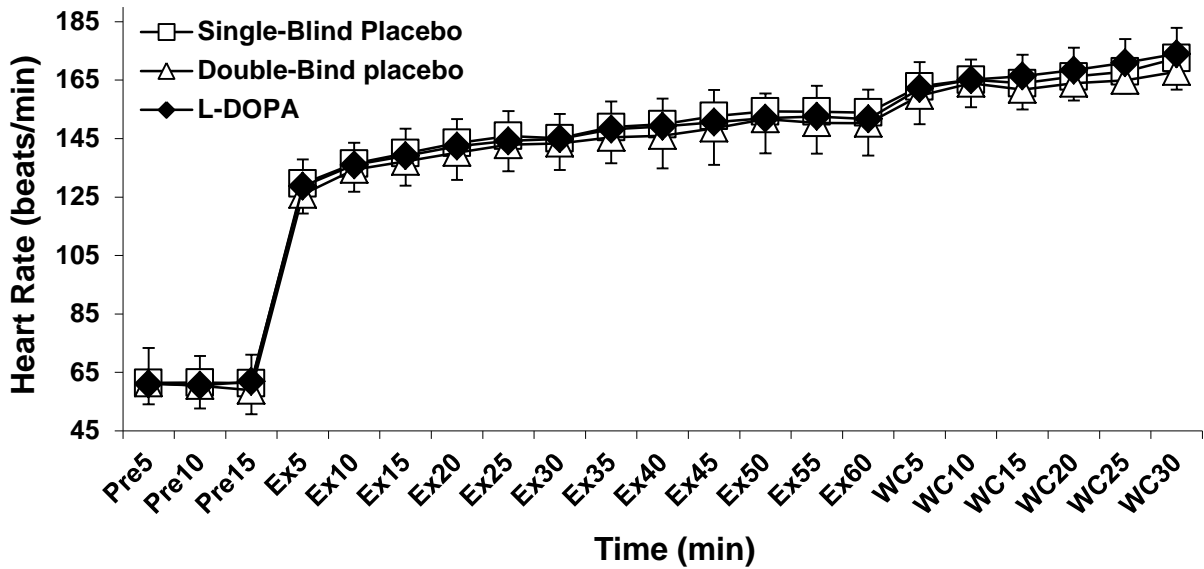


Figure 5.5 Heart rate throughout trials.

Rating of perceived exertion increased throughout exercise (figure 5.6), but no significant differences were observed between trials ($P=0.853$). Ratings of thermal sensation rose rapidly in the first 15 minutes of exercise, but remained stable during the prolonged exercise period (figure 5.7). During the workload challenge thermal sensation ratings rose more rapidly. No significant differences were observed between trials ($P=0.682$).

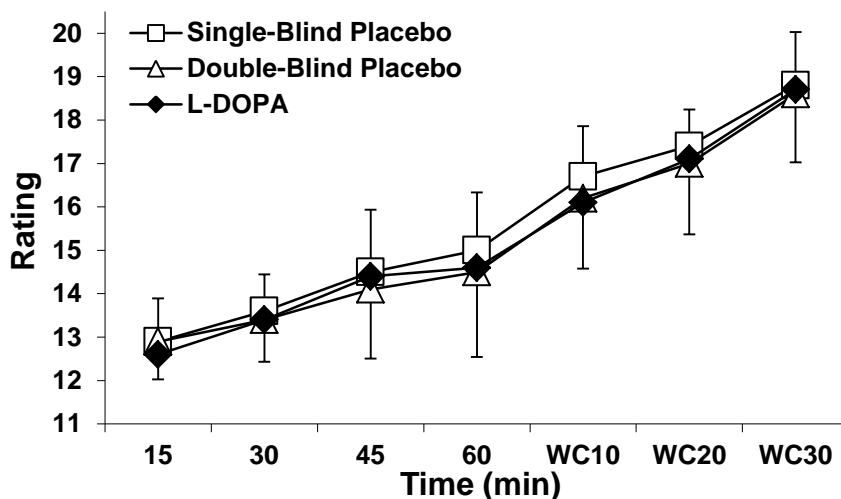


Figure 5.6 Ratings of perceived exertion throughout exercise.

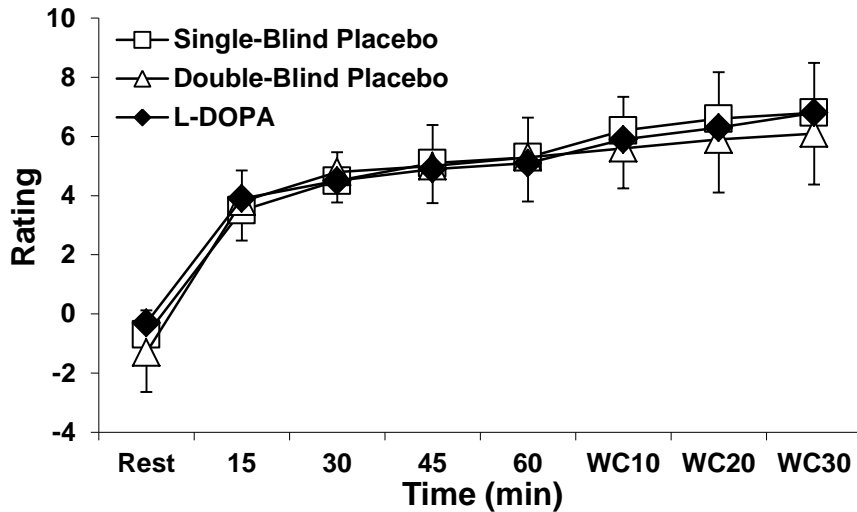


Figure 5.7 Ratings of thermal sensation throughout trials.

Prolactin concentrations were significantly elevated at the end of exercise in all trials ($P < 0.001$), but this response was significantly attenuated during the L-DOPA trial (11.4 ± 5.5 mIU/mL) compared to single-blind (23.6 ± 5.6 mIU/mL) and double-blind placebo trials (20.8 ± 3.3 mIU/mL; $P = 0.024$). Cortisol concentrations were significantly increased at the end of exercise in all trials ($P = 0.001$), but there was no significant difference apparent between trials ($P = 0.448$).

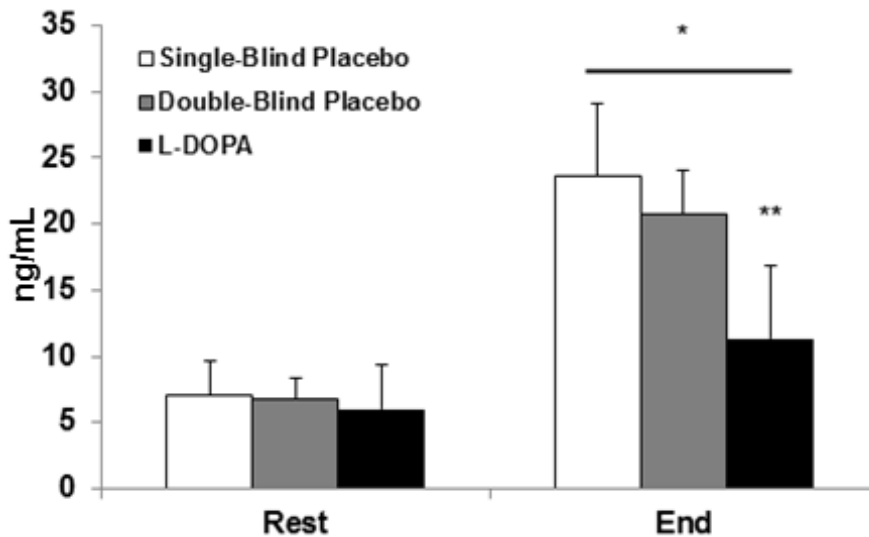


Figure 5.8 Serum prolactin at rest and after the workload challenge. * denotes significant difference from rest ($P < 0.001$). ** denotes significant difference between trials ($P = 0.024$).

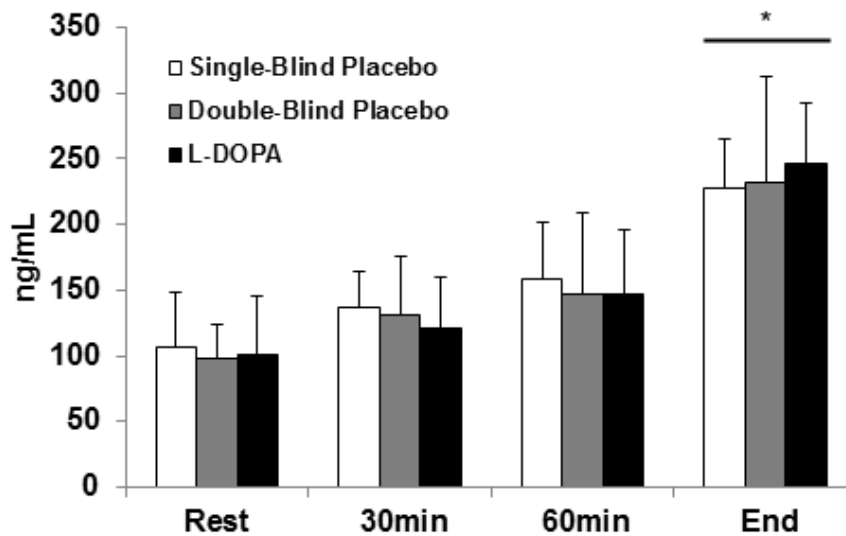


Figure 5.9 Serum cortisol throughout trials. * denotes significant difference from rest, 30min and 60 min (P=0.001)

A significant exercise effect was found for finger-tap performance for the dominant hand only (P=0.007). This was reflected in an increased number of taps after exercise. No significant difference was observed for finger-tap test between trials.

Table 5.1 Number of taps for dominant and non-dominant hands before and after exercise for each trial. ^a denotes significant difference after exercise (P<0.05).

	Single-Blind		Double-Blind		L-DOPA	
	Placebo		Placebo			
	Pre	Post	Pre	Post	Pre	Post
Dominant	74.4±9.1	77.5±9.0 ^a	74.7±8.3	76.1±11.1	74.4±9.3	77.2±10.6 ^a
Non	68.3±9.2	72.8±9.4 ^a	69.8±12.6	72.6±12.8	69.1±11.6	72±8.8

Table 5.2 Changes in haematocrit (Hct), haemoglobin (Hb), and glucose from rest to end of each trial. All values were significantly increased compared to rest ($P<0.05$). No significant differences between trials were observed.

		Single-Blind	Double-Blind	
		Placebo	Placebo	L-DOPA
Hct (%)	Rest	43.3 ± 1.9	43.4 ± 1.5	43.4 ± 1.4
	30min	45.2 ± 1.6	44.1 ± 0.9	45.4 ± 1.6
	60min	45.1 ± 1.6	44.6 ± 1.7	45.3 ± 1.3
	End	45.8 ± 1.7	45.2 ± 1.1	46.1 ± 1.1
Hb (g/L)	Rest	151.8 ± 5.8	151.6 ± 5.5	149.0 ± 7.7
	30min	159.3 ± 9.2	156.9 ± 5.9	159.4 ± 6.8
	60min	160.6 ± 7.2	162.0 ± 11.0	159.0 ± 6.6
	End	163.1 ± 7.3	160.2 ± 5.6	161.6 ± 6.2
Glu(mmol/L)	Rest	4.5 ± 0.9	4.5 ± 0.9	3.9 ± 0.4
	30min	4.5 ± 0.7	4.1 ± 0.5	4.1 ± 0.8
	60min	4.7 ± 0.7	4.3 ± 0.7	4.1 ± 0.8
	End	5.0 ± 1.1	4.6 ± 0.8	4.6 ± 0.8

Table 5.3 Percentage changes in blood volume (BV), cell volume (CV) and plasma volume (PV) at the end of each trial. Calculated using the method described by Dill and Costill(1974). All values were significantly increased compared to rest ($P<0.05$). No significant differences between trials were observed.

		Single-Blind	Double-Blind	
		Placebo	Placebo	L-DOPA
Δ BV%	30min	-4.6 ± 2.5	-4.2 ± 2.3	-6.3 ± 3.7
	60min	-5.5 ± 1.8	-5.8 ± 4.8	-6.2 ± 3.5
	End	-6.9 ± 1.7	-5.6 ± 1.9	-8.0 ± 3.8
Δ CV%	30min	-0.3 ± 3.3	-1.7 ± 2.8	-2.1 ± 5.6
	60min	-1.4 ± 1.9	-3.4 ± 4.7	-2.1 ± 5.3
	End	-1.3 ± 2.0	-1.5 ± 1.8	-2.2 ± 4.7
Δ PV%	30min	-7.8 ± 2.8	-6.0 ± 3.0	-9.4 ± 3.2
	60min	-8.5 ± 2.9	-7.6 ± 5.3	-9.3 ± 3.0
	End	-11.1 ± 3.0	-8.8 ± 2.8	-12.4 ± 3.4

No differences between were observed for sweat loss (figure 5.10) or steady state expired gas values between trials ($P>0.05$). Expired gas showed a steady increase in % VO_2 peak throughout the bout of steady state exercise, but there was no significant differences apparent between trials ($P>0.05$).

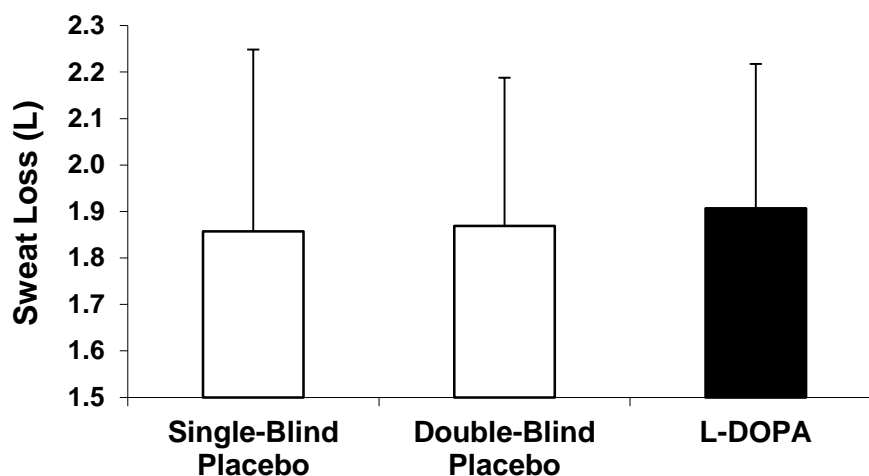


Figure 5.10 Mean sweat loss during experimental trials.

5.5 – Discussion

The results of the present study suggest that augmenting availability of a catecholamine precursor produces no effect on exercise performance, thermoregulation or sympathetic outflow. While an effect on brain catecholamine production was confirmed through a change in circulating prolactin concentrations, no other clear physiological effects were apparent, supporting the findings by Meeusen and co-workers (Meeusen *et al.*, 1997b). Inhibition of the prolactin response was evident in the L-DOPA trial, which is a common effect of L-DOPA treatment that has been well documented (Ben-Jonathan & Hnasko, 2001). This indicates that the dosing protocol produced the desired response: elevating brain catecholamine synthesis and release. The lack of performance response observed may be due to an insufficient increase in synaptic release elicited by the dose used in the present study. However, a study using similar dosages to the present study found an inverted-U dose-response to L-DOPA on cognitive function associated with the inverted-U dose-response to dopamine on PFC function (Onur *et al.*, 2011). In the present study, a finger-tap test was employed to examine possible fine motor control effects, which is often used as a diagnostic test in experiments concerning Parkinson's disease. No drug effect was observed, but an increase in finger-tap performance was observed after exercise. This may be due to increased relative arousal, sympathetic outflow or thermal effects on conductivity following intense exercise in the heat. While the pathological loss of dopaminergic function in Parkinson's disease provides a completely different environment in which L-DOPA can improve motor control, it is worth noting that L-DOPA has been found to exert an inverted-U dose-response on transcranial magnetic stimulation-elicited motor-evoked responses in healthy young men, using similar doses to those used in the present study (Monte-Silva *et al.*, 2010).

Because L-DOPA can increase central catecholamine stores and release without altering performance as reuptake inhibition does, perhaps there are regional cerebral changes that are affected differently by distinct mechanisms important in the control of central fatigue during prolonged exercise in warm conditions. For example, catecholamine reuptake inhibition changes the electrophysiology of the locus

coeruleus via autoreceptor mediated feedback inhibition (Nestler *et al.*, 1999). Despite L-DOPA actively taken up by locus coeruleus neurons for noradrenaline synthesis, it has no effect on locus coeruleus electrophysiology in healthy rats (Migueluez *et al.*, 2011). In the striatum, ventral and dorsal baseline dopamine levels are modulated by tonic activity from the VTA and substantia nigra pars compacta, respectively, and the ventral striatum is particularly sensitive to changes in tonic firing from the VTA due to a greater range in extracellular dopamine concentrations (Zhang *et al.*, 2009). Supporting this, recent evidence suggests that in the NAc, located in the ventral striatum, dopamine reuptake inhibition increased tonic stimulation of low-affinity post-synaptic receptors and results in desensitisation to phasic dopamine signals (Dreyer & Hounsgaard, 2013). In a study in humans, [¹¹C]-raclopride, a radioisotopic ligand for the D2 receptor, demonstrated that an acute dose of 100/25mg L-DOPA/carbidopa did not increase resting extracellular dopamine in young healthy human striatum at rest, but did during mental task performance (Floel *et al.*, 2008).

Low levels of baseline dopamine levels in the NAc shell are associated with decreased motivation and exertion of effort for food, whilst reward-seeking remains intact in rats (Salamone *et al.*, 2003). Tonic dopamine signals in the NAc modulate baseline extracellular dopamine and set an 'average reward' of current behaviour, which then determines the likelihood to exert effort and vigorous responding to reward cues (Niv *et al.*, 2007). Phasic dopamine signalling is dependent on synaptic vesicle exocytosis, whereas tonic dopamine signalling is dependent on non-vesicular efflux (Moquin & Michael, 2011). This is supported by evidence in rats that demonstrates L-DOPA-induced increases in dopamine release is primarily due to increased phasic dopamine quanta (Rodríguez *et al.*, 2007). This may explain why vesicular monoamine transporter inhibition by reserpine attenuates L-DOPA induced extracellular dopamine increases (Kannari *et al.*, 2000), but does not affect low dose amphetamine induced extracellular dopamine increases in vivo (Cadoni *et al.*, 1995). Indeed, phasic and tonic dopamine releases appear to be affected by two distinct, but related pools of presynaptic dopamine: cytosolic or the AMPT-sensitive dopamine pool and the vesicular or reserpine-sensitive dopamine pool. These two pools of dopamine appear to play a role in distinguishing drug actions and individual

variability in sensitivity and susceptibility to particular drug effects (Verheij & Cools, 2007). In the study by Rodríguez and co-workers (2007), L-DOPA-induced increase in dopamine release was found to be reliant on the reserpine-sensitive pool, via synaptic vesicle exocytosis.

In summary, provision of L-DOPA, resulting in increased central catecholamine synthesis may not be sufficient to alter prolonged exercise performance in warm conditions. Despite effects on cognitive function and motor cortex plasticity seen in other studies, neither exercise nor finger-tapping performances were affected by acute augmentation of central L-DOPA availability. Furthermore, because L-DOPA preferentially increases dopamine quantal release during phasic signalling, but does not alter exercise performance, it may be that the alteration in tonic neurotransmission by catecholamine reuptake inhibitors is more important to exercise performance. The significance of this distinction will be reviewed in greater detail in chapter 7.

Chapter 6

The Effect of One Week of Oral S-adenosylmethionine Supplementation on Cycling Performance and Thermoregulation in Warm Conditions

6.1 – Abstract

The onset of fatigue during endurance exercise in warm conditions appears to be at least partly mediated through mechanisms residing within the central nervous system. S-adenosylmethionine (SAM) is a primary methyl group donor, involved in the metabolism of several neurotransmitters. It has been used to treat depression, but it is not yet known what role SAM may have in exercise performance. With local ethics committee approval, 8 physically active males (Mean \pm SD age 26 ± 4 y; height 1.79 ± 0.07 m; body mass 76.3 ± 10.2 kg; $VO_2\text{max}$ 55.7 ± 4.0 mL/kg/min) were recruited to examine the effect of a week-long oral administration of SAM (2 x 800 mg/day) on time to exhaustion in a warm environment ($30.2 \pm 0.2^\circ\text{C}$, $50 \pm 1\%$ rh). Participants completed a $VO_2\text{max}$ test and a familiarisation trial before a randomised, double-blind, placebo-controlled crossover design was employed. Trials consisted of cycle exercise at a power output equivalent to 70% $VO_2\text{max}$ to volitional exhaustion. Heart rate, skin and core temperature, and ratings of perceived exertion and thermal sensation were recorded throughout exercise. Blood samples were collected at rest, every 15 min of exercise and at exhaustion. No difference in time to exhaustion was observed between the placebo (67.5 ± 12.4 min) and SAM (68.5 ± 12.0 min) trials ($P=0.857$). Serum prolactin concentration ($P=0.009$) and weighted mean skin temperature ($P=0.015$) were elevated during exercise in the SAM trial compared to placebo. No further differences were found between trials in the other measures and no order effects were observed. These results suggest that a week-long dosing protocol of SAM does not influence time to exhaustion in warm conditions, despite evidence supporting an effect on the CNS and thermoregulation.

6.2– Introduction

Endurance exercise capacity is reduced in warm conditions in a temperature- (Galloway & Maughan, 1997) and humidity-dependent manner (Maughan *et al.*, 2012). The main physiological difference to exercise in normal ambient conditions is the challenge to thermoregulation and, consequently, fluid balance (Hargreaves, 2008; Nybo, 2008; Maughan, 2010). This cumulative strain results in the eventual onset of fatigue and subsequent impairment in performance. This appears to be regulated largely by mechanisms within the CNS (as described in section 1.2). Manipulation of central catecholamines has produced consistent changes in the onset of fatigue and exercise performance in warm conditions (Roelands & Meeusen, 2010, Chapters 3+4). Accordingly, research points to an increase in use of drugs which act on central catecholamines amongst athletes (Machnik *et al.*, 2009). However, there are several over-the-counter alternative treatments for depression that may influence central neurotransmission and should be considered as well. SAM is a compound which has been researched for over 50 years for its unique versatility, efficacy and tolerability (Delle Chiaie, Pancheri, & Scapicchio, 2002). It has been used to treat depression (Papakostas *et al.*, 2003), osteoarthritis (Soeken *et al.*, 2002) and is considered potentially useful for liver disorders (Purohit *et al.*, 2007). While it is a prescription drug in Germany, Spain and Italy, it is available over the counter and online in the UK and USA. As the primary methyl donor in human physiology, SAM plays a vast number of important roles in the body (see figure 1.8). These include homocysteine metabolism, the synthesis of creatine, metabolism of several neurotransmitters and the regulation of DNA and RNA. Via the transsulfuration pathway SAM is involved in the synthesis of glutathione (Lu & Mato, 2008) and may effect on synthesis of glutamate and GABA as well. SAM also plays a role in the one carbon cycle, in which it is tightly co-dependent with folate and choline metabolism (Bottiglieri, 2002; Zeisel & Blusztajn, 1994). These are involved in acetylcholine and betaine synthesis (Zeisel & Blusztajn, 1994), as well as tetrahydrobiopterin synthesis, which is a cofactor for nitric oxide and monoamine synthesis (Bottiglieri *et al.*, 1992; Stahl, 2007).

While there have not been any exercise studies with SAM, related compounds such as homocysteine, choline, choline-containing phospholipids and betaine have been investigated. In recreational athletes prolonged exercise has been found to increase blood homocysteine and decreases folate and B12 (Herrmann *et al.*, 2003). Synthesis of SAM is directly related to homocysteine metabolism. SAM is synthesised by the addition of ATP to methionine by SAM synthetase. The transfer of the methyl group yields s-adenosylhomocysteine. S-adenosylhomocysteine hydrolase then removes adenosine from homocysteine. Chronically elevated homocysteine levels are associated with folate and B12 deficiencies as well as a number of disorders, including depression (Bottiglieri, 2005). The efficacy of SAM in the treatment of depression and schizophrenia are attributed to the role SAM plays in catecholamine metabolism, as the connection between catecholamines and these disorders is well established. This relationship to catecholamine metabolism is clearly demonstrated by the effects of L-DOPA treatment on SAM concentrations. In rats, SAM brain concentration decreases in response to L-DOPA infusions (Chalmers *et al.*, 1971). In humans, L-DOPA treatment decreases cerebrospinal fluid SAM concentrations (Surtees & Hyland, 1990). Similarly, patients receiving L-DOPA treatment for Parkinson's disease, showed a decrease in plasma methionine and SAM, while homocysteine was elevated (Müller *et al.*, 2001).

The decrease in plasma choline, loss of betaine through sweat and increase in homocysteine observe during prolonged exercise may reflect a decrease of on methyl group donors. Depending on the magnitude of the effect, nutritional status and genetics of an individual, this detriment to methyl group metabolism could have significant ramifications for exercise performance. While choline and betaine represent a significant pool of methyl-group donors, only SAM is recognised for clinical effects on various central nervous disorders. These most significant of these effects appear to be mediated by catecholamine metabolism. As central catecholamines appear to play a role in the genesis of fatigue during prolonged exercise in warm conditions, the aim of the present study was to determine if SAM would affect physiological and psychological measures as well as performance during prolonged exercise in a warm environment. Because SAM is involved in the synthesis of dopamine and noradrenaline, it is hypothesised that by increasing SAM

availability, prolonged exercise in warm conditions will be improved by increasing central catecholamine concentrations.

6.3– Methods

With the approval of the Loughborough Ethics Advisory Committee (reference no. R11-P99) eight male volunteers (age 26 ± 4 y; Ht 1.79 ± 0.07 m; mass 75.5 ± 10.9 kg; VO_{2peak} 57 ± 9 mL/kg/min) were recruited to participate in this randomised crossover design study. Volunteers visited the laboratories at Loughborough University on 5 separate occasions. The first visit was to complete an incremental exercise test to determine VO_{2peak} , which was used to determine the intensity of exercise to be undertaken during subsequent tests. A familiarisation trial was followed by a double-blind placebo controlled crossover design. Between experimental trials participants were asked to visit the labs to collect their supply of capsules at the end of the washout period. Experimental trials consisted of two 7 day periods, with a 7 day washout period between the first experimental trial and the beginning of the following dosing period. The dosing protocol consisted of ingesting 800mg SAM (Nature Made, USA) or placebo (approximately 1.2g glucose) twice daily for 6 days. On day 7, participants took either 800mg of SAM or placebo following an overnight fast having consumed 500mL of water and arrived at the laboratory for the experimental trial approximately 2 hours thereafter. Since many dietary supplements have been reported to contain additional compounds not included on the ingredients label (Geyer *et al.*, 2004), samples of the SAM supplement were sent to the Centre for Doping Research, German Sport University, Cologne for independent testing (analysis report no: S2011005796; see appendix). No additional steroid or stimulant compounds found to be present in the batch. The SAM content of the supplement was also confirmed, in house, by fluorimetric post-column derivitisation on reversed phase HPLC (Birsan *et al.*, 2008).

Upon arrival to the laboratory participants were asked to void their bladder and bowels, if necessary. Nude body mass was then recorded behind a curtain and participants were asked to position a heart rate monitor and rectal thermistor in private. Four skin thermistors were positioned and participants were asked to remain seated to establish baseline measurements for 15 minutes. At the end of this period, a cannula positioned in a superior forearm vein and blood samples were drawn as described in Chapter 2 at rest and every 15 minutes throughout the first hour of exercise and once upon the cessation of exercise. Participants were then asked to enter the environmental chamber, which was maintained at 30°C, 50%rh. Before exercise began participants were asked to complete a series of computer-based cognitive function tests. These included a visual search test, the Stroop word-colour test and a rapid visual information processing (RVIP) test. Upon completion participants were asked to cycle to volitional exhaustion at 70% VO_2peak , intended to last 60-90 minutes. This performance test was selected over a time trial or performance challenge as in previous chapters as it was desired to observe the effects of SAM on a pronounced state of mental fatigue. During this period skin and core temperature were recorded every 5 minutes. Participants were asked to consume 100mL of water every 10 minutes during exercise at which point RPE and ratings of thermal sensation were recorded. For the first hour of exercise, expired gas was collected every 15 minutes to confirm work rate (see figure 6.1). Whole blood was used to measure haematocrit, haemoglobin and glucose. Serum was used to measure prolactin and cortisol by ELISA. Hormone AUC calculations were made using the linear trapezoidal method.

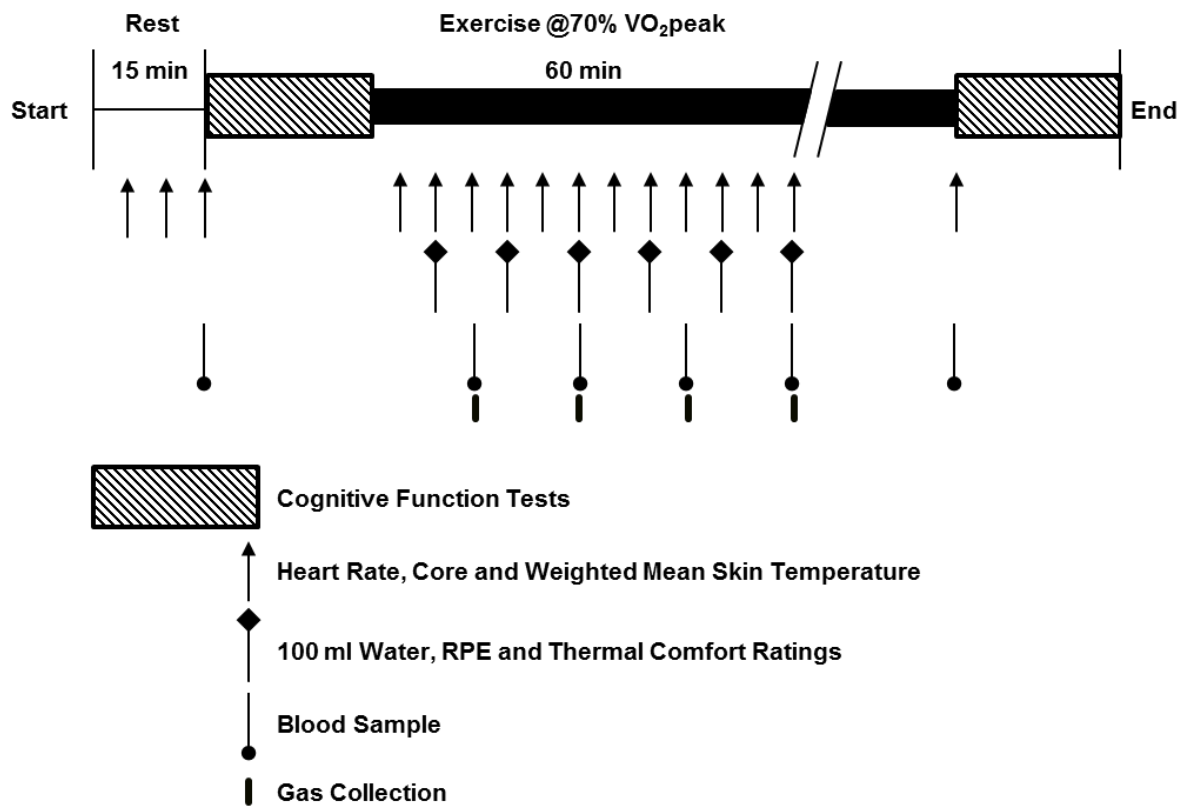


Figure 6.1 Schematic of the experimental trials.

6.4– Results

All participants completed all experimental trials. There was no difference in time to exhaustion (figure 6.2) between PLA (67.5 ± 12.4min) and SAM (68.5 ± 12.0min) ($P>0.05$). Two participants appeared to demonstrate a response to SAM supplementation, but this was in opposite directions (figure 6.3).

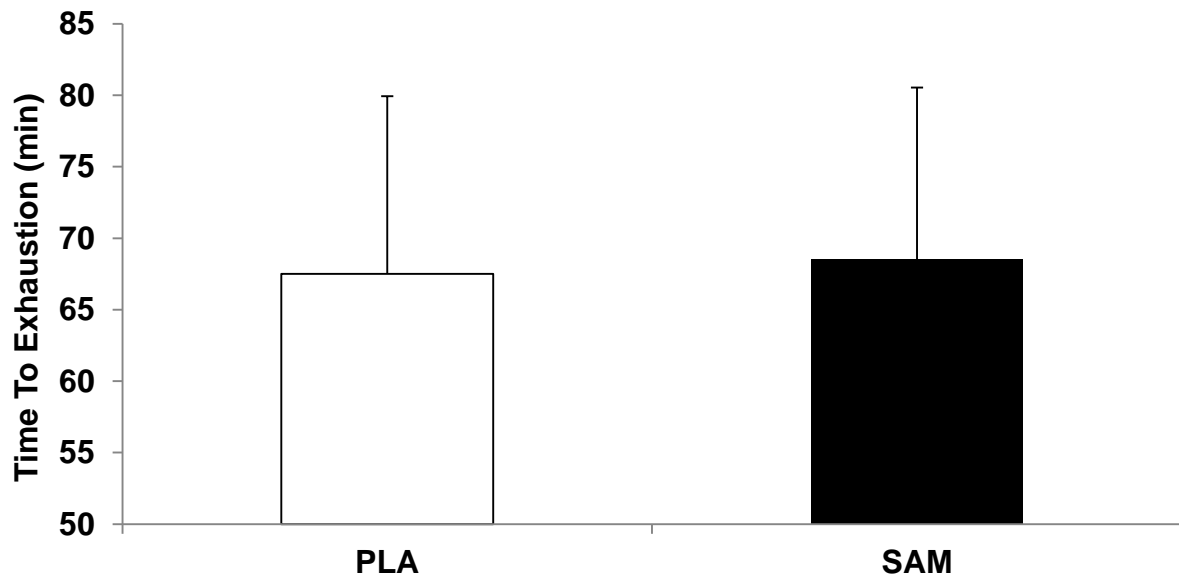


Figure 6.2 Mean times to exhaustion between trials. No significant differences were observed between PLA (67.5 ± 12.4min) and SAM (68.5 ± 12.0min) ($p>0.05$).

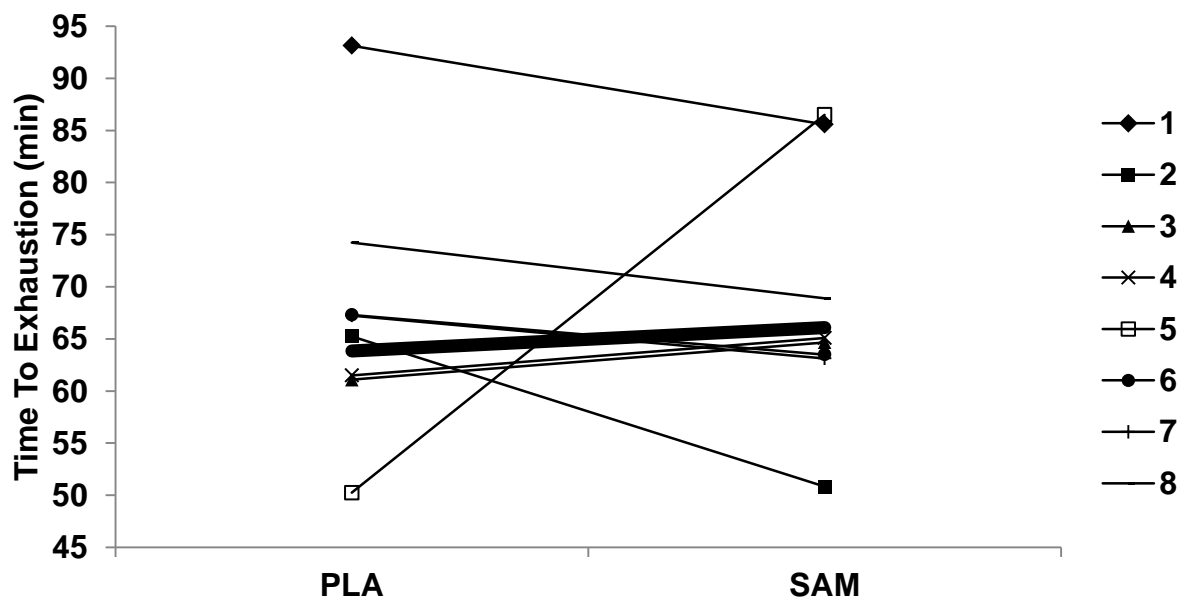


Figure 6.3 Individual changes in time to exhaustion.

Core temperature increased throughout exercise (figure 6.4A) with no differences apparent between trials. Weighted mean skin temperature rose rapidly during the first 15 minutes of exercise and remained relatively stable until exhaustion (figure 6.4B). Weighted mean skin temperature remained an average of 0.26°C higher throughout exercise in the SAM trial compared to placebo (P=0.015). Heart rate rose rapidly during the first 15 minutes of exercise and climbed steadily until exhaustion. No differences between trials were observed (P>0.05).

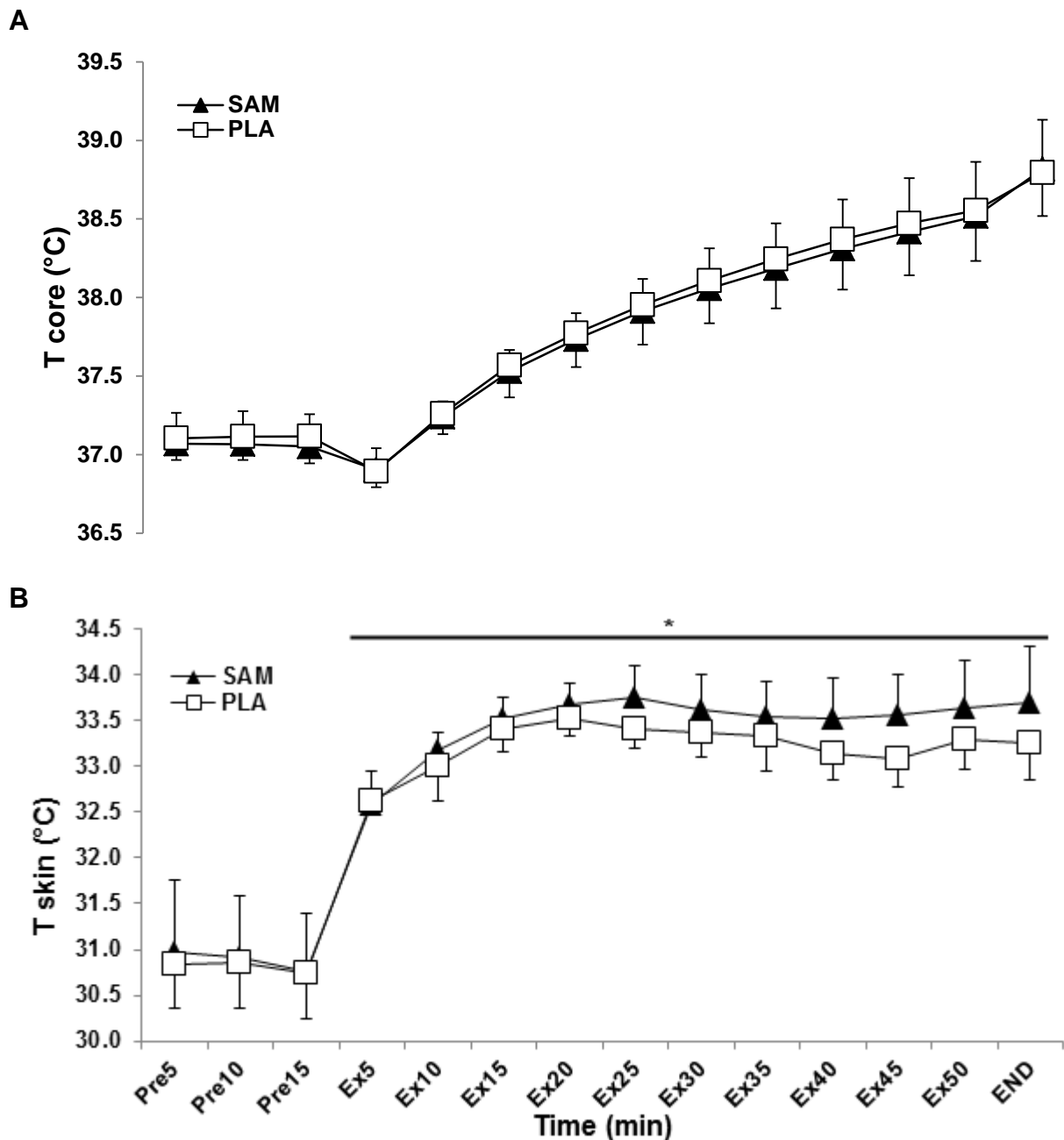


Figure 6.4 Core temperature (A) and weighted mean skin temperature (B) during experimental trials. * denotes significant difference (P=0.015).

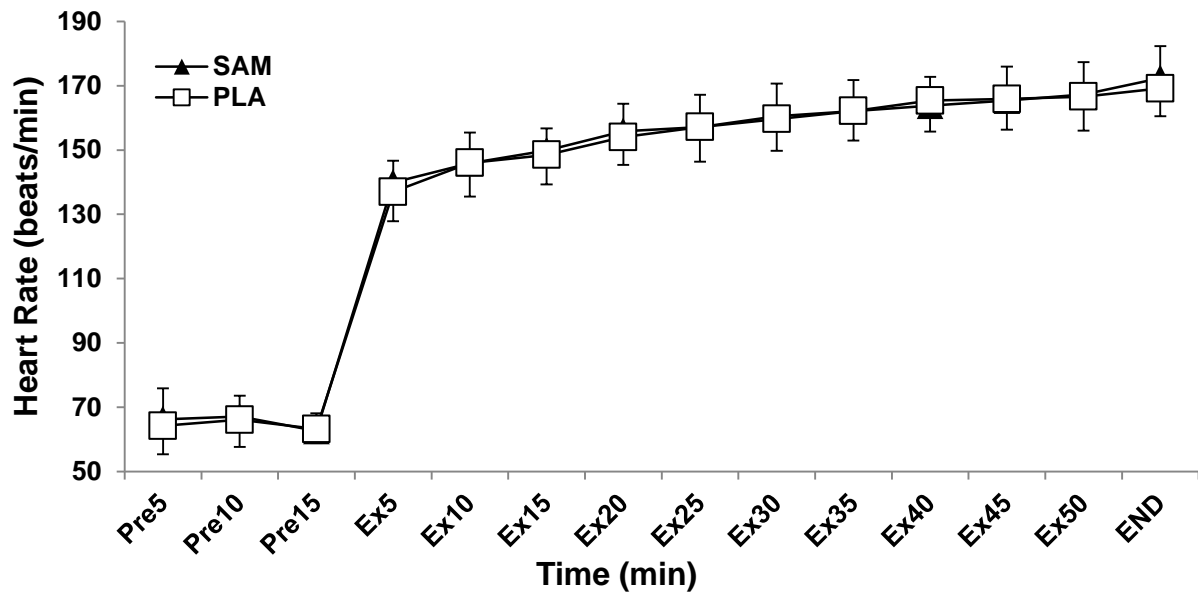


Figure 6.5 Heart rate during experimental trials.

Ratings of perceived exertion rose steadily throughout exercise, but there were no significant differences apparent between trials ($P=0.774$; figure 6.6). Ratings of thermal sensation increased at the onset of exercise and steadily increased until exhaustion. There were no significant differences between trials ($P=0.853$; figure 6.7).

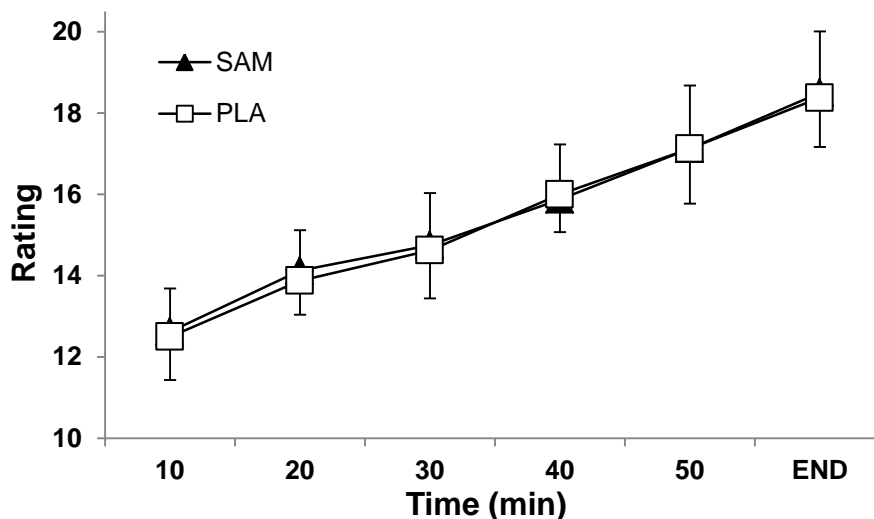


Figure 6.6 Ratings of perceived exertion during experimental trials.

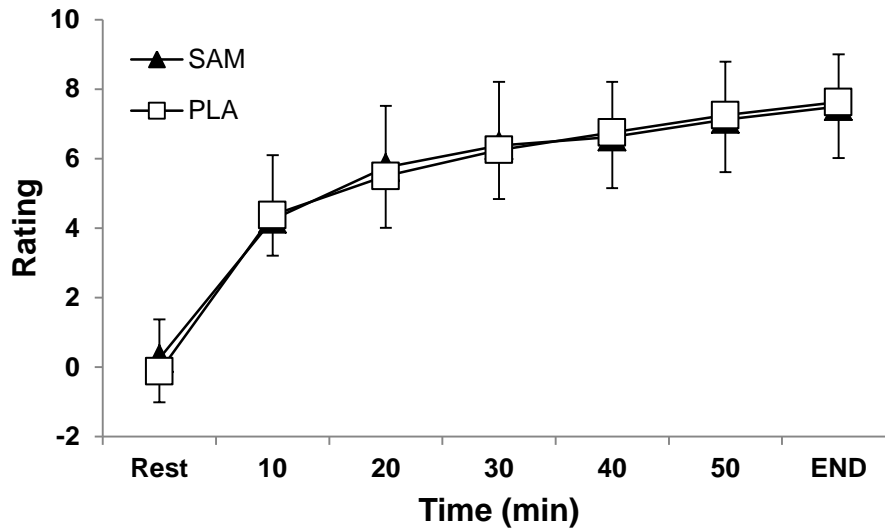


Figure 6.7 Ratings of thermal sensation during experimental trials.

Serum cortisol concentrations were significantly increased at the end of exercise compared to rest ($P < 0.05$), but no significant differences were observed between trials ($P = 0.371$; figure 6.8). Prolactin was significantly increased at the end of exercise compared to rest ($P < 0.05$) and prolactin AUC was significantly larger in the SAM trial ($537 \pm 152 \text{ mIU/hr/mL}$), compared to placebo ($453 \pm 158 \text{ mIU/hr/mL}$) ($P = 0.009$; figure 6.9).

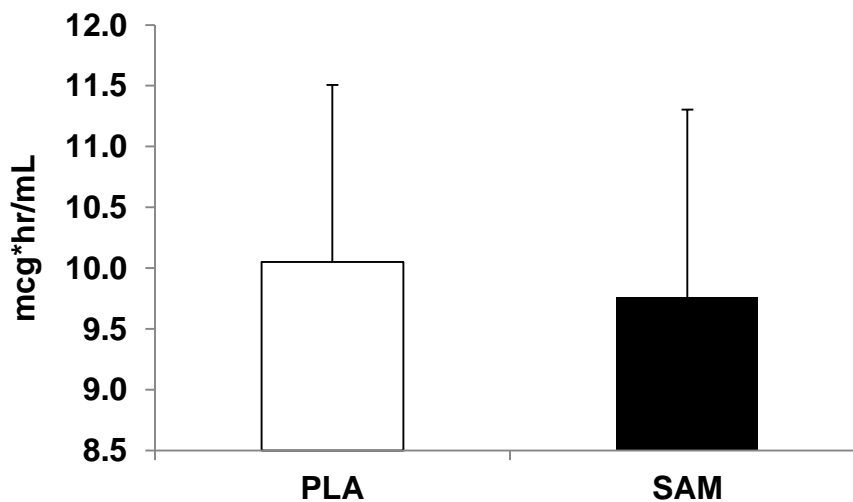


Figure 6.8 Serum cortisol area under the curve.

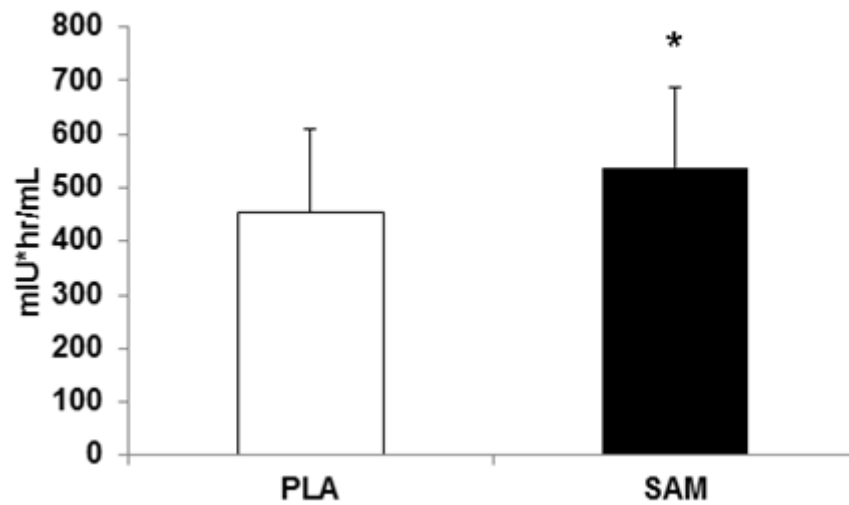


Figure 6.9 Serum prolactin area under the curve. * denotes significant difference ($P=0.009$).

Packed cell volume, haemoglobin were significantly increased compared to baseline at 30min and exhaustion ($P<0.05$) (table 6.1). No differences were observed between trials. Glucose was only significantly elevated at the exhaustion compared to baseline ($P<0.05$). No differences were observed between trials. Percentage change in blood and plasma volumes all increased compared to baseline at 30min and exhaustion ($P<0.05$) (table 6.2). Cell volume did not change significantly from baseline after 30min during the SAM trial ($P>0.05$), but did during placebo ($P<0.05$). No differences in sweat rate (figure 6.10) or total sweat loss were observed ($P>0.05$). Work rate as determined by percentage of VO_2 max increased significantly at exhaustion compared to the first 15 minutes of exercise ($P<0.05$), but this response was not different between trials ($P>0.05$).

Table 6.1 Haematorcrit (Hct), haemoglobin (Hb) and blood glucose concentrations. ^a denotes significant difference from baseline measure (P<0.05).

		Placebo	SAM
Hct (%)	Start	45.1 ± 1.9	45.3 ± 1.6
	30min	46.9 ± 2.5 ^a	47.3 ± 1.7 ^a
	End	47.2 ± 2.5 ^a	46.9 ± 1.8 ^a
Hb (g/L)	Start	157.5 ± 9.3	160.4 ± 6.8
	30min	165.6 ± 11.0 ^a	168.1 ± 8.6 ^a
	End	165.8 ± 11.5 ^a	166.9 ± 7.3 ^a
Glucose (mmol/L)	Start	4.0 ± 0.4	4.0 ± 0.2
	30min	3.9 ± 0.6	4.1 ± 0.5
	End	4.6 ± 0.7 ^a	4.8 ± 0.6 ^a

Table 6.2 Percentage changes in blood volume (BV), cell volume (CV) and plasma volume (PV).^a denotes significant difference from baseline measure (P<0.05).

		Placebo	SAM
ΔBV%	30min	-5.0 ± 2.8 ^a	-4.1 ± 2.6 ^a
	End	-5.1 ± 2.9 ^a	-3.9 ± 2.0 ^a
ΔCV%	30min	-1.4 ± 0.9 ^a	0.0 ± 1.3
	End	-0.9 ± 1.6 ^a	-0.4 ± 1.1 ^a
ΔPV%	30min	-8.0 ± 4.8 ^a	-7.6 ± 4.1 ^a
	End	-8.6 ± 4.4 ^a	-6.7 ± 3.8 ^a

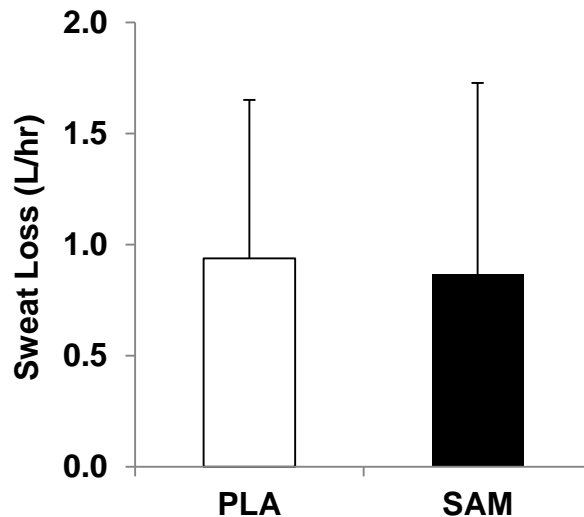


Figure 6.10 Mean sweat rate between trials.

No exercise effects were observed for visual search and no significant differences between trials were observed ($P > 0.05$). Percentage correct scores for RVIP were significantly decreased in the SAM trial compared to placebo for both before and after exercise ($P < 0.05$; figure 6.11). A non-significant trend ($P = 0.06$) was observed for exercise effect on RVIP percentage correct scores. A treatment effect ($P < 0.01$) and a treatment x time interaction effect ($P = 0.01$) were observed for reaction times during the word-match portion of the Stroop test, but not for the conflicting portion of the test (figure 6.12). SAM reaction times were significantly slower ($P < 0.05$) before exercise compared to placebo, but was similar to placebo after exercise.

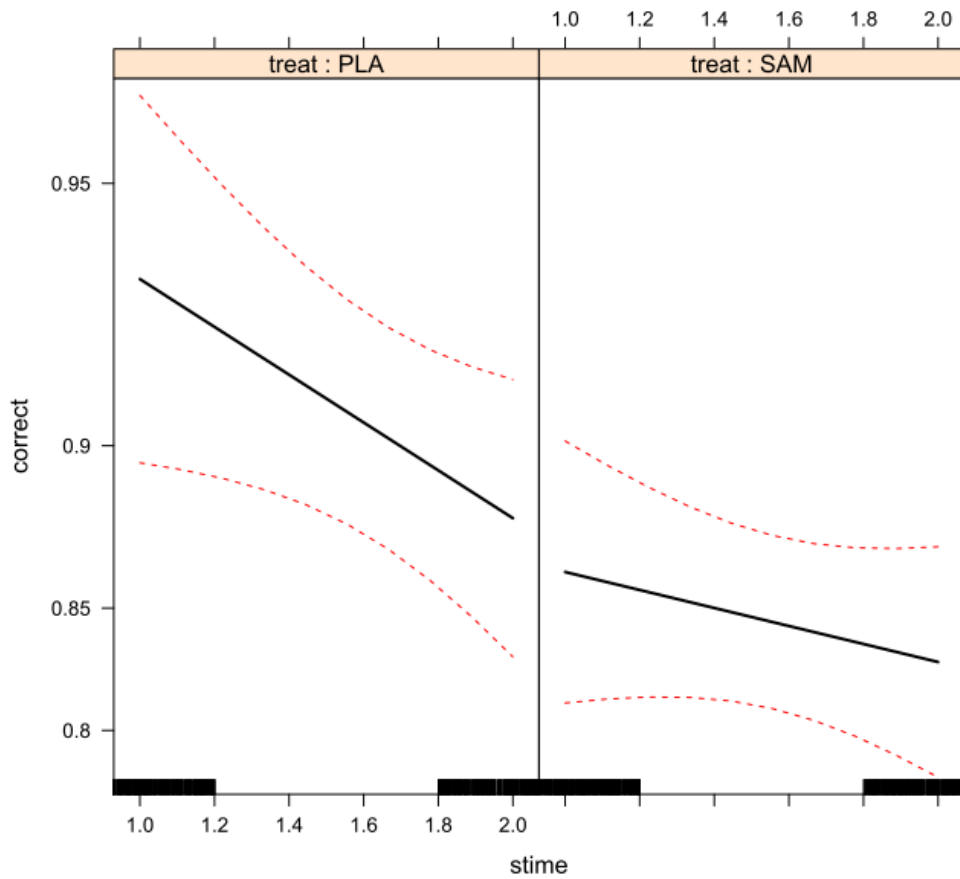


Figure 6.11 RVIP proportion correct. Treatment main effect $P=0.02$, time main effect $P=0.06$. stime 1 and 2 represent before and after exercise, respectively. Solid lines represent mean and dotted lines represent 95% confidence intervals.

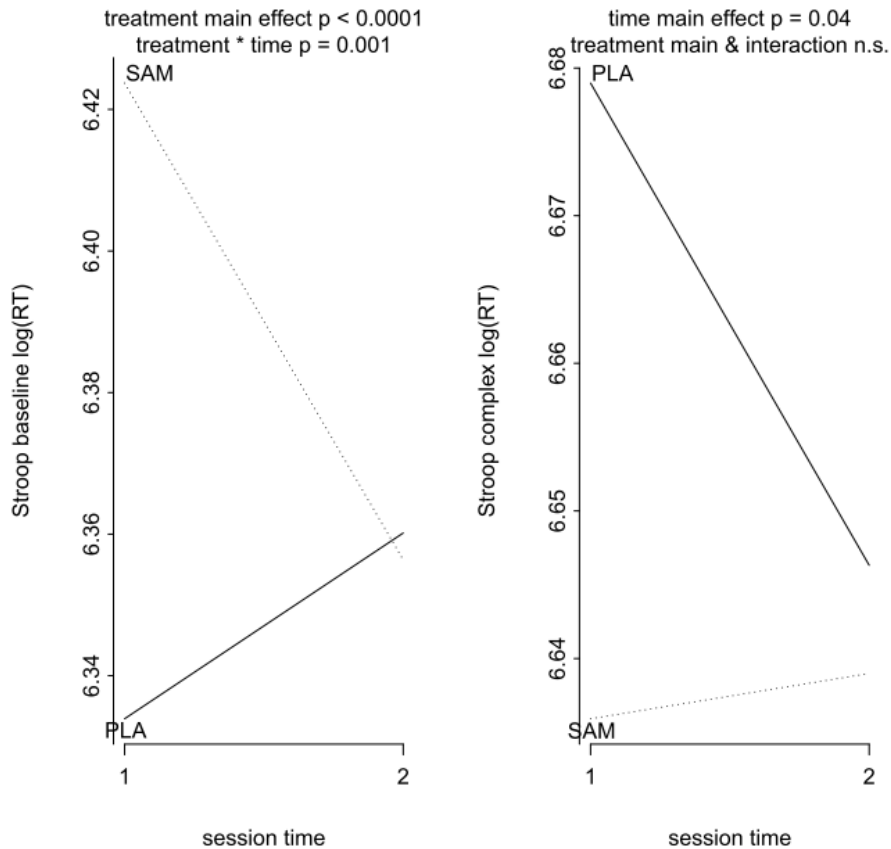


Figure 6.12 Stroop reaction times during the word match (left) and conflicted (right). Session time 1 and 2 represent before and after exercise, respectively.

6.5– Discussion

This is the first study to examine the effects of SAM supplementation on exercise. The complex and diverse actions of SAM make it difficult to determine the exact physiological mechanism, but effects on central monoaminergic effects are likely important. This is supported by evidence from human studies whereby SAM exerts therapeutic effects via central catecholaminergic metabolism. The present results demonstrate a week long dose of SAM does not affect prolonged exercise performance in warm conditions, despite increased skin temperature and impaired cognitive function. The small increase in skin temperature, increased prolactin and impaired cognitive performance, do not appear to have affected exercise performance, heart rate, core temperatures, RPE or thermal sensation in this study. Core temperature appears to be marginally lower during the SAM trial compared to

the placebo condition, possibly due to the increased heat loss at the skin, but this response was not statistically significant.

Previous studies have found that acutely increasing central catecholaminergic activity via dual dopamine/noradrenaline reuptake inhibitors can improve exercise performance in warm conditions compared to placebo. The neural networks associated with affect, cognition, and the cognitive/emotional experience of stress are interconnected and catecholamines are key neurotransmitters in their function (Ashby *et al.*, 1999). This includes the PFC, a key target of action for therapeutic doses of methylphenidate (Berridge *et al.*, 2006) and bupropion (Bares *et al.*, 2010). The present study, however, may provide evidence suggesting that lower PFC dopamine does not necessarily negatively affect prolonged exercise performance in warm conditions. Because the difference in Stroop reaction times normalised between trials at the end of exercise may indicate that at the point of fatigue during placebo PFC dopamine concentration had surpassed the apex of the inverted-U response. This stress induced switch to taking the PFC offline may serve to favour dorsal- and sub-cortical control over behaviour, effectively reducing executive control over behaviour (Arnsten, 2009), but does not appear to be a causative factor in the voluntary cessation of exercise *per se*.

Prolactin secretion appears to be positively correlated to skin temperature independently of core-temperature (Low *et al.*, 2005; Mündel *et al.*, 2006). Exogenous SAM increases COMT activity and COMT expression is much higher in the periphery, where COMT-dependent methylation of catecholamines is much more significant than in the CNS (Männistö & Kaakkola, 1999). Peripheral noradrenaline acts as a vasoconstrictor (Charkoudian, 2003), and by increasing COMT activity, increased SAM may have reduced peripheral noradrenaline, thereby disinhibiting cutaneous blood flow and temperature, which may explain the increased circulating prolactin during the SAM trial. This effect has been demonstrated in patients with COMT polymorphism (Kang *et al.*, 2010). However, monoamines tonically inhibit, while acetylcholine increases prolactin secretion (Freeman *et al.*, 2000). Because SAM increases monoamine turnover and may enhance cholinergic tone, a less

inhibited prolactin response to the increased skin temperature may explain the findings of the present study, at least in part. A study using a potent noradrenaline reuptake inhibitor found decreased prolactin and higher core temperature after exercise than placebo in normal ambient temperature despite a lower rate of exertion (Roelands *et al.*, 2008a). This could be due to decreased heat dissipation through the skin due to increased cutaneous vasoconstriction. However, no differences in skin temperature were detected in that study.

The source of the effects on cognitive function are difficult to pinpoint without more direct measurement of brain function and any rationalisation will rely heavily on speculation. Nonetheless, there is some evidence from previous studies investigating cognitive function to draw upon. Monoaminergic and cholinergic tone in the frontal cortex are determining factors for Stroop and RVIP performance. Cholinergic tone is positively correlated with cognitive function and cholinergic agonists improve performance (Callaway *et al.*, 1992). As SAM favourably affects cholinergic tone, changes in cholinergic neurotransmission cannot explain our results. Acute-tryptophan depletion and catecholamine depletion by alpha-methyl-para-tyrosine (AMPT) improve Stroop performance and decrease attention performance, respectively (Booij *et al.*, 2003). Therefore, changes in serotonergic neurotransmission cannot explain our results either. SAM has been found to improve cognitive function in schizophrenics with the low COMT-activity MET/MET genotype, whilst reducing peripheral noradrenaline, providing evidence of its capacity to increase COMT enzyme activity in humans (Strous *et al.*, 2009). Both noradrenaline and dopamine exert a concentration-dependent inverted-U effect on cognitive function in the PFC (Arnsten, 2007). COMT inhibition does not appear to affect dopamine release or turnover in the striatum, while it potentiates dopamine efflux in the PFC in rats (Männistö & Kaakkola, 1999). This does not appear to be accompanied by increased noradrenaline efflux however, which may be due to the relatively large number of noradrenaline transporters in the PFC (Tunbridge *et al.*, 2004). This regional selectivity may explain why central COMT inhibition does not appear to have reinforcing/addictive effects, but can enhance cognitive function (Apud *et al.*, 2007). The inverted-U relationship for prefrontal catecholamines is extended to COMT activity. This is demonstrated by studies assessing cognitive

function between COMT genotypes in which the less active MET/MET genotype exhibits faster reaction times during the Stroop test than the more active VAL/VAL genotype under normal conditions (Reuter *et al.*, 2005), but reverses after administration of amphetamine (Mattay *et al.*, 2003). Similarly, the opposite relative performance after exercise observed in this study may be explained by increased COMT activity in the SAM trial. As PFC function is impaired during stress via excessive catecholamine efflux (Arnsten, 2009), what was detrimental in the placebo condition may have ameliorated the condition of the SAM trial.

In conclusion, the results of the present study suggest that SAM supplementation for 1 week affects thermoregulation and central neurotransmission without altering exercise performance. The results of this study may have implications for athletes for whom sustained attention and reaction times are important (i.e.: tennis, team sports, etc.). SAM may be of benefit to athletes with the MET/MET polymorphism, to reduce elevated dopamine in the PFC, improving cognitive function without directly affecting exercise performance. While this study attempted to saturate the various physiological pools for the extensive metabolism of SAM by use of a week-long dosing protocol, future studies should determine the effects of an acute dose as the neurophysiological adaptations to sub-chronic exposure may alter any potential impact on exercise performance, as is the case with bupropion (Roelands *et al.*, 2009).

Chapter 7

General Discussion

7.1 – Background

Fatigue during prolonged exercise in warm conditions occurs long before muscle glycogen depletion (Parkin *et al.*, 1999; Febbraio, 2000). Prolonged exercise in warm environments places considerable strain on the thermoregulatory and cardiovascular systems (Hargreaves, 2008; Nybo, 2008; Maughan, 2010; Chevront *et al.*, 2010). Prolonged heat strain and fluid loss results in further diminished capacity to dissipate heat (Crandall & González-Alonso, 2010). This cumulative physiological strain may result in eventual fatigue via mechanisms residing within the CNS (Nybo, 2008). Central catecholaminergic signalling coordinates the behavioural response to stress (Chrousos & Gold, 1992) and coping (Pascucci *et al.*, 2007; Snyder *et al.*, 2012; Cabib & Puglisi-Allegra, 2012). Pharmacological inhibition of dopamine/noradrenaline reuptake has consistently improved performance during prolonged exercise in warm conditions, whereas studies attempting increase catecholamine metabolism have not (Roelands & Meeusen, 2010). The aim of the work described in the present thesis was to further characterise the role of central catecholamines during prolonged exercise in warm conditions.

7.2 – Effects of Central Dopamine/Noradrenaline Reuptake Inhibitors on Prolonged Exercise Performance in Warm Conditions

The results of Chapters 3 and 4 are in agreement with similar research in which dual dopamine/noradrenaline reuptake inhibitors at therapeutic doses improves exercise performance during prolonged exercise in warm conditions (Watson *et al.*, 2005a; Roelands *et al.*, 2008d). In all of the above studies, exercise performance was improved without significant changes to heart rate, core temperature, skin temperature, RPE, or thermal sensation, during steady state exercise. Changes in core temp and HR during the time trial were a consequence of the maintenance of an increased power output. Together these findings support the hypothesis that dopamine/noradrenaline reuptake inhibitors improve performance during prolonged exercise in warm conditions through central mechanisms rather than affecting changes in the periphery. Since the increased power outputs during time trial/workload challenge were not accompanied with increased RPE, it appears that

dopamine/noradrenaline reuptake inhibitors may alter the function of the teleoanticipatory system as proposed by St. Clair Gibson and co-workers (2006).

The results of Chapter 3 suggest that these effects are extended to women in the follicular phase of the menstrual cycle; during which circulating sex hormones concentrations are at the lowest point. This was the first study to investigate the effects of central catecholamine manipulation during prolonged exercise in warm conditions in female participants. This strengthens the argument for a common underlying central mechanism for the decrease in power output observed during prolonged exercise in warm compared to temperate conditions. A similar core temperature and heart rate response was observed during the workload challenge compared to that observed for male participants during the time trial in previous studies (Watson *et al.*, 2005a). Despite a slightly larger dose compared to those in the studies using male participants (9.4 vs. 8.0-8.2mg/kg), there was a similar effect size for exercise performance. As oestrogen and progesterone alter thermoregulation (Janse de Jonge, 2003) and may interact with drug effects (Young & Becker, 2009), future studies should determine the effect of bupropion and other centrally-acting dopamine/noradrenaline drugs also persist during the luteal phase.

The results of Chapter 4 suggest that there is a threshold point where reuptake inhibition facilitates performance during prolonged exercise in warm conditions. This study investigated a dose-response relationship for bupropion on exercise performance, and was the first exercise study to use less than the maximum therapeutic dose. Only the maximum dose appeared to significantly influence exercise performance. This may be explained by a level of redundancy in the expression of monoamine transporters, such that partial blockade may not sufficiently affect reuptake to alter neurotransmission (Blier, 2008). Egerton and co-workers (2010) found who found that 150mg bupropion, which produces a striatal DAT occupancy of about 26% (Learned-Coughlin, 2003), had no effect on extracellular dopamine concentrations in humans. Similarly, Volkow and co-workers (2002) induced DAT occupancy of 60% with methylphenidate and found insignificant increases in extracellular dopamine. However, striatal DAT occupancy levels may

not be the best predictor of effects on exercise. For example, Swart and co-workers (2009) found that 10mg methylphenidate, which has been found to produce 40% occupancy (Volkow *et al.*, 1998), improved exercise performance in temperate conditions. In contrast, neither 300mg bupropion (Watson *et al.*, 2005a) or 20mg methylphenidate (Roelands *et al.*, 2008d), which has been found to induce 54% DAT occupancy (Volkow *et al.*, 1998) found no improvements in performance in temperate conditions. Volkow and co-workers (Volkow *et al.*, 2005) later demonstrated that striatal dopamine was more dependent on dopaminergic neuron activity and dopamine release, during an engaging task, for example.

However, the apparent inconsistency in DAT occupancy as a predictor of exercise effects may be due to differences in methodology instead. Swart and co-workers (2009) used time to exhaustion, while Watson and co-workers (2005) and Roelands and co-workers (2008) used time trials as a measures of performance. As time trial performance is more susceptible to individual pacing strategies than time to exhaustion (Hinckson & Hopkins, 2005), it may be possible that time trials aren't quite as robust or sensitive in detecting performance changes made by interventions (Laursen *et al.*, 2007). However, more studies are required to determine the relative sensitivity of time to exhaustion versus time trials to the effects of dopamine/noradrenaline reuptake inhibition. Nonetheless, the difference in results underscores the contribution of heat strain to the centrally regulated component of performance. Another component of dopamine/noradrenaline reuptake inhibitors not characterised by striatal DAT imaging studies are the effects on NAT, which is the primary transporter for dopamine in the PFC, a key area for their therapeutic effects.

Low, clinically relevant doses of psychostimulants preferentially increase extracellular catecholamines in the PFC (Berridge & Arnsten, 2012) while also slightly decreasing locus coeruleus cell firing by inhibiting reuptake (Stahl *et al.*, 2004; Devilbiss & Berridge, 2006)(see figure 7.1). This includes methylphenidate (Berridge *et al.*, 2006) and bupropion (Bares *et al.*, 2010) and amphetamine (Berridge & Arnsten, 2012). DAT expression appears to be correlated with D2-like receptor expression (Hall *et al.*, 1999). The PFC expresses greater concentrations of

D1-like receptors than D2-like; accordingly, there is little DAT expression in cerebral cortical areas (Hall *et al.*, 1999). Whereas reuptake inhibition normally increases stimulation of D2 autoreceptors to control dopamine release via feedback inhibition, the mesoprefrontal dopamine neurons do not express D2 autoreceptors (Lammel *et al.*, 2008) and PFC D1 receptors uniquely appear to be targets for tonic dopamine (Dreher & Burnod, 2002; Thurley *et al.*, 2008). The increase in PFC dopamine by these drugs appears to be mediated by blockade of PFC NAT, which acts as the primary transporter for dopamine in the PFC (Morón *et al.*, 2002). Dopamine may also be co-released from noradrenergic neurons in the PFC (Devoto & Flore, 2006). Low-dose psychostimulants also reduce the metabolic cost required for optimal mental performance, improve connectivity within cortical executive areas and reduce interference from irrelevant stimuli and interruption of focus (Swanson *et al.*, 2011). This may suggest that these drugs improve task performance by increasing executive control over attentional and behavioural resources.

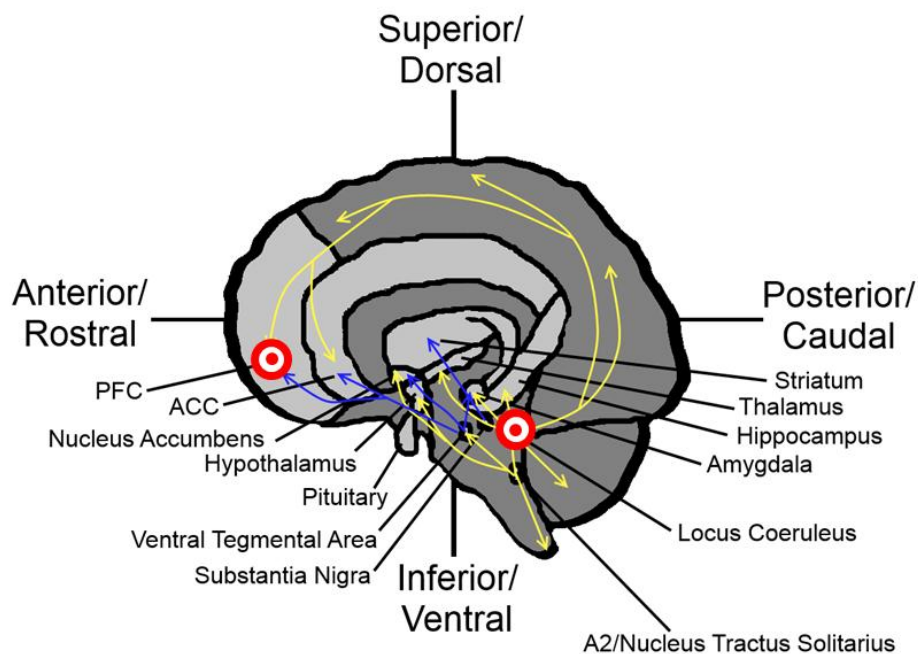


Figure 7.1 Key neuroanatomical targets for dopamine/noradrenaline reuptake inhibitors at therapeutic doses represented by red targets. Blue and yellow arrows represent dopaminergic and noradrenergic projections, respectively.

PFC noradrenaline has been shown to be involved in mediating amphetamine induced behavioural activation and dopamine efflux in the NAc (Ventura *et al.*, 2003; McKittrick & Abercrombie, 2007). Novel stressors increase noradrenaline release within the mPFC which parallels the enhancement of mesoaccumbens dopamine release (Pascucci *et al.*, 2007). However, dopamine in the mPFC inhibits stress-induced dopamine release in the NAc (King *et al.*, 1997; Pascucci *et al.*, 2007), while noradrenaline is necessary for NAc dopamine release by stress or by amphetamine (Darracq *et al.*, 1998; Ventura *et al.*, 2003; Mitrano *et al.*, 2012). Tonic dopamine in the NAc appears to mediate stress-induced aversive behaviour, and inhibition of this stress-induced increase in tonic dopamine prevents this withdrawal behaviour (Cabib & Puglisi-Allegra, 2012). However, diminished tonic dopamine in the NAc shell also results in larger phasic signals, which promote behavioural switching and reflect increased salience of motivationally relevant stimuli. The ventromedial PFC appears to exert control over both phasic and tonic signalling in the NAc shell, by suppressing phasic dopamine and increasing tonic dopamine signalling, both acting to prevent selection of task-irrelevant behaviour (Ghazizadeh *et al.*, 2012). Insufficient tonic dopamine in the NAc shell potentiates the impact of phasic D1 signalling from limbic inputs and facilitates behavioural flexibility, while excessive tonic dopamine inhibits PFC input to the NAc (Goto *et al.*, 2007). This relative ratio between tonic and phasic dopamine depends on the state of the organism, but tonic dopamine levels appear to represent the utility of on-going behaviour, with decreases resulting in concomitant reduction of vigour of effort (Niv *et al.*, 2007).

A recent study in humans using arterial spin labelling MRI also investigated the effects of methylphenidate on the blood oxygen level dependent signal in a large number of extrastriatal structures (Marquand *et al.*, 2012). The authors found a decreased signal in many of the structures involved in the stress-response including the NTS, hypothalamus and amygdala and an increase in activity in mesocorticolimbic structures. This supports both top-down and bottom-up alterations in activation, which is reflected in behavioural and EEG tests with effective ADHD treatments (Kenemans & Kähkönen, 2011). The increased prefrontal catecholamines may enable better executive control over sub-cortical structures involved in generating stress-mediated behaviours (as described in section 1.11). The

noradrenergic brainstem nuclei, the amygdala, and the NAc all receive afferent projections from the PFC. In rats, medioventral PFC inactivation (Amat *et al.*, 2005) and activation (Amat *et al.*, 2008) has been found to increase and decrease the impact of stress, respectively, by afferent control of brainstem and limbic structures (Maier & Watkins, 2010). Improved executive control via the PFC may facilitate the maintenance of desired behaviour by strengthening the input to the insular cortex or ACC, possible candidates for the input and output centres of the teleoanticipatory system, respectively. Alternatively, or concomitantly, a decreased reactivity of the noradrenergic nuclei may occur due to increased autoreceptor stimulated feedback inhibition as a result of reuptake inhibition. This might result in reduced or delayed stress-signalling within the CNS and a prevention of the associated behavioural shift. Whether by PFC-mediated top-down effects or by inhibition of bottom-up stress signalling, these neurobiological mechanisms provide a possible framework within the teleoanticipatory model for how power output is increase without changes in perceived exertion.

7.3 – Effects of a Catecholamine Precursor on Prolonged Exercise Performance in Warm Conditions

The results of Chapter 5 suggest that despite observing changes in central neurotransmission, as measured by the inhibition of the prolactin response, no differences were observed to performance, core or skin temperature, heart rate, RPE, thermal sensation, blood glucose, haemoglobin or haematocrit. Central catecholamines appear to play a greater role in the development of fatigue during prolonged exercise in warm conditions than in temperate conditions. Subsequently, studies in warm conditions should theoretically be more sensitive to changes induced by catecholamine precursors. Only one study has found improved performance in warm conditions with a catecholamine precursor (L-tyrosine)(Tumilty *et al.*, 2011), while a more recent study using the same exercise conditions, but with a more robust increase and maintenance of plasma L-tyrosine concentrations found no difference in performance or cognitive function (Watson *et al.*, 2012). The results of the latter study and the findings in Chapter 5 support the conclusion that acute oral administration of catecholamine precursors does not improve prolonged

exercise performance in the heat. This is in agreement with the majority of studies using L-tyrosine in temperate conditions, in which no effect on exercise performance has been found (Meeusen *et al.*, 2006a). The lack of effect of catecholamine precursors on exercise performance may be related to the lack relative clinical efficacy in treating either ADHD or depression. Although catecholamine precursors appear to produce changes in cognitive function (Owasoyo *et al.*, 1992; Onur *et al.*, 2011) and can produce changes in striatal dopamine in humans (Floel *et al.*, 2008), they do not appear to consistently affect exercise performance. This may suggest that in the majority of healthy individuals catecholamine synthesis is not a determining factor in exercise performance. While genetic factors may determine the effect of precursor availability, they would similarly affect changes to reuptake inhibition.

The effects of drugs with therapeutic efficacy for treatment of ADHD, which include amphetamine, methylphenidate, and bupropion (Wilens, 2006), do not share the same neuropharmacological effects as L-DOPA. Specifically, drugs which are effective in treating ADHD affect behavioural and EEG measures of top-down control and selective attention, while L-DOPA does not (Kenemans & Kähkönen, 2011). The reasons for these differences are not fully understood, but may be due to the relative differences in combined changes of dopamine and noradrenaline transmission. For example, although L-DOPA is actively taken up by locus coeruleus neurons for noradrenaline synthesis, it has no effect on locus coeruleus electrophysiology in healthy rats (Miguelé *et al.*, 2011), while both acute bupropion and methylphenidate slightly decrease locus coeruleus activity (Cooper *et al.*, 1994; Devilbiss & Berridge, 2006). Further differences may be due to relative changes between tonic and phasic neurotransmission (Sikström & Söderlund, 2007). Evidence in rats suggests that L-DOPA-induced increases in dopamine release is primarily due to increased phasic dopamine quanta (Rodríguez *et al.*, 2007). There is supporting evidence from human imaging studies for this; the capacity of dopamine synthesis as measured by striatal [¹⁸F]fluorometatyrosine uptake observed with PET appears to be predictor of outcome-specific reversal-learning performance, which is determined by phasic dopamine (Cools *et al.*, 2009). Another PET study found that acute treatment with L-DOPA did not increase striatal D2 receptor binding as measured by [¹¹C]raclopride at

rest, but during motor learning, a small increase associated with phasic spiking was observed (Floel *et al.*, 2008). A similar effect has been observed for methylphenidate in PET imaging of [¹¹C]raclopride binding (Volkow *et al.*, 2005). Although both appear to increase striatal dopamine during engaging tasks, L-DOPA does not appear to influence performance, suggesting that striatal dopamine may not be a good predictor of the performance enhancing effects of dopamine/noradrenaline reuptake inhibition. This supports the notion that extrastriatal catecholamines may be more important for the performance enhancing effects of bupropion and methylphenidate. Supporting this, it is the effect of these drugs on extrastriatal catecholamines which appears to be more important for their efficacy in the treatment of ADHD and depression.

7.4 – Effects of S-adenosylmethionine on Prolonged Exercise Performance in Warm Conditions

SAM is a widely available nutritional supplement with clinical antidepressant qualities related to monoamine metabolism. The study in Chapter 6 appears to be the first exercise study investigating the effects of SAM supplementation on performance. It was thought at the onset of the study that a protocol to ensure the saturation of the various metabolic pathways related to SAM would be more conducive to providing results, but it is possible that this approach may have precluded any definitive effects on performance; the week long supplementation period may have resulted in an adaptation to the CNS, altering the response to the intervention. This neurobiological response is commonly reported when centrally-acting drugs are used in the management of many psychiatric disorders (e.g. depression) and a similar effect was observed when bupropion was administered chronically by Roelands and co-workers (2009). Despite the apparent changes in cognitive performance in tests that are influenced by cortical catecholamines, no clear effect on exercise performance was observed. The present results does suggest that supplementation of SAM for one week influences baseline measures of cognitive function, associated with attention, as well as elevating the skin temperature and prolactin concentrations during prolonged exercise in warm conditions, without altering performance. However, the

diverse metabolic fates of SAM make it difficult to precisely determine the physiological cause of these effects.

The antidepressant qualities of SAM are comparable to typical tricyclic antidepressants, both in efficacy (Mischoulon & Fava, 2002) and the effects on EEG (Saletu-Zyhlarz *et al.*, 2002). These EEG patterns are distinct to methylphenidate (Saletu *et al.*, 2006) and appear to be more similar to imipramine (Anderer *et al.*, 2002; Saletu *et al.*, 2010), a tricyclic antidepressant with primarily serotonergic and noradrenergic effects (Lee *et al.*, 1982). The changes in P300 amplitude and latency indicate effects on the locus coeruleus and phasic noradrenergic cortical signalling (Nieuwenhuis *et al.*, 2005). Supporting this, small decreases in α and β power observed by Saletu-Zyhlarz and co-workers (2002) suggests a decreased level of arousal, which could be due to decreased cortical noradrenaline (Berridge & Morris, 2000). This is also reflected in the increased reaction times and decreased attention, similar to what we observed in our study. After exercise, these effects appeared to be normalised, perhaps due to stress-induced arousal and cortical catecholamine efflux (Arnsten, 2009). To date there is no evidence of effects for SAM at receptors or transporters, meaning any changes in signalling are likely a result of changes in metabolism.

SAM has been found to improve cognitive function in participants with schizophrenia with the low COMT-activity MET/MET genotype, whilst reducing peripheral noradrenaline, providing evidence of its capacity to increase COMT enzyme activity in humans (Strous *et al.*, 2009). This may explain the increased skin temperature and increased prolactin release observed in this study. Similarly, participants with less active MET/MET genotype exhibits faster reaction times during the Stroop test than the more active VAL/VAL genotype under normal conditions (Reuter *et al.*, 2005), but reverses after administration of amphetamine (Mattay *et al.*, 2003). This suggests the inverted-U relationship for prefrontal catecholamines described by Arnsten (2007) may be regulated, in part, by COMT activity. This is supported by evidence suggesting COMT plays a greater role in the degradation of catecholamines than MAO in the PFC, than elsewhere in the brain (Matsumoto *et al.*,

2003; Tunbridge *et al.*, 2004; Seamans & Yang, 2004). The opposite relative performance after exercise observed in this study may be explained by increased COMT activity in the SAM trial, although this is merely speculation at this point. As PFC function is impaired during stress via excessive catecholamine efflux (Arnsten, 2009), what was detrimental in the placebo condition may have ameliorated the condition of the SAM trial. The results in Chapter 6 indicate that future studies are required before a definitive conclusion can be made regarding the effects of SAM on exercise performance. Primarily, the same tests should be conducted using an acute dose to determine if the central effects of this drug are connected to performance.

7.5 – Conclusion and Future Research

Pharmacological inhibition of dopamine/noradrenaline reuptake has consistently improved performance during prolonged exercise in warm conditions, whereas studies attempting increase catecholamine synthesis have not (Roelands & Meeusen, 2010). The studies contained within this thesis have attempted to further characterise this apparent difference. Central catecholaminergic signalling coordinates the behavioural response to stress (Chrousos & Gold, 1992) and coping (Pascucci *et al.*, 2007; Snyder *et al.*, 2012; Cabib & Puglisi-Allegra, 2012). Therapeutic doses of dopamine/noradrenaline reuptake inhibitors used in human exercise studies preferentially effect the PFC and the dopaminergic and noradrenergic nuclei which innervate it: the VTA and locus coeruleus, respectively (Berridge & Arnsten, 2012). This may result in improved executive control, a reduced impact of stress on behavioural switching, or a combination of both. The difference between catecholamine precursors and reuptake inhibitors may be due to differences in combined changes of dopamine and noradrenaline transmission or changes to tonic and phasic release, which would have differing effects on autoreceptor mediated feedback inhibition and subsequent signalling dynamics. The results of the studies presented in this thesis support the distinction in the effects of reuptake inhibition compared to increased synthesis of catecholamines on exercise performance. This suggests that future research be conducted with more consideration on the roles of catecholamines in particular areas of the brain.

Unfortunately, imaging of human brain activity during exercise remains impractical due to the numerous constraints of the imaging equipment. Therefore, future research could investigate the effects of bupropion, amphetamine or methylphenidate on EEG during prolonged exercise in warm conditions, to determine whether the changes in cortical activity reflect the performance enhancing effects of these drugs. Because all of these drugs are effective treatments for ADHD, the connection between ADHD, central catecholamines and prolonged exercise in warm conditions should be explored. Further pharmacotherapies for ADHD could be investigated, such as Atomoxetine. Studies comparing differences in prolonged exercise in adults with ADHD on and off their medication may further develop our understanding of the impact of catecholamines on the regulation of prolonged exercise. The importance of genetic differences in catecholamine metabolism and signalling, such as COMT polymorphism, could be explored. To further categorise gender differences, a study to investigate the effects of dopamine/noradrenaline reuptake inhibition on prolonged exercise in warm conditions in women during the luteal phase would help elucidate the impact of the menstrual cycle on exercise performance and the drug effects. Animal models provide the most flexibility and power for directly measuring changes within the CNS, but it may be difficult to devise an appropriate animal model for examining central fatigue, as motivation and stress will be difficult to control for in the context of goal-direction and executive control of behaviour. Forced behaviour alters the impact of stress on catecholamine systems (Cabib & Puglisi-Allegra, 1994), therefore, it would be necessary to develop a model in which animal exercise is self-motivated. This might be achieved by conditioning a fixed-ratio schedule or fixed-interval for rewards dependent on exercise performance or duration, respectively.

Key points:

- 1) The effects of bupropion on exercise performance in warm conditions appear to be similar in both men and women.
- 2) There is a threshold for dosage at which the effects of bupropion on exercise performance manifest.

- 3) Increased availability of central catecholamine precursor does not appear to improve performance during prolonged exercise in warm conditions. This suggests site-specific changes in signalling within the CNS are responsible for the effects of dopamine/noradrenaline reuptake inhibitors, rather than a simple increase or decrease of neurotransmitter availability.

- 4) 7-day SAM supplementation does not influence exercise capacity in the heat. To further characterise the role of methyl-group donors in the synthesis of catecholamines and how this may affect exercise performance, future SAM studies employing acute dosing protocols are required.

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Appendix

Thermal Sensation 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 body 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**



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Analysis Report S2011005796

Sample N2011040650

Client: School of Sport, Exercise and Health Sciences, Loughborough University
Product name: SAM-e Complete
Date of receipt: 07.11.2011
Charge: 1034930 best before end: --
Form of presentation: Tablets Amount: 24 pcs.
Brief description: Yellow tablets in blister pack with black imprint

Analysis for anabolic androgenic steroids:

Aliquots of the sample were analysed by gas-chromatography/mass-spectrometry for the following substances (reporting level 10 ng/g):
19-Nor-4-androstene-3 β ,17 β -diol, 19-Nor-5-androstene-3 β ,17 β -diol, 19-Nor-4-androstene-3,17-dione, 19-Nortestosterone, 4-Androstene-3 β ,17 β -diol, 5 Androstene-3 β ,17 β -diol, 4-Androstene-3,17-dione, Dehydroepiandrosterone (DHEA), Testosterone, 5 α -Androstan-3 β ,17 β -diol, Androstadien-3,17-dione

Analysis for stimulants:


Aliquots of the sample were analysed by liquid-chromatography/mass-spectrometry for the following substances (reporting level 100 ng/g):
Amphetamine, Metamphetamine, Dimetamphetamine, Methylenedioamphetamin, Methylephedrine, Methypseudoephedrine, Ephedrine, Pseudoephedrine, Norephedrine, Norpseudoephedrine, Strychnine, Methylenedioxymetamphetamine, Methylenedioethylamphetamine, Benzphetamine, Fenfluramine

Results:

None of the listed anabolic androgenic steroids was detected.

None of the listed stimulants was detected.

Cologne, 22.11.2011


Dr. Maria Kristina Parr
(Senior Chemist)

Disclaimer: The analysis report refers exclusively to the sample listed above. The laboratory is not responsible for sampling. Without written permission by the Centre for Preventive Doping Research, German Sport University Cologne the analysis report may not be copied or published, not even partially.

