Manipulations of Neural Fatigue

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MANIPULATIONS OF NEURAL FATIGUE

by

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Dedicated to the memory of my father, James Ian Hunter, who will always be one of my strongest influences.

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ABSTRACT

The aims of this thesis were to investigate the neural manipulations of fatigue, by examining cycling fatigue under differing conditions. This data should provide more understanding to the neural involvement in exercise fatigue.

In the first two studies of this thesis we established the most effective electromyographic (EMG) methodology for analysing neural recruitment patterns during submaximal cycling. In the first study we analysed the effect of different EMG capture rates during maximal voluntary contraction (MVC), submaximal and maximal dynamic cycling activity on EMG amplitude and signal characteristics. We began by testing Peak power output (PPO) and MVC, followed by a progressive cycle ride on a cycle ergometer, in 10 healthy subjects. EMG data was simultaneously captured during the MVC and cycling activities at frequencies of 32, 64, 128, 256, 512, 1024 and 1984 Hz. Significant differences in amplitude were found ($P < 0.01$) between MVC, submaximal (SUB) and maximal cycling activities (PWATT) for all capture rates. Asymptote values for integrated EMG (IEMG) amplitude occurred at EMG capture rates of 1604 $\pm$ 235.6 Hz during MVC, 503.1 $\pm$ 236.2 Hz during PWATT and 326.2 $\pm$ 105.4 Hz during SUB cycling activity and were significantly different ($P< 0.01$). Non significant differences were found for force/EMG ratios between PWATT and MVC at 1984 Hz capture rates (3.8 $\pm$ 1.7 N/V vs 2.5 $\pm$ 0.9 N/V) while significant differences occurred at 32 Hz capture rate (6.2 $\pm$ 3.8 vs 16.0 $\pm$ 8.0; $P < 0.01$). Insignificant low correlations were found between EMG activity captured at 1984 Hz during PWATT and lean thigh volume ($r = 0.36$) and MVC ($r = 0.32$). It was concluded that

asymptote values indicated that data captured below 326 Hz for SUB, 503 Hz for PWATT and 1604 Hz for MVC are not reliable. Therefore apparatus capturing EMG data at low frequencies from these values cannot be used for quantitative data analyses. From this conclusion it was decided that the highest capture rate on our EMG equipment of 1984 Hz would be used for all data collection in this thesis. This would enable inter activity comparison and ensure that it is at least twice as high as the maximum amplitude generated.

The second study aimed to determine the most effective EMG normalisation method for cycling fatigue protocols to compare intersubject data. Ten healthy subjects performed 5-second MVC at a knee joint angle of 60°; fixed cycle pedal contraction at knee joint angles of 60° (60°A), and 108° (108°A); and a dynamic single maximal revolution of a cycle pedal (1REV). IEMG data was recorded for all contractions and power output recorded during MVC and 1REV. Mean IEMG for MVC was significantly ($P < 0.01$) greater than 60°, 108° and 1REV. There were no significant differences between MVC and 1REV power output/EMG relationship. It was concluded that MVC will record a higher IEMG than 60°A, 108°A and 1REV. As IEMG was greatest during MVC, and the relationship between IEMG and power output was not different between MVC and 1REV, normalisation against maximal possible recruitment potential is most likely during MVC. From this observation we decided that MVC would be most appropriate for normalising all EMG data.

Once the most effective EMG methodology had been established we could then study exercise fatigue in different conditions. In the third study we wanted to use an environment that would affect both central and metabolic mediated

fatigue. Accordingly, we analysed the effect of hot (35 °C) and cold (15 °C) environments on EMG signal characteristics on, skin and rectal temperatures and heart rate during progressive endurance exercise. Eight healthy subjects performed three successive 15 min rides at 30%, 50% and 70% of their peak sustained power output and then cycled at increasing (15 $W.min^{-1}$) work rates to exhaustion in both 35°C (HOT) and 15°C (COLD) environments. Skin and rectal temperatures, heart rate and EMG data were measured during the trials. The skin temperatures and thermal comfort values were higher in the hot group ($P < 0.01$). Although rectal temperature was higher in the hot group this was not significant. Heart rate was significantly higher in the hot group (between groups $P < 0.05$). PPO (267.4 $\pm$ 67.7 W vs 250.1 $\pm$ 61.5 W) and time to exhaustion (55.7 $\pm$ 16.7 min vs 54.5 $\pm$ 17.1 min, COLD vs HOT) were not different between groups. There were no differences in IEMG or mean power frequency spectrum (MPFS) between groups. Rating of perceived exertion increased similarly in both groups over time. It was concluded that a number of factors affect exercise under hot and cold conditions. However, in this study the HOT protocol caused changes in skin temperature and heart rate, but not in rectal temperature. EMG was not altered in the presence of elevated skin temperature and thermal comfort. RPE was the same for both HOT and COLD, suggesting that peripheral mechanisms resulted in unchanged central drive. Therefore, it appears that during HOT, effective peripheral thermoregulation mechanisms control core temperature, resulting in an unchanged neuromuscular recruitment strategy.

In the fourth study we used a condition that would enhance fatigue by limiting exercise performance, therefore analysed the effect of selective ß1-blockade on EMG signal characteristics during maximal progressive endurance exercise. Seven healthy subjects ingested a selective adrenergic receptor blocker, acebutolol (200mg b.d.) (for one of two cycling trials). On the last day of acebutolol subjects performed an MVC. This was followed by three successive 15 min rides at 30%, 50% and 70% of their PPO. Subsequently, work rate increased by 15 $W.min^{-1}$ work rates until they fatigued. Force output, heart rate, submaximal VO_2, rate of perceived exertion (RPE), electromyographic (EMG) data and blood lactate were captured during the cycling activity. Peak work rate (270 $\pm$ 111 W vs 197 $\pm$ 75 W, control group (CON) vs BETA, $P < 0.01$), time to exhaustion (49.7 $\pm$ 23.2 min vs 40.3 $\pm$ 23.7 min, CON vs BETA, $P < 0.05$) and heart rate (mean, for the full ride 135.5 $\pm$ 38.3 $b.min^{-1}$ vs 111.5 $\pm$ 30.0 $b.min^{-1}$ CON vs BETA, $P < 0.05$) were significantly lower for BETA compared to CON. Although not significant, submaximal VO_2 was reduced in BETA during the ride, while RPE was significantly higher during the ride for BETA ($P < 0.01$). Mean integrated electromyography (IEMG) was higher in the BETA group although these differences were not significant. Mean power frequency values (MPFS) of the BETA group showed a significant ($P < 0.05$) shift to the upper end of the spectrum in comparison to the control group. Lactate values (11.7 $\pm$ 3.5 $mmol.l^{-1}$ vs 7.1 $\pm$ 4.1 $mmol.l^{-1}$ CON vs BETA) were significantly lower ($P < 0.05$) at exhaustion in BETA. In conclusion, significant reductions in cycling performance were found when subjects ingested ß1-blockers. This study has shown significant shifts to the upper end of the EMG frequency spectrum after

ß1-blocker ingestion, which could be caused by a change in neuromuscular recruitment strategy to compensate for the impaired maximal capacity.

For the fifth study we wanted to prolong the onset of fatigue by using a condition that stimulates the physiological system. Also, in the third and fourth studies we used open loop protocols whereupon cadence was dictated to, consequently we decided it was necessary to use a closed loop protocol that required the subject to employ a pacing strategy to finish the distance in the fastest time possible. Therefore in the fifth study we analysed the effect of caffeine ingestion on performance during a 100 km cycling time trial that included bouts of 1 and 4 km sprints. Eight highly trained cyclists participated in 3 separate trials: either placebo ingestion before exercise with a placebo carbohydrate solution and placebo tablets during exercise (Pl) or placebo ingestion before exercise with a 7% carbohydrate drink and placebo tablets during exercise (Cho), or caffeine tablet ingestion before and during exercise with a 7% carbohydrate (Caf). Placebo (twice) or 6 $mg.kg^{-1}$ caffeine was ingested 60 minutes prior to starting one of the three cycling trials during which subjects ingested either additional placebos followed by further placebo ingestion or a maintenance dose of 0.33 $mg.kg^{-1}$ every 15 minutes until the completion of the trial. The 100 km time trial consisted of five 1 km sprints after 10, 32, 52, 72 and 99 km, as well as four 4 km sprints after 20, 40, 60 and 80 km. Subjects were required to complete the 100 km time trial and all sprints in the fastest time possible. Plasma [caffeine] was significantly higher during Caf (0.43 $\pm$ 0.56 μM and 1.11 $\pm$ 1.78 μM and 47.32 $\pm$ 12.01 μM and 72.43 $\pm$ 29.08 μM pre and postride for Pl and Caf respectively). Average power, time to complete the sprints as well as total time to complete the 100

km were not different between trials. However, in the Caf group, work output and sprint times were marginally faster in the first section of the trial with slower times and lesser work output in the latter section. Heart rate (HR) was higher during the entire bout in Caf than in the other groups. No significant differences were found between groups for IEMG or MPFS. It was concluded that despite markedly elevated serum [caffeine] concentrations, subjects ingesting caffeine did not perform better during a 100 km cycling time trial that included repeated sprinting. Hence caffeine may be without ergogenic benefit during endurance exercise in which the athlete begins exercise with a defined, predetermined goal measured as speed or distance. We conclude that performance during these trials is controlled by a centrally-mediated pacing strategy.

The final study analysed the effects of supramaximal exercise on EMG signal characteristics as this high intensity protocol still leaves investigators unsure of the mechanisms that mediate the fatigue. Ten healthy subjects completed the Wingate cycling protocol whilst EMG data and rate of fatigue was recorded throughout the cycling. Mean rate of fatigue was 44.5 $\pm$ 8.6 %, no significant change in IEMG and a highly significant drop off ($P < 0.01$) in MPFS over the 30-second period. It was concluded during the Wingate, MPFS was attenuated with no decline in IEMG, which could be caused by a drop off in central command or from an accumulation of metabolites in the periphery. However, it is possible that there is a reduction in central drive, but the feedback loop between central drive and neural recruitment does not have enough time to act within the 30 second period of the Wingate.

In summary, this thesis demonstrated the most effective EMG methodology to use for cycling exercise to fatigue. From there it was shown that first, efficient thermoregulation will enable similar neural recruitment patterns in hot conditions to enable maintenance of performance times. Second, ß- blocker ingestion, showed the possibility of increased central drive through altered neuromuscular recruitment patterns to compensate for the reduced exercise capacity. Third, caffeine ingestion clearly had a stimulatory effect, shown by an elevated heart rate, however this failed to improve 100 km cycling time trial, we therefore conclude that performance during these trials is controlled by a centrally-controlled pacing strategy. Finally, during the Wingate MPFS was attenuated with no decline in IEMG, which could be caused by a drop off in central command or from an accumulation of metabolites in the periphery. The findings from this thesis therefore show the neuromuscular and performance response to exercise fatigue in different conditions. It appears that in some conditions there is a distinct change in neural recruitment strategy, possibly to serve as a protective mechanism, however in others there appears to be no change either because of an effective haemodynamic response that acts as a protective mechanism or because of an ineffective neural response mechanism that fails to protect the body.

LIST OF ABBREVIATIONS

1REV – one dynamic maximal cycle pedal revolution

5 HIAA – 5-hydroxyindole acetic acid

5-HT – hydroxytryptomine

60°A – isometric cycle pedal at a knee angle of 60°

108°A - isometric cycle pedal at a knee angle of 108°

ATP – adenosine triphosphate

ADP – adenosine diphosphate

BCAA – branched chain amino acids

BETA – experimental group who have ingested ß-blocker

BW – body weight

Caf- experimental group who have ingested 7% carbohydrate solution with 6 mg/kg of caffeine

Ca_2 - Calcium

CAR – central activation ratio

CFS – chronic fatigue sufferers

Cho - experimental group who have ingested 7% carbohydrate solution with placebo tablet

CMAP – compound muscle action potential

CNS – central nervous system

COLD – ambient temperature of 15°C

CON – control group

Cr – creatine

CV – coefficient of variation

DA – dopamine

DLPFC – dorsolateral prefrontal cortex

EC – excitation contraction

EMG – electromyography

FDI – first dorsal interosseous

FFA – free fatty acid

FI – fatigue index in Wingate protocol

FORCE – Newtons

f-TRP – plasma free tryptophan

GABA – gamma-aminobutyynic acid

H^+ - hydrogen

HFF – high frequency fatigue

HOT – ambient temperature of 35°C

HPLC – high performance liquid chromatography

HR – heart rate

IEMG – integrated electromyography

IMP – inosine monophosphate

IOC – International Olympic Committee

$J.Mv^{-1}$ – work/IEMG

K^+ - pottasium

LDH – lactate dehydrogenase

LFF – low frequency fatigue

LTV – lean thigh volume

MEP – motor evoked potentials

MFCV – muscle fiber conduction velocity

MPFS – mean power frequency spectrum

MU – motor units

MUAP – motor unit action potential

MVC – maximal isometric voluntary contraction

Na^+ - sodium

NH_3 – ammonia

Pi – inorganic phosphate

PCr - phosphocreatine

PI – group who have ingested sports elctrolyte solution containing a sweetener (1.7% carbohydrate)

PWATT – maximum force output at exhaustion

RF – rectus femoris

RPE – rating of perceived exertion

RPS – revolution per second

RQ – respiratory quotient

SR – sarcoplasmic reticulum

TC – thermal comfort

TCA – tricarboxylic acid

T_{hyp} – hypothalamic temperature

TIME – time to exhaustion

TMS – transcranial magnetic stimulation

T_{rec} – rectal temperature

TRP - tryptophan

TT – time trial

V_m – resting membrane potential

VO_{2MAX} – maximum oxygen consumption

WAT – Wingate Anaerobic Test

INTRODUCTION

Muscle fatigue has been defined as the inability to maintain a given work load during exercise (75). Fatigue is a multifaceted phenomenon that is task specific and will vary according to the conditions in which it occurs. Historically, exercise scientists have believed that causes of exercise fatigue involve "peripheral" mechanisms or exclusively physiological processes (386;407;463). Peripheral fatigue has, however, also been described as "the decrease in force generation capacity of the muscle due to accumulation of metabolites in the presence of an increasing motor drive or motor command" (488). Indeed, increasing evidence and theories are emerging of central involvement in exercise fatigue (408;497). Davis and Bailey (130) defined central fatigue as the failure to maintain the required or expected force output when the dysfunction could not be reasonably explained in the muscle itself. An abundance of evidence showing the existence of central fatigue comes from the study of neuromuscular recruitment activity during the fatiguing process (48;534). However, most of these studies have been performed on isolated human muscle or dissected or anaesthetized animal models. While these studies have provided the basis of understanding for neuromuscular recruitment pattern changes, the relevance of these studies to human exercising to fatigue is questionable. There are a number of factors that will affect the workings of the central nervous system which will include thermoregulation, electrolyte changes, substrate depletion, oxygen availability, haemodynamics and pH changes, to name but a few. These factors will depend on the conditions in which the exercise fatigue process occurs, such

as hot and cold environments, both depression and stimulation of the physiological system and the intensity of exercise performance. To date, few studies (285;481) have examined the effect of submaximal exercise fatigue on neuromuscular recruitment.

Accordingly, it is the aim of this thesis to investigate the role of neuromuscular recruitment in the fatigue process under specific conditions that stress the physiological system. In reviewing the literature on central, peripheral fatigue and the effect of different conditions on neuromuscular recruitment pattern, we have gained further understanding of the mechanisms involved. From this we have identified questions that need to be addressed for a more complete understanding of the role of neuromuscular recruitment in the fatigue process.

CHAPTER ONE

LITERATURE REVIEW

1.1 INTRODUCTION

The unpleasant variety of symptoms that are associated with intense fatigue has been the topic of research for many years. The paucity of the literature and data is conflicting and sends out mixed messages, often clouded by the adopted paradigms of different researchers. It is this uncertainty that poses challenging questions for the physiologist as a result of the intricacy involved in determining the mechanisms responsible for the inability to sustain performance, as occurs in fatigue.

The majority of research has focused firstly, on the peripheral mechanisms of fatigue, rather than central or neural mechanisms and secondly, on isolated skeletal muscle, which tends to ignore the multitude of influencing variables existing whilst undergoing sustained dynamic exercise. To date, little research has concentrated on examining neuromuscular recruitment during dynamic submaximal and maximal exercise resulting in fatigue.

Accordingly, the aim of this literature review is to gain further insight into the mechanisms that affect neuromuscular recruitment during both dynamic submaximal and maximal exercise to fatigue. Consequently, it is necessary to research both central and peripheral fatigue and the different contributions of each condition that can affect fatigue in order to elucidate what the responsible mechanisms might be. However, the object was not to review the effect of each condition on exercise fatigue in its entirety, rather how the differing conditions influence neural recruitment strategies during exercise.

1.2 CENTRAL FATIGUE

Fatigue during exercise is a complicated occurrence that is affected by a multitude of factors. Historically, researches have mainly focused on factors that cause the dysfunction of the intramuscular contraction process (peripheral fatigue), with little regard for the function of the central nervous system (CNS). Most researchers who endorse the involvement of CNS in fatigue are usually unsuccessful in providing a reasonable explanation for the biological mechanisms and consequently describe the involvement as a "black box" occurrence that is hard to support (130). The evidence surrounding the origins of CNS fatigue is indeed nebulous and still under debate. It has been shown, however, that CNS fatigue results in post exercise depression of motor evoked potentials (MEP's), which originate from a reduced neural drive adjacent to the anterior horn cell in the spinal cord (70;71). Other investigators postulate that the frontal cortex and basal ganglia may also be involved in this process (435).

Noakes (408) has questioned the traditional models of fatigue and has proposed that a central governor, rather than a limited supply of oxygen and the accumulation of metabolites, is the major cause of fatigue during exercise. The central governor would force the exercising body to slow down to prevent any cardiac ischaemia, muscular rigour or cellular damage from occurring.

Noakes' hypothesis was born out of the traditional research, which has focussed on the function of maximal aerobic capacity (VO_{2MAX}) as the main

factor in causing fatigue during maximal high intensity endurance exercise. This model is based on the assumption that the capacity of the heart to pump large volumes of blood and oxygen to the working muscles determines termination of endurance performance. The muscles will then be allowed to achieve greater work rates before exhausting the oxygen availability and resulting in muscle anaerobiosis (30;406-408). Noakes (408), however, questions this model and illustrates that if oxygen utilization of working skeletal muscle is limited by the pumping capacity of the heart, then the first organ to be affected by any postulated oxygen delivery will be the heart. If indeed this were the correct mechanism, the reduction in coronary blood flow would subsequently reduce the heart's pumping ability, resulting in a cycle of irreversible and progressive ischaemia (408), which does not occur in healthy athletes (429). Noakes (408) then proposed that the limitation of maximal exercise is more likely to be a synchronized process via the CNS before the absolute maximum coronary blood flow and cardiac output are attained (see figure 1.1). Ironically, it was Hill et al. in 1924 (255;256) who originally suggested that there might be a mechanism acting as a governor by sustaining a high degree of oxygen saturation of the blood (408).

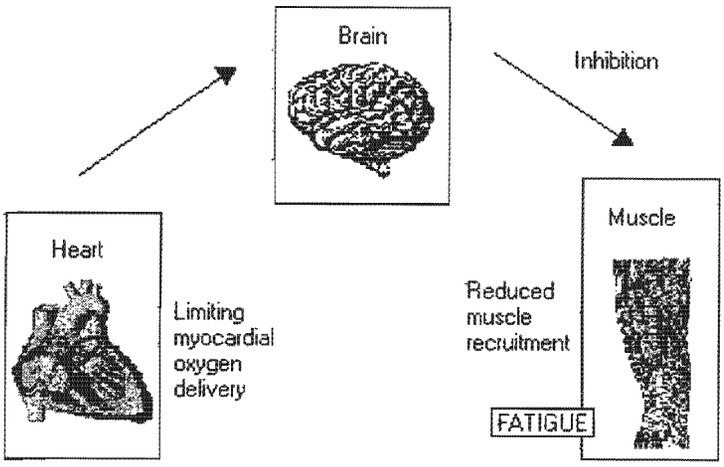


Figure 1.1. The central governor as postulated by Hill (255;256) and later defined by Noakes (408). Limiting myocardial oxygen causes a reduced activation of the skeletal muscle by the cerebral cortex.

Noakes (405) also proposes that the plateau observed in VO_2 during maximal testing is not a physiological consequence, but rather an artefact of testing protocols. Recent studies have shown that more than 50% of trained individuals do not reach a plateau during VO_{2MAX} testing and terminate exercise whilst VO_2 values are still increasing (11;145;480).

Noakes' hypothesis is supported by studies on skeletal and cardiac muscle performed at altitude, which suggest that for the heart or skeletal muscle to become deficient in oxygen during maximal exercise, its manifestation will be more recognisable during conditions of altitude when the available oxygen is less (407).

However, maximum exercise with increasing altitude results in a reduction in peak blood lactate (150), cardiac output and heart rate (486). Edwards (150) surmised that a protective mechanism was disabling the ability to accumulate large amounts of lactate at altitude by preventing an already low arterial saturation from decreasing further. Moreover, Kayser et al. (287) showed that with increasing altitude, skeletal muscle electromyographic (EMG) activity at peak exercise falls, but increases after oxygen administration. From this Noakes (408) then postulated that the heart possesses receptors, which ascertain the sufficiency of coronary blood flow and oxygen delivery. Before a predetermined limit is reached, an afferent signal is sent to the motor cortex, which then sends an efferent command to reduce skeletal muscle activation. The reduction in skeletal muscle activation therefore reduces myocardial demand for oxygen and consequently, prevents the risk of myocardial ischaemia.

Davis and Bailey (130) have defined CNS fatigue as failure to maintain the required or expected force output when the dysfunction could not be reasonably explained within the muscle itself. It could be suggested that the reduction in CNS drive to the motorneuron is due to a decrease in the efferent (corticospinal) impulses reaching the motor neurons and/or an inhibition of motor neuron activity by neural afferent command originating from the muscle. Most of the evidence suggests afferent feedback inhibition at the spinal cord level (130). This is supported by Bigland-Ritchie et al (49) who hypothesised that motor unit firing rates are inhibited from a reflex involving mechanoreceptor feedback or group III and IV free nerve endings which are

sensitive to muscle metabolism throughout fatigue. Similar to Noakes (408), Enoka and Stuart (158) have suggested that during fatigue the CNS attempts to optimise the maximal force produced by skeletal muscle to allow a safe and economical recruitment pattern to occur.

Direct evidence for decreases in central drive during fatigue is provided from studies that have shown the rate of discharge for primary motor cortex neurons often decreases during repeated isometric contractions (348). Moreover, transcranial magnetic stimulation (TMS) in humans has been used to determine CNS activity from the motor cortex to the alpha-motor neuron (71). The results of these studies showed that the level of motor responses generated in the muscle by TMS was briefly decreased after fatiguing exercise. It was then concluded that the decreased central drive probably involved the collection and reduction of the neurotransmitters in the CNS pathways that are situated before entering the corticospinal neurons. Furthermore, Rollnik et al. (436) showed that activation of the dorsolateral prefrontal cortex (DLPFC) is essential in central motor control. This study showed that activation of the DLPFC using rapid rate repetitive TMS may induce motor evoked action potential depression. Thus, these authors concluded that the DLPFC is able to reduce motor cortex activity. Moreover, Taylor et al. (490) studied supraspinal fatigue during sustained MVC by examining the EMG responses to TMS changes. This study showed by a series of intermittent MVC's that there is a supraspinal component to central fatigue, however the authors did conclude that it could not fully account for the failure of voluntary fatigue.

1.2.1. Chronic fatigue

There have been a number of studies performed on individuals who suffer from chronic fatigue. These studies showed minimal decrements in maximal force production for both voluntary contraction and electrical stimulation in comparison to healthy age matched controls (28), despite having marked symptoms of fatigue at rest (201;292;326). It has been shown that the amount of corollary discharge from the motor cortex that projects to the primary somatosensory cortex is the main influence for sense of effort in humans (158;353), and may be altered in individuals with chronic fatigue. Results from studies with chronic fatigue sufferers (CFS) have shown that there is an elevation of the serotonin metabolite 5-HIAA, when compared with normal subjects (135). Also, a possible upregulation of 5-HT receptors in CFS patients has also been suggested (20).

1.2.2. Serotonin

The prime intermediary of CNS fatigue is serotonin (5-hydroxytryptamine [5-HT]). A rise in brain 5-HT enhances sleepiness, lethargy, muscular fatigue and perceptions of effort (543). It has been suggested that increased concentrations of brain 5-HT can cause deterioration in exercise performance by impairing CNS function (398). Elevated brain 5-HT synthesis occurs in reaction to an up-regulated delivery to the brain of blood-borne tryptophan (TRP), an amino acid precursor to 5-HT. This mechanism occurs because no

enzymes involved in 5-HT synthesis are saturated under physiological circumstances (398).

Table 1.1: Summary of studies examining brain 5-HT and fatigue

Author	Protocol	Key results
Barchas and Freemen (27)	3 hours of low intensity swimming of the rat	↑ brain 5-HT
Romanowski and Graiec (437)	90 mins of treadmill running	↑ brain 5-HT
Chaouloff et al. (98)	60 mins of treadmill running	↑ brain f-TRP, 5HIAA
Chaouloff et al. (97)	90 mins of treadmill running	↑ midbrain, hippocampus striatum 5-HT and 5-HIAA
Blomstrand et al. (54)	180 mins of treadmill running of the rat	↑ brain 5-HT, 5HIAA
Bailey et al. (19)	60 mins of treadmill running of the rat	↑ brain 5-HT, 5HIAA ↓dopamine

The studies shown in Table 1.1 display a positive relationship between exercise fatigue and increased brain 5-HT, but do not provide a causal link. The strength of the findings will remain questionable until methods are developed to directly measure CNS fatigue in humans during dynamic exercise (130).

Various studies have investigated the physiological mechanisms responsible for elevated brain 5-HT. Inhibition of the dopaminergic system by increased levels of serotonin could cause fatigue (19;97), thereby reducing motivation and arousal to perform strenuous exercise (543). Moreover, investigators have concluded that serotonergic activity may influence the hypothalamic –

pituitary-adrenal axis, pain, thermoregulation and mood in both humans and rats (3;5;170;544).

To study the effects of fatigue on regional brain concentrations of 5-HT and metabolites it is only ethically possible to use the rat model. However, Wilson and Maughan (533) used a 5-HT agonist, paroxetine, on human subjects which showed a reduction in exercise time in comparison to the placebo trial (533). Most studies, however concerning human subjects and central fatigue have focused on nutritional factors that effect availability of TRP to the brain, which could act as a marker of CNS fatigue (19).

1.2.3. Branched chain amino acids supplementation

Blomstrand et al. (53) (51;52) performed studies that investigated the lowering of branched chain amino acids (BCAA) during submaximal exercise. It was postulated that CNS fatigue could be delayed by BCAA supplementation in an attempt to lower the plasma free tryptophan (f-TRP) ratio and most probably 5-HT synthesis, due to a reduction in f-TRP transport across the blood brain barrier (129). Therefore, because f-TRP compete with BCAA's for the same transport locations across the blood-brain barrier, a decrease in this ratio will subsequently reduce the quantity of f-TRP entering the brain, consequently limiting 5-HT synthesis (398). Accordingly, Blomstrand et al. (53) supplemented athletes with BCAA before and during sporting events, and showed improvements in performance. However, these studies have invited wide spread criticism and results have limited value due to lack of

experimental control. Varnier et al. (508) and Verger et al. (510) have also investigated BCAA supplementation under controlled laboratory experiments and have showed no improvements. Nevertheless, Davis (129), suggested that the negative effects of ammonia accumulation on both brain and muscle function, could counteract the potentially beneficial effect of BCAA supplementation on brain 5-HT.

1.2.4. Carbohydrate supplementation

Fritzsche et al. (186) recently studied prolonged moderate intensity exercise in a warm environment, which showed that the ingestion of water combined CHO delayed the onset of fatigue to a greater extent than ingesting plain water. The ingestion of CHO alone did not have any effect on fatigue development. These results indicate a higher level of central drive when water alone and water with carbohydrate are ingested.

Noakes (408) also supports this theory by questioning the energy depletion model. Few of the studies that claim carbohydrate loading provides athletes with an ergogenic effect were conducted with an adequate placebo group. Of the two trials that satisfied these requirements, improvements in performance were not significant (83;241). Interestingly, the fast reversal of hypoglycaemia by carbohydrate ingestion allows exercise performance to continue (105) even though it would take considerably longer to convert the carbohydrate into readily available glycogen. These studies indicate that liver glycogen depletion is a form of energy reduction that can reduce the fatigue threshold, which

demonstrates the likely involvement of a central neural mechanism, possibly by glucose concentrations acting directly on a "fatigue centre" in the brain (see section 1.1.).

1.2.5. Dopamine

Dopamine (DA) is another neurotransmitter investigated for its possible role in CNS fatigue. Investigators have shown a decrease in whole rat brain DA metabolism post fatiguing exercise (19). Further analyses have shown that DA metabolism is increased during exercise in the hippocampus, striatum, midbrain and the hypothalamus (96;251). Previous investigators have discovered increased levels of DA by the ingestion of amphetamine to rats (38;199;251). Furthermore, Bailey et al. (19) suggested that fatigue in the rat is related to a reduction of dopamine metabolism in the midbrain and brain stem and when dopamine synthesis is maintained fatigue becomes delayed. Chaouloff et al. (95;99) hypothesized that increased brain DA activity may assist in delaying fatigue due to its capacity to alter brain metabolism and inhibit 5-HT synthesis.

Parkinson's sufferers are known to have a deficiency of DA in the brain and are consequently treated with Levodopa, a naturally occurring amino acid that forms an intermediate stage in the synthesis of catecholamines, including dopamine, adrenaline and noradrenaline. Ziv et al. (548), showed enhanced skeletal muscle fatigue during motor performance in patients with Parkinson's disease. Their study showed a significant reduction in the fatigue status after

the administration of Levodopa. It was concluded there was a possible relationship between Parkinson's disease fatigue and central dopamine deficiency. This finding indicates an important association between DA and fatigue.

1.2.6. Acetylcholine

Another neurotransmitter that may be involved in CNS fatigue is acetylcholine, which is essential for the generation of muscular force as it is the main neurotransmitter at neuromuscular junctions (130). Within the CNS, acetylcholine has been associated with temperature regulation and memory awareness. Similar to 5-HT, the availability of the precursor choline determines the rate of synthesis for acetylcholine. It has been postulated that fatigue throughout prolonged exercise may be instigated by a decrease in cholinergic activity as a result of depletions in choline availability (110;111;453;539). However, researchers have shown conflicting results. A field study on athletes supplemented with plasma choline citate showed the time for them to complete the Boston Marathon was significantly faster (110), whereas, Spector et al. (476) investigated choline supplementation in the laboratory and found no improvements in time to exhaustion and no depletion in serum choline. From these studies it can be concluded that further research is needed before the role of acetylcholine in fatigue can be clearly defined.

1.2.7. Cytokines

Fatigue related to viral infections could be a consequence of one or more substances released from immune cells called cytokines. Exercise tolerance is reduced during acute infection and the convalescent phase (125;184). These cytokines have been shown to influence fatigue at the CNS level (238;327;328;340). Although the precise mechanism for performance decrements when undergoing a viral infection are not known, lack of available metabolic substrates, lactic acidosis or depleted fuel stores do not appear to be the limiting factors (292). In addition, Smith (472) proposed the cytokine hypothesis of over training. It was suggested that over training could induce a musculoskeletal response that begins as micro trauma, develops into local acute inflammation, then into local chronic inflammation where it evolves into a systemic immume/inflammatory response. This immune/inflammatory response entails activation of circulating monocytes, which could synthesize large amounts of inflammatory cytokines that act on the CNS and induce a variety of "sickness" type behaviours such as depression or reduced appetite that will force the body to slow down and allow recuperation to take place. However, further research is needed in this field to test this hypothesis before any conclusions can be made.

1.2.8. Ammonia

Bannister and Cameron (24) studied the effect of ammonia during exercise. Ammonia is released into the blood by skeletal muscle during exercise and is

known to be toxic to the brain (24). Elevated levels of ammonia can change CNS function by firstly, acting on certain brain centres (428) or secondly, by altering the brain membrane permeability to designated amino acids that are precursors to neurotransmitters, or thirdly, by changing reactions involving glutamate and a-ketoglurate and their consequent impact on the metabolism of neurotransrnitters (24). Increased blood ammonia accumulates quickly in the brain and is produced throughout both submaximal and maximal exercise (24). It is hypothesised that the increase in ammonia emanates from stimulation of the purine nucleotide cycle, which is primarily found in the type II skeletal muscle (369). It is possible that fatigue during exercise can induce a condition of ammonia toxicity, which although reversible and transient, could be acute enough in parts of the CNS system to inhibit performance (24). Conversely, significantly higher levels of ammonia have been found in a high glycogen human leg compared to the low glycogen contralateral limb during the fatigue of both legs, suggesting that it may prolong as oppose to shorten, the time to fatigue (55). However, even if the glycogen is depleted, it is unlikely to have a significant effect on nonrobic metabolism (375). Also, Guezennec et al. (221) showed higher levels of ammonia and decreased gamma-aminobutyric acid (GABA) and glutamine levels in trained compared to untrained rats at exhaustion. It was concluded from this study that enhanced brain ammonia throughout exercise stimulates glutamine synthesis as a detoxication mechanism. It was also suggested that a relationship exists of central command between cerebral changes in neurotransmitters and excitatory amino acids, such as glutamate and GABA, and central fatigue. Also, Snow et al. (474) first showed that plasma ammonia and muscle

ammonia accumulation were higher whilst exercising at 40°C in comparison to exercise at 20°C. However, the author's concluded that the metabolic alteration could be related to reduced performance during exercise at 40°C as opposed to ammonia toxicity per se. Second, Snow et al. (473) showed that carbohydrate ingestion reduced the amount of muscle ammonia production from amino acid degradation, which suggests that ammonia will contribute to fatigue during hypoglycaemia. However, the amount of research involving exercise and ammonia and its mechanistic properties remains limited, therefore further study surrounding this topic is required before firm conclusions can be made.

1.2.9. Heat

It is possible that the fatigue occurring during exercise in hot environments is likely to be influenced by a reduction in central drive as this type of fatigue cannot be interpreted by any other mechanism (408). It has been suggested that rather than circulatory failure, it is core temperature that is the critical factor limiting exercise capacity in the heat (192) and that each athlete can store only a limiting amount of heat before being forced to decrease exercise intensity or completely terminate the exercise (400-402). Brown et al. (77) observed a decline in exercise duration at 80% of VO_{2MAX} when ambient temperature was increased from 20 to 35°C. They concluded that this was the effect of an increased temperature, resulting in an increase in the oxygen independent contribution to energy production. However, Noakes (408) concluded that there is no evidence from any studies of exercise to fatigue in

the heat that display an association with skeletal muscle energy depletion or "anaerobiosis". Noakes (406) surmised; "there is sufficient evidence to suggest that a reduced central nervous system recruitment of the active muscles terminates maximal exercise at high altitudes (and probably also at sea level)". The same mechanism is likely to terminate exercise in the heat when the body temperature reaches some limiting maximum and also when hypoglycaemia develops.

In support of this, Walters et al. (516) recently studied both hypothalamic (T_{hyp}) and rectal temperatures (T_{rec}) in moderate exercising rats in a ambient temperature of 35° C. Prior to exercise the rats were administered microwave irradiation to achieve preheated T_{hyp} of 41.5, 42.5 and 43.5°C. The main finding of this study was that the exercising rats fatigued at a T_{hyp} and a T_{rec} of 42.1°C and 42.4°C respectively, despite the difference in temperatures at the start of exercise. This study shows clear support for the hypothesis that a critical internal temperature limits submaximal exercise in the heat. Previous studies have also linked impaired physical performance with internal temperature (94;190;372;372;434;489). The two main methods used to examine the question of whether a critical exercise limiting internal temperature exists to limit performance is firstly, by altering the rate of heating throughout exercise by varying ambient temperature (190;236;489). This method is limited because the length of time needed to precipitate a significant rise in baseline temperature predominantly leads to the onset of confounding variables such as cardiovascular drift, electrolyte imbalances and dehydration that accelerate fatigue throughout exercise. The second method is by heating (187;190) or cooling (190;337) subjects prior to exercise in order

to alter the initial temperature. In Walters et al.'s (516) study the use of microwave irradiation avoided the problems of manipulating environmental temperature due to the brief period of time needed to pre heat the rats (3-8 min). As previously suggested, a critical temperature exists, which imposes a limit from which exercise is not possible. This mechanism has been proposed to protect the animal from attaining a lethal level of heat stress (94;208;434). It has been shown that rats will run to the point of heatstroke leading to death in studies designed to assess lethality of external heat stress (413). However, it is not only the temperature that decides the lethality of heat stress, but rather the thermal load, adjusted by the level of hyperthermia and the extent to which the exercise is continued (233). Walters et al. (516) concluded from their study that fatigue was reached before the onset of lethal thermal load, thus providing strong evidence that exercise is terminated when critical internal temperature is attained. In addition, the energy depletion model cannot account for termination of exercise as glycogen is not depleted by high ambient or internal temperature (65). Therefore, a central component must be operating.

In all these examples, reduced central recruitment of muscle would function to prevent organ damage. The effects of both hot and cold environments on neuromuscular function will be discussed in greater detail in section 1.4.1.

1.2.10. CNS involvement in force production

Evidence for CNS involvement in force production is provided from various studies. Enoka (155) showed that when strength training is performed on the muscles of one limb, the rested contralateral limb also increases its strength by 10-15% for both isokinetic and electrical stimulation (90;315). Narici et al. (393) showed that when a group of healthy subjects began a strength weight training programme, initial gains in strength were reported without any change in the cross-sectional areas of the muscles involved. It was suggested that training heavy resistance enhances the synthesis of protein, but there was no change in the cross sectional area of the skeletal muscle until the programme had been continued for a minimum of eight weeks (393). Other studies have shown that muscular strength adaptations are task dependent and increases in maximal voluntary contractions (MVC) do not transfer to comparable increases in other tasks (271;289). Finally, Yue and Cole (545) studied imagined contractions and showed that strength increases could be achieved without repeated muscle activation. These authors concluded that force gains appear to result from practice effects on central motor programming.

It appears that strength training affects the function of motor unit synchronization during force production. Isometric contractions reduce the coactivation of the antagonistic muscle and also enhance activation of the synergist muscles (155). This function may occur at the level of descending drive from supraspinal centres (265;392)

Koh et al. (298) have illustrated one of the most convincing mechanisms for neural involvement during maximal contractions. This study showed that when a fatiguing muscle starts to decrease force output, the homologous muscle in the contralateral limb becomes concurrently activated. This is also known as the bilateral deficit, the magnitude of which is approximately 10% (298). This deficit can be altered with training, however, and subjects who exercise both limbs concurrently usually display a bilateral facilitation, which equal activation in both limbs as oppose to a deficit (260).

1.2.11. Diaphragmatic fatigue

CNS involvement in exercise fatigue is also shown in diaphragmatic fatigue studies. Bellemare and Bigland-Ritchie (32) induced fatigue of the diaphragm by administering bilateral shocks to the phrenic nerves between breaths and during voluntary contractions to detect the reduction in central drive. Evidence of fatigue was shown by the failure of subjects to achieve either 50% or 75% of maximal transdiaphragmatic pressure (Pdi), which concurred with a decline in the central activation ratio. Although there was no impairment in neurotransmission it was concluded that half of the fatigue was caused from a reduction in central drive while the other half was caused from peripheral muscle contraction failure. In addition, Luo et al. (336) recently examined the effect of diaphragmatic fatigue on neural respiratory drive. In this study subjects were instructed to swallow (via the nasal passage) an EMG electrode. This electrode was designed to measure neural recruitment during respiratory function. EMG measurements were taken during inspiratory

resistive loading, which involved subjects inspiring through an inspiratory resistance at maximal effort. The results showed both reduced EMG amplitude and Pdi. The authors suggested that this occurrence could be resulting from reduced central drive to the diaphragm to serve as a protective mechanism to avoid severe peripheral fatigue (336). These studies provide some interesting evidence to the involvement of CNS in the exercise fatigue process, however further research is needed to determine the involvement of diaphragmatice fatigue during exercise fatigue as opposed to just inducing diaphragmatic fatigue on static subjects.

1.2.12. Muscle reflex and CNS control of the neuroendocrine system, glucose homeostasis and haemodynamcs

Recently, Vissing (512) produced some interesting findings and hypotheses involving muscle reflex and central motor control of neuroendocrine activity, glucose homeostasis and circulation during exercise. Previous studies (370;475;518) have suggested that during exercise the regulation of glucose homeostasis is governed by neural control mechanisms that are unrelated to depleted glucose availability (512). It has been proposed that increasing neural drive with increasing work-loads could be related to increased afferent command from mechano- and chemoreceptors in the working muscle or could be associated with augmented action in the motor centres of the brain that stimulate neuroendocrine activity in accordance with contracting skeletal muscle (512). Vissing (512) performed a series of animal studies on hypothalamic regions, which showed that motor centres situated in the brain

might instigate metabolic and hormonal responses throughout exercise by the CNS. During exercise the activity in these regions could be integrated in the cortex, which transfer to the hypothalamus in conjunction with the excitation of muscle activity, therefore providing a concurrent drive of the autonomic and somatomotor circuitry.

Vissing (512) described that static muscle will cause reflexes to increase sympathetic outflow to the chromaffin cells in the adrenal medulla. It was suggested that the mechanoreflexes caused this reflex, which in turn stimulate group III and IV muscle afferents, resulting in glucoregulatory hormone and glucose production changes similar to that seen in prolonged submaximal exercise (512). From this Vissing (512), went on to form the hypothesis that there is a neural feedback regulation of neuroendocrine responses and fuel mobilization from chemo and mechanosensitive receptors in the contracting muscle. If this mechanism exists, fuel can be utilized instantaneously at the commencement of exercise by mechanical response from the contracting muscle, and can be altered throughout prolonged exercise to the energy requirements of the exercising muscle by signals from chemosensitive nerve endings in the muscle (512). This hypothesis of feedback regulatory control helps to justify the ideas by Ulmer (497) on "teleoanticipation" (see section 1.2.16.3).

1.2.13. Perceived exertion

Perceived exertion is a human effort response related predictably to stimulus intensity (409). The standard measurement for perceived exertion that is widely used by exercise scientists and clinicians alike during exercise is the Borg scale (67). This rating is thought to measure an active process in which many external and internal inputs are arranged in the cerebral cortex (409). There are two different types of RPE scales: first, the Borg 6-20 scale (66), which is largely used with exercise protocols and is based on linear assumption of parallel increases in HR, VO_2 and exercise intensity (66). Second, the Borg CR10 scale is designed to measure pain, which should increase according to a positively accelerating function (427) and therefore has been designed to increase curvilinearly. Indeed, in our laboratory Kay et al. (285) used the Borg 6-20 scale as one of the determinants of central drive when examining power outputs and EMG during a 60 minute cycling time trial. However, further study of RPE as a measure of central fatigue is needed before it can be widely used as an exclusive tool in this regard.

1.2.14. Motor unit recruitment

Throughout voluntary muscular contractions, muscle strength is increased by an additional recruitment of motor units and an increase in the firing rate of the already active units (160).

1.2.14.1. The size principle

Henneman et al. (249) defined "the size principle" by examining motor unit synchronization in the stretch reflex of the cat. These authors discovered that the smaller motorneurons had lower activation rates when compared to the larger ones, while Adrian and Bronk (1) determined that the contraction force was related to the motor unit action potentials. High recruitment threshold motor units have reportedly larger surface EMG amplitudes (319;345;346;373). However, studies illustrating these differences could only quantify motor unit action potentials on low force EMG recordings because of technical problems in analysing motor unit action potentials (MUAP) of high threshold motor units. Accordingly, a decomposition technique was established whereupon high threshold units were quantified (143). This technique is limited, however, (29;134) because it is dependent on the basic supposition that the same motor units are recorded throughout contraction (2). Akaboshi (2) found a relationship between the motor unit recruitment threshold and duration to be the same as the Henneman's size principle in EMG. Interestingly, different muscle groups possess different recruitment strategies producing muscle force. De Luca et al. (134) showed that in the first dorsal interosseous (FDI), nearly all motor units were recruited at a force of 50% MVC, after which the firing rate increased to obtain 100% MVC. In the rectus femoris (RF), however, changes in firing rates were not seen until 100% MVC. This was because new motor units were recruited until 100% MVC was reached (131). It can therefore be assumed that in contractions of 0 to 50% of MVC, it is predominantly type I fibers, with small diameters, that are

recruited in the RF, as both types are recruited in the FDI and consequently the MUAP is higher in the FDI than the RF. When comparing MUAP duration, the amount of muscle fibers that is governed by a single motor unit is an important consideration.

Burke (84) discovered a correlation between axonal excitability and motor neuron excitability in the cat and Kernall et al. (294) showed that type I motor units were activated first and type II units later at a higher threshold. Furthermore, motor units recruited in the early stage of the voluntary contraction have a smaller twitch tension than those activated at higher thresholds (1;374). The EMG MUAP replicates the size of the motor unit and displays a positive correlation with the recruitment threshold (483). From this Henneman (248) concluded that in conventional EMG recordings, motor units recruited later have larger MUAP amplitudes and longer durations than those units recruited earlier. Ertas et al. (160) questioned the size principle by performing EMG analyses with monopolar and concentric electrodes from the deltoid muscle. Their observations showed that there were only slight differences in the area and amplitude between the consecutively recruited motor units. Obvious differences were shown in the pooled values suggesting the Henneman's size principle is perhaps only relevant in pooled material. By observing the comparison of electrode uptake area relative to the location of motor units it is unlikely that the conventional EMG recordings will detect differences of total motor unit distribution and its territory. It is only when mean values for group EMG are analysed, can differences be observed. This then becomes relevant for conventional EMG analyses.

The power produced by a single motor unit is dependent on the tension and cross section of its muscle fibres and innervation ratio (85). Doherty and Brown (142) examined the twitch contractile properties of age related single motor units, which they activated by graded percutaneous electrical stimulation. The results of this study suggested an age related increase in pooled motor unit size and slowing of contraction speed (493).

In conclusion, Henneman's size principal of an orderly recruitment of motor units has been shown to occur with pooled motor units only, therefore is only relevant when analysing group EMG data. Further technique development is required for the tracking of individual motor units before any firm conclusions can be made for motor unit recruitment strategy during fatiguing exercise.

1.2.14.2 Muscle wisdom

"Muscle wisdom" has been described as being the decline in motorneuron firing rate (343), caused by a decrease in conduction velocity of the neural signal, which compresses the frequency content of the EMG signal (48;534). Marsden et al. (344) later defined "muscle wisdom" as being the sensitive adjustment of motor unit firing rates that utilize muscle fiber conduction velocities. Muscle wisdom is a central command to execute the most economical way to perform the contraction, whether it is at a submaximal or maximal fatiguing intensity. An example of this would be a high initial instantaneous firing rate to "kick start" the contraction, followed by a decrease

in the firing rate to minimize the onset of fatigue on the motor unit and benefit from the assisting increases in contraction times as its firing continues (112).

Sacco et al. (444) suggested that the possible cause for a reduced firing rate was firstly, a reduction in motorneuron excitation due to a diminished response from muscle spindle afferent signals. The diminished response may occur as a result of a declined fusimotoneuron discharge as the voluntary contraction progresses (228;229). Secondly, ionic conductances from the cell membrane have also been shown to decrease motorneuron excitability (295;296). Finally, evidence for an inhibitory reflex originating in the contracting muscle comes from studies on muscle fatigue with arterial occlusion. These studies have shown that the depression in EMG activity of plantarflexor and dorsiflexor muscles continues until the arterial cuff is released from around the thigh, despite the contraction being from voluntary or electrical contraction to induce fatigue (196;534). Sacco et al. (444) concluded that the suppression of motorneuron excitement is from the accumulation of metabolites and/or the imposed anoxia.

Throughout high frequency fatigue it has been suggested that motor unit firing rates could be regulated by a reflex response within the muscle from fatigue-induced changes (49). As mentioned earlier (see section 1.2), it is also possible that group III and/or IV muscle receptors are responsible for the reflex afferent signal (48). Evidence for an inhibitory reflex has been displayed from fatiguing one muscle, which is followed by depression of EMG activity in a nonfatigued synergist. This serves to dampen motoneuron excitability

throughout fatigue (444). Windhorst and Boorman (534) have reviewed the various types of spinal interneuron and afferent fiber signals that could be involved in neural reflexes. Limb ischaemia has been shown to prolong the firing rate (538), which also suggests group III and IV involvement in response to metabolic and/or contractile changes within the fatigued muscle (242;360;440).

Magnetic stimulation of the motor cortex has showed that motor evoked potentials are maximal, even whilst central fatigue was present. It was concluded from these studies that motor drive was reduced due to the neural events "upstream" from the motor cortex (193).

In summary, muscle wisdom is the sensitive adjustment of motor firing rates that can utilize muscle fiber conduction velocities, from signals via both CNS and type III and IV afferents. It is the central command to execute the most economical way to perform the contraction.

1.2.14.3. Rate coding

Contractile force can be adapted by altering the firing rates of active motor units, which has become known as "rate coding" (112). Rate coding and recruitment are not independent and the association between these two mechanisms varies depending on the size of the muscle and type of contraction (112). An illustration of this comes from larger muscles such as the biceps brachii, whereupon motor unit recruitment operates over the

complete force range and rate coding will only come into effect towards the upper end of the spectrum (112;133). Furthermore, these studies have shown that in the smaller muscles, such as the adductor pollicis, at forces greater than 50%, there was no motor unit recruitment, with the majority of recruitment occurring below 30% MVC. At contractions higher than 50% further increases in force are entirely governed by rate coding (304).

Needle electrode EMG techniques can determine firing rates of single units, but this has been shown to be a difficult measurement (113). Although, individual motor unit twitch tension is significantly related to the motor units size, the tension/size relationship can be confused because of the varying depths of the motor unit action potential within the muscle when using EMG techniques. For example, a small shallow unit may have a large motor unit action potential, whereas a large deep unit may have a small motor unit action potential (112). Conwitt et al. (113), however, found that consistently sampling smaller motor unit action potentials is unreliable due to the recruitment of larger motor units and difficulty of the interference profile.

In summary, "rate coding" is the alteration of active motor units for the most economical means of force production. The relationship between rate coding and recruitment will largely depend on muscle size and type and the kind of contraction being performed.

1.2.14.4. Inhibitory reflexes

The excitatory drive from higher motor centres will primarily regulate the discharge patterns of higher motor unit centres as a result of voluntary effort (538). This neural response to voluntary effort may also change at supraspinal and spinal levels, by afferent signals from the contracting muscle (538). Throughout a sustained MVC, motoneuron rates decrease (44;49), which could largely result from processes within the CNS, since modifications in motorneuron excitability have been significantly shown (295). It has also been postulated that reflexes originating in the muscle, when responding to exercise fatigue, inhibit motoneurons (444). It has been shown that local ischaemia keeps the muscle in its fatigued state with no improvement of either contractile speed or force (526), which suggests that motoneuron rates are inhibited from a fatigued induced reflex somewhere in the muscle (538). Ischaemia is also known to precipitate transmission failure (482), as well as activating painful mechanisms in the legs. These factors may prevent the subject from making full maximal contractions during these conditions. Ischaemia is also known to cause failure of neurotransmission (482). The association of a reflex was originally proposed when investigators showed a decrease in firing rates throughout MVC, which was paralleled by a decline in muscle contraction speed (43;44). Interestingly, no MPFS depression was seen after a cat's skeletal muscle had been exposed to regular long-term stimulation. It was thus concluded that exercise training could improve impulse propagation efficacy as well as the function of other parts of the motor system (153). Kernell (293) also found that the long term electrical stimulation

fatigue test caused almost the same loss in force for deafferented and hemispinalized cats who received long term chronic stimulation and normal, untreated cats, regardless of the improvement in maintaining the M-wave amplitudes in the experimental group. Woods et al. (538) thus concluded that, firstly, the decrease in M-wave amplitude was as a result of changes in the muscle sarcolemma properties and not because of a neuromuscular junction blockage. Secondly, a significant M-wave amplitude decrease could occur at the muscle surface without altering force production.

Accordingly, it has been suggested that there is a large safety margin in the combination between mechanical and electrical responses of the muscle (538). In experiments on the adductor pollicis, it has been shown that recovery of MVC firing rates are prevented if the fatigued state of the muscle is preserved by local occlusion of blood supply and logically, when the blood supply to the muscle is restored, full recovery follows shortly (538). Blood supply restriction to the fatigued muscle averts MVC firing rates at a time when they have usually recovered, which again suggests the possible involvement of a reflex mechanism (538).

In conclusion, the majority of evidence, particularly studies that induced ischaemia, suggest the existence of a neural inhibitory reflex to voluntary effort at supraspinal levels by afferent signals from the contracting muscle. It is likely that this inhibitory reflex is a safety mechanism to prevent the skeletal muscle from developing cellular damage.

1.2.14.5. Motor unit substitution

In addition to the Henneman size theory is the concept of motor unit substitution, which has been surmised as a way of offsetting the effects of fatigue (419;524). The theory behind this concept suggests that higher threshold motor units are recruited to replace lower-threshold units that have stopped firing, similar to motor unit rotation (452). Westgaard and DeLuca (524) showed that during isometric contraction of the Trapezius muscle, that low-threshold units showed periods of inactivity and were substituted by motor units of higher recruitment threshold. It was then proposed that the substitution phenomenon guards motor units from extreme fatigue when there is a requirement for continued sub maximal muscle activity (524). The main limitation of this study, however, was the use of the EMG decomposition technique. The reason for this limitation was that this method is reliant on a basic supposition that the same motor units are monitored throughout the contraction. The MUAP shape of each unit has to be the same in all four channels throughout the contraction to ensure that the location of the needle in relation to surrounding muscle fibers is steady so the same motor units can be followed (2).

In summary, motor unit recruitment is a controversial issue debated by many neuroscientists, and because of the intricacy and complexity involved in achieving accurate detection of motor units, conclusions are often based on supposition.

1.2.15. Neural control during isometric fatigue

Du Bois-Reymond first studied isometric voluntary contractions in 1849 (29). Most research studying neural contributions during muscular fatigue have used invasive or surface EMG techniques throughout isometric submaximal or maximal voluntary contractions, or by twitch interpolation techniques (29). Isometric contractions are the most controllable non-invasive method to isolate a muscle group and minimize motion artifact.

The limitation of using isometric contractions as a method for determining muscular fatigue is that the mechanisms occurring are different to those in dynamic activity. For example, during isometric contraction there is a restricted blood flow, often resulting in ischaemia, which can cause accumulation of metabolic byproducts and decrease in intracellular pH (347). The change in intracellular states can affect changes in muscle fiber conduction velocity and mean power frequency spectrum (MPFS) (347). Consequently, previous findings of electrical activity during isometric contraction are not wholly relevant when applying them to the contractile function of muscle during dynamic contractions.

1.2.15.1 Amplitude changes

Integrated EMG (IEMG) has been defined as a measure of the whole electrical signal sent to the muscle from the CNS (157;371). EMG studies using isometric voluntary contractions (137;381) showed that the amplitude of

myoelectric signals from surface electrodes increased progressively over time throughout sustained submaximal contractions. This was generally considered to be a function of additional motor units (MU) being recruited to compensate for the loss of contractility due to impaired fatigued MU's. However, during sustained maximal contractions, the same rise in EMG amplitude could not always be demonstrated (44;46;378;422). Moritani et al. (379) studied EMG amplitude and frequency on subjects for both 50% MVC and 100% MVC. The results showed a decrease in MPFS for both groups but this was accompanied by a rise and decline in IEMG during 50% MVC and 100% MVC respectively (see Figure 1.2). It has been postulated that in order to maintain force, the rise in IEMG during 50% MVC occurs because of a progressive recruitment of additional MU's, perhaps with type IIA and B fibers (379). It has also been shown that during submaximal fatigue, the increases in IEMG are paralleled by increases in the discharge frequency of the existing active MU's (425). It can thus be concluded that a muscle group comprised largely of type I fibers could adjust the force output by "rate coding" throughout fatigue (379). Alternatively, muscle glycogen studies have also shown a rise in IEMG throughout submaximal fatigue indicating that the glycogen content of types IIab and IIb fibers remain unaltered throughout the initial section of exercise. There are also decreases in type IIa and then type IIb recruitment, indicating a decrease in the fiber recruitment threshold force (514). The decrease in IEMG during 100% MVC may be from attempts to maintain a high MU firing frequency, resulting in impaired excitation contraction coupling, probably associated with the depletion of extracellular Na^+ depletion (45;151;378). It has been suggested that the reduced IEMG during 100% MVC may be an

attempt to obtain the optimal force by preventing peripheral transmission failure (42;43;47). This occurrence may be because of a "fail-safe" mechanism to protect the muscle from developing rigour as a result of a depletion of ATP (303;394).

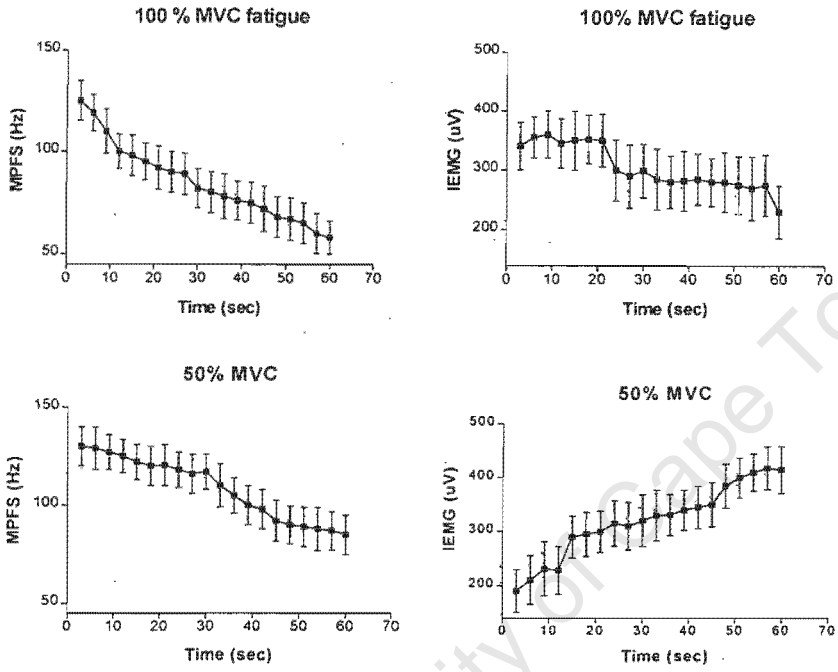


Figure 1.2. Time course changes of mean power frequency spectrum (MPFS) and integrated EMG amplitude (IEMG of surface EMG during sustained maximal voluntary contraction (MVC) for 50% and 100% intensity (means + SE) (379).

1.2.15.2. Frequency spectrum changes

In all instances of fatiguing isometric contractions a decline in MPFS is seen during the contraction, although the rate of decline is greater in maximal than submaximal contractions (42;230;300;383;422). This rate of decline for MPFS

may be related to the metabolic state of the fatiguing muscle (300;378;390). It was suggested that the decrease in MPFS was due to an accumulation of metabolic byproducts (383) such as lactate, which would cause a lowering of pH, thereby causing a reduction of membrane excitability, due to the sodium-potassium pump dependency on pH (275). This accumulation of lactate is thought to cause muscle fiber conduction velocity to decrease (74;132;363;383) resulting in a decrease in the spectral frequency (324;485). However, Brody et al. (73) electrically stimulated the hamster diaphragm, whilst decreasing the bath pH. This resulted in a significant decrease in both spectral power and muscle fiber conduction velocity (MFCV). The conclusion was then made that the MPFS and MFCV association was situational and reliant on specific biochemical changes. Furthermore, throughout a prolonged isometric contraction the change in MPFS was caused by more than just changes in pH and MFCV. Moreover, Linssen et al. (325) showed that MFCV did not decline throughout MVC in patients with McArdle's disease, when the spectral frequency had decreased. This would suggest that factors other than increasing lactate would influence MFCV. Finally, Naeijie and Zorn (388), Lindström et al (324), Stulen and DeLuca (485) and Hägg (232) have shown that an MPFS change may occur without a concomitant change in MFCV which also suggests that other mechanisms may be responsible.

MPFS is also affected by muscle temperature, by speeding up conduction velocity under hot conditions and slowing it down during colder environments, however this will be discussed in greater depth in section 1.4.1.

1.2.15.3. Electrical stimulation

Muscle fatigue induced by electrical stimulation is a useful tool to examine the electrophysiological occurrence related to force failure (291). EMG and electrical stimulation have been used as a means of elucidating the influence of central command during voluntary isometric contractions.

The three main methods used by previous investigators to determine changes in central activation are firstly, comparing changes in central activation ratio (CAR) pre and post exercise (291). The definition of CAR is:

$$CAR = \frac{\text{peak MVC}}{\text{Peak total force (peak MVC + electrical stimulation)}}$$

Secondly, is by examining the decline in voluntary force with the decline in tetanic force (45;364), by determining the change in MVC/tetanic force ratio. When this ratio decreases, it will reflect a decrease in voluntary force, thus indicating central activation failure. Finally, by comparing the change in IEMG during contraction with the change in the compound muscle action potential (CMAP). The CMAP amplitude is a measurement of the excitability of the muscle membrane and the transmission of the signal across the neuromuscular junction (189;198;371).

Kent-Braun (290) showed a 16% decrease in CAR and a difference of 15% between voluntary and tetanic fatigue. It could be estimated from these results that of the 78% decrease in MVC, 15-16% was due to central fatigue. Also,

the CMAP data displayed no failure of neuromuscular transmission, therefore it was suggested that remainder of the fatigue was due to intramuscular sources (290). There was a significant correlation between the rise in pH and IEMG, which would suggest a feedback loop between central motor drive and intramuscular metabolism throughout fatigue. Also, when comparing changes in CMAP and IEMG amplitude there was a significant reduction in IEMG with no decrease in CAMP. This finding indicates a reduction in spectral frequency as oppose to a decrease in motor unit recruitment (43;49). Kent-Braun (290) concluded that although intramuscular metabolic milieu ($[H^+]$) contributed to approximately 80% of the reduction in force, central fatigue was responsible for the remainder.

Electrically stimulated contractions recruit a higher proportion of type II fibers (26) when compared to voluntary muscle contractions. During short duration exercises where fatigue develops within 1-2 minutes, this is not a serious consideration as type II fibers are predominantly active during this time anyway. However, in electrically stimulated contractions lasting 15 mins (432), the recruitment shows a reversed recruitment pattern, which suggests that comparisons between voluntary contractions and electrically stimulated contractions are invalid (23).

In summary, the three main methods used to determine changes in central activation through electrical stimulation are firstly, comparison of changes in central activation ratio pre and post exercise, secondly the decrease in the MVC/tetanic force ratio will indicate the level of central activation failure and

finally by comparing the change in IEMG with the change in CMAP during contraction. Electrical stimulation should only be used for contractions of a short duration (1-2 mins), due to the preferential recruitment of type II fibers.

1.2.15.3.1. High frequency fatigue

It has been shown that fatigue develops during high frequency activation, more rapidly when compared to low frequency stimulation (197). The effect of high frequency stimulation could be from an impaired excitation of the muscle fibers (152).

To obtain maximum force from a muscle it is necessary to stimulate it at approximately 50 Hz, however the duration of the contraction has shown to be limited at this high level (269). To judge the loss of drive throughout electrical stimulation combined with voluntary contraction, choosing the correct frequency has become a complex decision (45). It has also become evident that the fatigue rate is reliant on stimulation frequency. Investigations of the electrical and mechanical responses to different stimulation frequencies have resulted in a number of high frequency characteristics being recognized. Firstly, the loss of force is accompanied by a reduction in amplitude and slowing of the muscle action potential waveform, secondly, the loss of force is intensified if extracellular potassium concentration ($[K^+]$) is increased or serum sodium concentration ($[Na^+]$) decreased. Finally, it has been shown that the loss of force is quickly reversed after decreasing the frequency (46;49;267). Jones (269) has hypothesised that these characteristics are a result of the

active muscle releasing K^+. This free K^+ accumulates in the extracellular spaces, thus preventing action potential propagation along the membrane surface. The slowing of the waveform and reduction of amplitude of the action potential, suggests that transmission across the surface membrane is influenced during high frequency fatigue, as the gross muscle action potential is affected by the surface, as opposed to t-tubular, electrical actions. It would be enticing to suggest that if extracellular $[K^+]$ were adequately elevated to avert conduction across the surface membrane, then the conduction across the t-tubules would be buffered, due to the increase in extracellular $[K^+]$. This $[K^+]$ would then be larger than the volume of the tubule will allow. However, Green (216) suggests that once the K^+ has accumulated in the intracellular spaces, it is then directed back into the cell and expels excess sodium ions (Na^+), which enter continuously during the action potential, back into the intracellular space. An electrogenic pump will control the re-establishment of the electrochemical gradients, which expends energy via ATP to pump both $[K^+]$ and $[Na^+]$ against their concentration gradients. Conversely, we conclude that studies on the role of K^+ within the t-tubules suggests otherwise (see section 1.3.2.4 on K^+ in t-tubules).

High frequency fatigue (HFF), caused by high frequency stimulation can result in large force losses, however it is debatable whether this is a normal fatigue occurrence. Available evidence indicates that the firing rates of individual motor units during prolonged isometric contractions commences at approximately 30Hz and then reduces as the contraction continues (49). However, most HFF studies will cause fatigue at a frequency of 50 Hz after

only 10 or 20 seconds of stimulation, which will show small changes in the waveform during stimulated MVC (269). However, a reduced amplitude continuing for a minimum of 10 minutes throughout recovery, post HFF, has also been shown (14;18;266;274) as well as in situ in animal studies (126). Furthermore, Matsunaga et al. (349) showed that through intermittent stimulation, the high frequency (100 Hz) fatigued less than the low frequency (20 Hz) stimulation. As mentioned earlier, the loss of force during HFF is quickly reversed after the contraction and inducing intermittent stimulation with regular rest periods will allow this recovery to occur.

It has previously been shown that there is typical failure of the muscle action potential at 50 Hz stimulation, while showing minimal changes in the action potential waveform throughout MVC. This indicates that HFF is not relevant during normal contractions. Jones (269) studied the reduction in performance of the tibialis anterior when stimulated at 30 Hz. The results of this study were similar to that of HFF of stimulated shortened muscle. Conversely, it has also been reported that HFF occurs throughout stimulation frequencies of 30 to 40 Hz (33) when Jones (269) categorised 30 Hz as low frequency fatigue. Jones (269) concluded that there is little evidence to suggest that HFF is a significant cause of fatigue. However, the contributing mechanisms warrant further examination to develop a greater understanding of peripheral fatigue. Interestingly, it has been shown that from HFF, muscles with a higher percentage of type II fibers display larger changes in muscle action potentials during and after contraction (275;302;307).

By using in situ muscle preparations, a significant reduction has been shown in M-wave amplitude throughout and immediately after electrically induced fatigue in muscle (46;126;366;454). These effects are significantly higher and the recovery of twitch amplitude faster during HFF. Moreover, changed M-wave formation with reduced amplitude occurred after HFF, but not low frequency fatigue (LFF) in both type I and II muscle fibers (18). These results support those of Darques and Jammes (126), who performed studies on TA rabbit muscle. These studies also showed a significant decline in M-wave amplitude during and after HFF, whereas LFF showed minimal or non-existent changes. Furthermore, it has previously been shown that changes in the muscle action potentials are more predominant in type II than in type I muscle fibers (18;275).

Finally, Baider et al. (18) have proposed another mechanism that suggests that calcium enters into the muscle after HFF trials for both TA and SOL muscles, and that the size of calcium influx is proportional to the reduction in M-wave amplitude. Accordingly, this represents an exclusive biological change, which may cause M-wave changes post HFF.

HFF characteristics can be summarized as being responsible for impaired excitation of muscle fibers and large force losses, however it is uncertain if this is a normal fatigue occurrence because of minimal changes in action potential waveform and muscles may not have such high frequency physiologically. Using HFF in laboratory conditions to study fatigue can be

advantageous due to the quick recovery periods the level of stimulation allows.

1.2.15.3.2. Low frequency fatigue

LFF is considered to occur during submaximal fatigue and has been characterised by (i) slow recovery after LFF, (ii) this effect continues without large electrical or metabolic disturbances to the muscle, and (iii) LFF affects force to a greater extent compared to HFF (269). However, fatigue can also be caused by a number of other types of activity, reflected by a loss of force when using low frequency stimulation, but is not necessarily caused by LFF. The characteristic of LFF, which is set aside from all other forms of fatigue, has generated interest. It has been speculated that the cause of this phenomenon could be from damage to the muscle fiber structure and the excitation-contraction coupling mechanism (268). Moreover, it is believed that this kind of fatigue is caused by activity that forces the exercising muscle to be stretched while simultaneously contracting (397), or during isometric contractions when the muscle is extended at a long length (270). Jones (269) concluded that this slow recovery is because of a procedure of renewal and repair, the duration of which is determined by protein turnover rather than metabolite resynthesis. Through direct measurements of intracellular Ca^{2+} from a specified stimulation frequency, it has been shown that there is a decrease in Ca^{2+} in the fatigued muscle fibers (523). The authors conclude that there was no evidence of changed intracellular buffering of calcium and the association between intracellular Ca^{2+} and tension. This suggests, that the

cause of LFF was from a decreased release of Ca^{2+} from the sarcoplasmic reticulum as opposed to reduced Ca^{2+} binding to tropinin.

The traditional potassium hypothesis for exercise induced fatigue is defined as potassium loss from the muscle, which accumulates in the interstitial space and consequently obstructs the excitation-contraction coupling procedure (21;92;470). Therefore, fatigue induced alterations in the M-wave could be caused from the transformation in extracellular ionic composition (18). Nevertheless, reduction in excitation induced activation of the Na^{+}-K^{+} pump could be a feedforward phenomenon that protects the functioning membrane in the active skeletal muscle. Furthermore, it has been shown that during LFF in rabbit hindlimb muscles an exogenous increase in extracellular potassium was produced which reversed the fatigue (227).

In conclusion, LFF takes on more similar characteristics than HFF to submaximal fatigue, but with a greater level of muscle damage and longer recovery periods, which makes repetitive low frequency stimulation for research on fatigue difficult.

1.2.16. Exercise protocols

An array of exercise protocols has been used to determine the cause of fatigue. The selection of the protocol will be based on the type of fatigue the investigator wishes to instigate and the information they wish to gain from it.

1.2.16.1 Maximal intermittent sprint activity

High intensity exercise has been investigated for many years in an attempt to determine the cause of fatigue. Fatigue at high intensities has been suggested to be related to hydrogen and inorganic phosphates (391;528). Consequently, these changes cause muscle excitation-contraction coupling and muscle action potential propagation, resulting in muscle fatigue from mechanical manifestations (42;378;380). To effectively study fatigue at high intensities, investigators have chosen to use intermittent sprint protocols to display the accumulative effects of both central and peripheral fatigue.

1.2.16.2. Fixed intensity endurance exercise

Laboratory protocols that have employed the use of exercise time to exhaustion at a fixed submaximal workload appear to have poor reliability and do not represent competitive situations where athletes have to use a pacing strategy to complete the distance economically (458).

1.2.16.3. Prolonged submaximal exercise of set duration

Most previous research trials on fatigue during submaximal exercise have used set duration (closed loop) exercise protocols to determine the metabolic cause of fatigue. The main cause of fatigue has been considered to be due to a lack of substrate availability, such as muscle glycogen (36;68;83;105;118;120;173;496).

However, Burke et al. (83) showed in a placebo-controlled 100-km stochastic cycling time trial, that there was no difference in total muscle glycogen utilization, time to complete the ride and the mean power output. In this study, the placebo group were fed a normal diet whilst the experimental group were loaded with additional carbohydrate, as opposed to examining actual carbohydrate depletion per se. This indicates that the effects of carbohydrate loading failed to improve cycling performance. Nevertheless, there have been a number of studies that have shown that glycogen depletion is not the limiting factor in submaximal exercise. Coyle et al. (119) showed that exercising athletes had the same glycogen content after 3 hours as they had at exhaustion 1 hour later.

When using the closed loop model, subjects are aware of the time, duration and number of high intensity sprints to be performed throughout the trial. Experienced cyclists intuitively know how to "pace" themselves throughout the trial to ensure the most economical ride in the fastest time possible. Ulmer (497) has suggested that a "central programmer" calculates the necessary speed required to complete the sprints and endurance activity in the most efficient way. This may be made possible by afferent command signals to skeletal muscles controlling temporal pattern of motion and metabolic rate by adjusting power output. This regulation, also known as "teleoanticipation", would then allow the individual to successfully complete the activity without damage to the cellular structures (Figure 1.3).

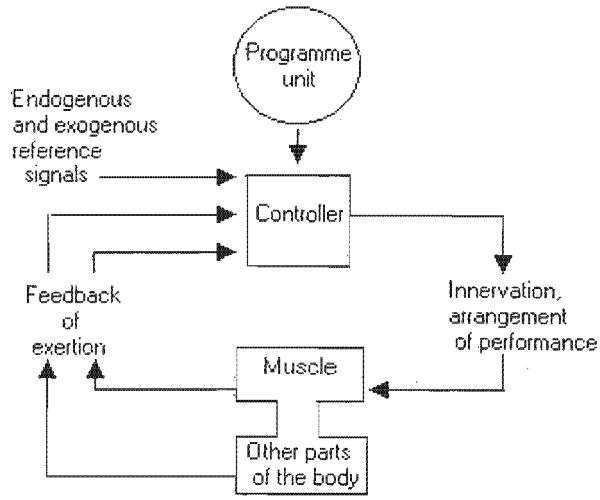


Figure 1.3. Hypothetical model of a control system for optimal adjustment of performance during heavy exercise, including teleoanticipation by a programmer as proposed by Ulmer (497).

Kay et al. (285) showed that during one hour of stochastic cycling, IEMG activity and power output decreased during sprints two to five, out of six in total. However, both IEMG activity and power output increased during the final sprints, demonstrating the possibility of a central command mechanism that was controlling power output changes. This finding indicates that the decrease in IEMG could not be exclusively explained by conductivity, temperature or electrode changes that may have occurred during the trial.

1.2.16.4. Prolonged incremental submaximal exercise of undetermined duration

Unlike closed loop protocols, many of the submaximal exercise protocols of undetermined duration (open loop) do not require pacing strategies to complete the task. The investigator will usually dictate the cadence and power output the subject has to perform. Open loop protocols are often used for obtaining maximal power output values by means of an increasing ramp of intensity (240). Maximal protocols such as this are invariably blind, so the subject will not be able to aim towards a goal power output, thus enabling them to fatigue at the point of severe discomfort only. It could be argued that this method has a comparatively reduced level of central drive, as there is no incentive to achieve true maximal power. However, most investigators will verbally encourage the subjects to push as "hard as possible" which has been suggested to be an incentive. Conversely, Moffatt et al. (377) showed that verbal encouragement only had an effect on untrained subjects for attaining VO_{2MAX} and that no significant differences were shown in trained athletes. Therefore, it becomes questionable when using open loop protocols, if subjects are actually achieving their true maximal power output compared to a race situation where peer competition and or monetary reward provide real incentive.

1.3. PERIPHERAL FATIGUE

Peripheral fatigue has been defined as the decrease in force generation capacity of the muscle due to accumulation of metabolites in the presence of an increasing motor drive or motor command (488). Bigland-Ritchie and Woods (48) identified the main factors involved in peripheral fatigue as being excitation–contraction coupling failure, failure of the muscle contractile mechanisms, sarcolemma excitability or metabolite accumulation. Another factor contributing towards peripheral fatigue is substrate depletion such as low blood glucose/hypoglycaemia and muscle glycogen.

1.3.1. Excitation–contraction coupling and contractile mechanism

Although excitation-contraction (EC) failure is not an isolated mechanism, it has been traditionally recognized as one of the many causes of fatigue in skeletal muscle. There are a number of processes involved in this mechanism.

Within skeletal muscle, the contractile apparatus that causes the development of force is governed by the intracellular concentration of calcium ($[Ca^{2+}]$), which is initially effected by the release and re-uptake of Ca^{2+} by the sarcoplasmic reticulum (SR) (310). Throughout the EC coupling process, action potentials depolarise the t-tubular system, thereby activating voltage sensor molecules. This activation causes the opening of the ryanodine

receptor/Ca^{2+} release channels in the adjoining SR membrane facilitating the efflux of Ca^{2+} into the cytoplasm (359).

Lamb et al. (311) postulated that fatigue continuing after LFF maybe a result of the Ca^{2+} dependent uncoupling process causing a reduction of Ca^{2+} release. Moreover, it has previously been shown that high intracellular [Ca^{2+}] results in uncoupling regardless of whether ATP is present or not. Nevertheless, Booth et al. (64), showed that prolonged exercise fatigue reduced muscle Ca^{2+} uptake, however this did not cause a slower relaxation of evoked contraction.

Westerblad et al. (523) showed that the resting [Ca^{2+}] in mouse type II fibers remained elevated by ~40% for more than 30 min after the induction of LFF. It was suggested this was a result of a 2-fold decline in SR pumping rate of [Ca^{2+}] and ~2.5 fold elevation in SR Ca^{2+} leakage. In another study, following extended exercise in rats, the Ca^{2+} uptake of the SR from both type I and II muscles decreased by approximately 20 to 40% without alteration in SR ATPase activity (119). It was concluded that Ca^{2+} transport during prolonged fatiguing exercise by the SR ATPase may become 'leaky' or uncoupled. The large [Ca^{2+}] reached throughout activity may have caused a long enduring elevation in the rate of Ca^{2+} leakage from the SR, developing into a reduced net Ca^{2+} uptake ability and an enhanced resting [Ca^{2+}] (310).

Lamb and Cellini (310) induced large release of Ca^{2+} into the SR, which resulted in a large force response of skinned skeletal muscle fibers of the rat,

depending on the amount of Ca^{2+} and the quantity and type of Ca^{2+} buffering present in the cytoplasm. However, the generation of high force levels were dependent on the Ca^{2+} signal being translated through the regulatory proteins, tropomyosin and tropinin, leads to a transformation of actomyosin from a weak binding to a powerful and strong binding, force producing condition (384).

During dynamic activity in particular, it is clear that all excitation and contraction processes must be well synchronized. It is also important that contraction processes are able to interpret high frequencies of impulse activity at each level, and predominantly produce a mechanical reaction consistent with the purpose of central command. The metabolic pathways differ in the rate at which ATP can be synthesized and subsequently are specialized to provide the energy needs to a specific action. During single repetitions of intense contractile function, such as the hydrolysis of phosphocreatine, this function will act a major source of regeneration of ATP from ADP (158).

The t-tubular system and sarcolemma are often the proposed sites for the development of fatigue (463). The fatigue development is considered to be causally associated to changed rise in $[K^+]$ (174;311;356;358;462).
The scale of these changes will depend largely on the intensity, contraction mode and duration of each contraction. There are several different theories of processes linking the loss of K^+ from a working muscle. Similarly, the effects of changes in $[K^+]$ on electrical properties are multifaceted, and as yet, there

are no straightforward associations between force development and $[K^+]$s (for review see (463)).

In conclusion, EC failure could be caused by firstly, a failure of the coupling mechanism between calcium release and action potential, secondly, failure of action potential transmission along the t tubular system and surface membrane or thirdly, a failure of calcium regulation at the point of the contractile elements (269).

1.3.2. Sarcolemma excitability

Alterations in sarcolemma function may induce muscle fatigue by inhibiting and/or preventing cell activation (322;468). This is caused by K^+ efflux and inhibition of the Na^+-K^+ pump resulting in reduced action potential amplitude, cell depolarisation and in some cells total inactivation (173). Cell depolarisation may provide a safety mechanism, which acts to protect the cell against Ca^{2+} accumulation and ATP depletion (151). The prevention of activation of ATP-utilizing steps and the increases in intracellular Ca^{2+} are both caused by the uncoupling of activation at the initial steps of EC coupling rather than the cross bridge cycle. The cross bridge cycle may stimulate phospholipases and Ca^{2+} release, resulting in the disruption of intracellular organelle and the sarcolemma (12). However, it has been shown that moderate increases in $[K^+]$ may contribute to force development, but at increasingly higher $[K^+]$ (i.e., higher than 8-10 Mm), the force becomes reduced (8;69;91;102;104;313;430). Interestingly, Sejersted and Sjogaard

(463) questioned the hypothesis that K^+ is the major determinant for muscle fatigue, as these high $[K^+]$s are not considered to occur during exercise. Nevertheless, $[K^+]$ should not be viewed in isolation, as a sequence of changes transpires in the in vivo situation, which may support changes in $[K^+]$, resulting in changes in force production (463).

1.3.2.1. Potassium and cell depolarisation

Exercise results in an increase of plasma potassium concentration ($[K^+]$), caused by a net efflux of K^+ from the contracting muscle (468). Consequently, this causes a change in the intracellular-to-extracellular $[K^+]$ gradient, which has been suggested to contribute to muscular fatigue by depolarising single muscle fiber membranes, thereby slowing the force generation ability of the muscle. The Na^+-K^+ pump of the sarcolemma not only resists the passive K^+ and Na^+ fluxes across the cell membrane (39;103) but because of its electrogenic nature, also assists the membrane potential of the skeletal muscle (253).

Skeletal muscle contains a resting membrane potential (V_m), which is predominantly a K^+ dependent potential (258). Therefore any alteration in the K^+ concentration, or conductance gradient across the sarcolemma, will influence V_m. Fatigue in both frog and mammalian muscle is related to an increase in chlorine (Cl^-), Na^+, and water concentrations and a reduction in intracellular potassium ($[K^+]$) (171). Water and ion shifts are greater in type II, than type I fibers and augments electrical stimulation (484). Investigators have

shown that fatigue could be related to alterations in the K^+ gradient across muscle cells and quick recovery after brief rest periods may represent partial reinstatement of the latent membrane potential (322;323;468;471).

In conclusion, the exercise-induced increase in extracellular [K^+] could have predictable effects on the polarization and, ultimately, excitability, of the muscle membrane. The K^+-induced depolarisation of the muscle membrane and subsequent breakdown in EC forms the foundation of the K^+ muscle fatigue hypothesis (468).

1.3.2.2. Sodium-potassium pump

It has been hypothesised that the sarcolemma Na^+ -K^+ pump is unable to sustain the ionic incline for Na^+ and K^+ that is required for the preservation of cell excitability and the V_m. (323;469) at high exercise intensities. However, it has been suggested that the Na^+ -K^+ pump may be restricted by inadequate quantities of ATP (461). Throughout exercise, a combination of catecholamine release, contractile activity and increases in intracellular [Na^+] and [K^+], excite the Na^+-K^+ pump. This increase in [Na^+] and [K^+], shows that the pump capacity is inadequate, otherwise the intracellular [Na^+] and [K^+] would be effectively pumped out to preserve both cell excitability and V_m. Furthermore, the density of the pump may not be large enough to completely compensate for the ionic influxes throughout the action potentials (461).

1.3.2.3. Sarcolemma action potential

The association between muscle fatigue and disturbances in either the sarcolemma or t-tubules has been extensively researched (173). The milestone for this area of research was reached in the 1980's when investigators showed a parallel reduction in muscle force and IEMG (262). This finding led to the supposition that excitation failure caused fatigue. But, as described previously, reduced EMG amplitude may also be indicative of reduced central drive rather than Na^+ -K^+ pump failure.

Muscle fatigue is often related to changes in the sarcolemma action potential, which is displayed by a prolonged duration, reduced amplitude and increased amplitude of the initial negative afterpotential (211;211;234;235;268;313;366). However, there are conflicting studies regarding whether these changes inhibit the muscle's ability to produce force or not. Rios and Gonzalez (433) suggested that if the amplitude of the action potential were reduced sufficiently, it would be unable to initiate or decrease the t-tubular charge movement, consequently inhibiting Ca^{2+} release and SR Ca^{2+} channel opening. Conversely, Grabowski et al. (211) showed that a decrease in the spike amplitude had little or no effect on peak force. Moreover, Metzger and Fitts (366) discovered that the peak force generating capacity of a muscle was significantly reduced after high frequency stimulation compared to low-frequency stimulation, when the action potentials decreased to the same level. In their study, the recovery of the action potential was significantly faster than the force output (366). It has also been shown that there are no permanent

associations developed between force output and the size of the action potential. This could be explained as a possible safety mechanism to protect the muscle from the amount of depolarisation required for full activation (455). Fitts (173) hypothesised that fatigue is related to the exercise-induced rise in $[K^+]$. Consequently, the sarcolemma and t-tubular depolarisation would cause a rise in excitation threshold, decrease the conduction velocity and action potential spike height and thereby result in a possible total inactivation (268).

1.3.2.4. t-tubular system

It appears that the t-tubular network functions as a microtransportation system for distributing the action potential from the outer membrane of the fiber, inward to the deep areas of the cell (351). Over 80 % of the inward rectification of the skeletal muscle membrane is found in the t-tubules (15), the ramifications of which have not been postulated (462). Wallinga et al. (515) recently studied the function of K^+ accumulation in the t tubules. The authors (515) concluded that their stimulation study of mammalian skeletal muscle fibers showed the high Cl^- conductance and K^+ accumulation in the t-tubules resulted in the K^+ equilibrium potential becoming positive in relation to the membrane potential. Therefore, the clearing of K^+ from the t-tubules was largely caused by an inward K^+ current through the inward rectifier. However, the role of the Na^+ -K^+ pump has been somewhat overlooked as an increased pump activity was not included in their study. Furthermore, as the pump stimulation would have a large hyperpolarizing power, it would have the ability to enhance the uptake of K^+ via the inward rectifier. Fitts (173) postulated that

the function of the voltage sensor may be more reliant on the $[Ca^{2+}]$ in the t-tubular lumen. Nevertheless, it remains unclear as to whether tubular $[Ca^{2+}]$ decreases or increases throughout muscle activation. It has been shown that Ca^{2+} reduction in the t-tubules may be a significant fatigue factor (93), whereas evidence also exists for increased levels of Ca^{2+} within the t-tubules being a factor contributing to fatigue (40). In conclusion, the evidence for the role of K^+ in the t-tubules is conflicting and further research is required before a satisfactory explanation can be provided.

1.3.3. Metabolite accumulation

Traditionally, it has been considered that the prime source of acid production in the skeletal muscle is via the oxygen independent production of lactic acid produced during the reduction of glucose and glycogen (204;282;445-447). Wassermen and McIlroy (517) initially developed the theory that a crucial threshold exists whereupon the metabolic requirement for oxygen in the muscle exceeds the ability of the cardiopulmonary system to supply them. This is then followed by an abrupt increase in oxygen independent metabolism resulting in the formation of lactate in the muscle. From this theory, the concept developed that lactate was the main source of fatigue during exercise and was also a contributing factor to post exercise muscle soreness (386). The cornerstone for the lactate hypothesis emanates from Fletcher and Hopkins (176), who suggested that lactic acid was produced during contractile activity and was responsible for muscle fatigue. Their study showed that resting muscles with an adequate oxygen supply produced

minimal lactate, whilst during high intensity, oxygen independent exercise conditions, produced a higher quantity of lactate, a finding that was supported by a number of later studies (16;35;282).

Studies have shown that the association between lactic acid quantity and muscle fatigue is mediated obliquely through the related decline in cell pH (148;202;368). It has been well established that type II muscle fibers and fast motor units fatigue more quickly and to a greater extent than type I muscle fibers with slower motor units (86;149;314). Furthermore, it has been shown that after both static and dynamic contractions to exhaustion, type II fibers contain significantly higher lactate concentrations than the adjacent type I fibers (162). A significant correlation has also been seen between fatigability and type II fiber content (494). In both in vitro and in situ studies, the muscle groups consisting of a higher proportion of type II fibers fatigue faster and display higher lactate concentrations and lower pH levels than muscle groups consisting predominantly of type I fibers (495). Furthermore, a decrease in pH and increase in lactate concentrations are both significantly correlated with a decrease in force production. Fitts (175) and Spriet et al. (477), have also showed that work requiring high glycolytic rates results in a decrease in force that is correlated to the increase in muscle lactate. Studies have also shown that when external pH and buffer solutions are reduced, lactate efflux rate also decreases, resulting in a higher accumulation of lactate (339). It has been suggested, however, that it is the accumulation of H^+ rather than the accumulation of lactate during glycolysis that contributes to fatigue, as large

concentrations of H^+ lower the blood pH, thereby negatively effecting muscle contraction and energy production (386).

Throughout prolonged submaximal exercise the net lactate becomes released from the working muscle. As the exercise progresses the net lactate production declines (272) until to a point of no measurable output, the rate of decline will depend on the duration and intensity of the exercise work output. Regardless of the amount of lactate produced during exercise, there seems to be a concurrent and constant level of lactate uptake in the muscle. Therefore, throughout a prolonged period of submaximal exercise, the rate of muscle lactate uptake is constant whereupon the muscle net lactate release declines significantly throughout exercise (504). Interestingly, the arterial lactate continues to increase, which suggests that even with a steady lactate uptake and a declining lactate release, the capacity for the uptake of lactate by muscle is limited, therefore an attempt to compensate is made by decreasing the level of lactate release. This mechanism could occur via an afferent command that gauges the level of arterial lactate concentration, from which triggers an efferent command to adjust the amount of lactate release or uptake accordingly by reducing force output and metabolic rate.

It is now widely accepted that the inverse correlation between force and lactate is largely reliant on the correlation between free H^+ and lactate, and that the force reducing agent is not lactate but H^+ (367;445). Conversely, Favero et al (169) examined the effects of lactate on (i) Ca^{2+} and caffeine stimulated Ca^{2+} release, (ii) [3H]ryanodine binding to SR vesicles and (iii),

caffeine stimulated release from SR vesicles. It was concluded, from this study, that severe muscle activity generating high quantities of lactate will "disturb" excitation-contraction, which may lead to a decline in Ca^{2+} transients. This would then encourage a decrease in muscle tension and thus the promotion of muscle fatigue. This study's relevance to human peripheral fatigue is limited, however, as it was performed on the hind leg of a rabbit with lactate concentrations of 10 to 30 mM. This is a considerably higher level than maximal lactate concentrations recorded in humans.

A summary of the above section on metabolite accumulation is:

i) It was traditionally thought that lactate production was the main source of fatigue throughout exercise.

ii) It has been shown that the association between lactic acid quantity and muscle fatigue is mediated obliquely through the related decline in cell pH.

iii) A decrease in pH and increase in lactate concentrations are both significantly correlated with a decline in force production.

iv) It is likely that the accumulation of H^+ during glycolysis that contributes to fatigue as large concentrations of H^+ lower the blood pH thus negatively effecting muscle contraction and energy production.

In conclusion, it appears that the accumulation of H^+ in skeletal muscle causing a reduction in pH contributes towards peripheral fatigue, rather than the accumulation of lactate acid, which eventually gets utilized as an energy

source to contribute towards contractile function. However, because lactate concentrations are significantly correlated with a decline in force, obtaining lactate samples from exercising subjects will still operate as an effective marker of peripheral fatigue.

1.3.3.1. Adenosinetriphosphate-phosphocreatine

It is essential for Adenosinetriphosphate (ATP) levels in the muscular tissue to be maintained in order to avoid fatigue (173). ATP provides the instantaneous source of energy for contractile function by the myosin cross bridges. Type II fibers contain a significantly higher levels of phosphocreatine (PCr) and ATP than type I fibers (172). Also, Adenosine 5'-triphosphate is required for the functioning of the Na^+ -K^+ pump, which is crucial in the preservation of a normal sarcolemma and t-tubular action potential. Moreover, ATP is a substrate of the SR and therefore necessary in the Ca^{2+} reuptake by the SR (173). Thus, a disruption in any of these developments may lead to symptoms of muscle fatigue.

The changes in creatine (Cr) and inorganic phosphate (P_i) with contractile function displays an inverse association with PCr (172;308;449), which is illustrated by the following:

$PCr + ADP + H^+ \rightarrow Cr + ATP$

consequently, the creatine kinase reaction is driven by cell utilization of ATP

$ATP + H_2O \rightarrow ADP + P1 + H^+ + energy$

Aside from large fluctuations in energy needs, muscle ATP remains stable and the controlling is accurate in altering the rate of the ATP generating procedure to the requirement of the muscle. This mechanism consists of a complex interchange between both feed-forward and feedback mechanisms (451). Sahlin et al. (451) postulated that during prolonged submaximal exercise, there is a point at which ATP expenditure exceeds the rate of ATP generation. At this point, a portion of the adenine nucleotide pool is deaminated to ammonia (NH_3) and inosine monophosphate (IMP). IMP can either be degraded further to urate, hypoxanthine and xanthine, or reaminated to produce AMP. This then provides useful markers for energy deficiency as increases in the degredation products of ATP can be detected by increases in muscle IMP and NH_3 (451) or in the blood as hypoxanthine and NH_3 (247). Therefore, scientists have been able to correlate muscle fatigue with signs of energy deficiency under differing conditions. Muscle fatigue has been shown to correlate with signs of energy deficiency under differing conditions, for example increases in blood hypoxanthine (247) and muscle IMP (451). However, this correlation does not necessarily suggest a causal relationship.

During high intensity exercise the maximal rate of energy expenditure cannot surpass the action of the ATP hydrolysing enzymes such as muscle ATPase activity (451). The maximal amount of CO^{2+} activated ATPase is three- fold higher in type II than in type I fibers (163). It is possible that the release of energy throughout short bursts (< 5 s) of high intensity exercise is due to limitations in ATP hydrolysis rather than the rate of ATP supply. This

hypothesis is supported by studies showing a higher degree of PCr depletion (257) and plasma NH_3 accumulation (231) during (< 5 s) the opening phase of sprinting. Although muscle PCr content decreases after the first 10 seconds of exercise (257;395), PCr breakdown can contribute to ATP supply for over 20 s because ATP also comes from other energy sources. This would explain the rapid force decrements in the Wingate protocol (see section 1.4.4). Moreover, both in vitro (114) and in vivo (532) studies have shown that increases in Pi acts as a significant fatiguing agent. It has also been found that with a decline in PCr there is a concomitant rise in Pi. This suggests that the correlation between force and PCr during both exercise and recovery could be as a result of increased Pi and not entirely from energy depletion (451). Traditionally, the hypothesis of increased H^+ ion concentration has been used, which suggests lowered pH will interfere with the contraction process (173). Spriet et al. (477;478) studied ATP release in skeletal muscle during electrical stimulation and postulated that extreme acidosis could inhibit muscular contraction and thus prevent ATP from decreasing to critically low levels that will cause rigor development. This may be from an indirect effect of acidosis on performance, which is mediated through the disruption of the ATP producing mechanisms (451). There are a number of enzymes involved in glycolysis that display distinct pH sensitivity and it has also been shown that hydrogen ions manipulate the creatine kinase equilibrium (451). Therefore, it is possible that acidosis hinders the contractile mechanisms indirectly via its influence on energy metabolism (451).

A large amount of evidence suggests that the failure of the energetic processes to produce ATP at the required rate, thereby resulting in fatigue is incidental and a direct causal relationship has not been identified (451). As muscle ATP remains relatively constant during exercise, the hypothesis that fatigue is caused by energy depletion has been questioned (173;215). Sahlin et al. (451) questioned this argument by showing that because both spatial and temporal gradients of adenine nucleotides are found in the active muscle and this mechanism could be associated with increases in the products of ATP hydrolysis (AMP, ADP or P_i) as opposed to a reduction in ATP per se. Relatively large increases in ADP and AMP are caused by a small decrease in ATP, because of much lower concentrations of both ADP and AMP in the contracting muscle (451). Increased ADP has been shown to interfere with force production throughout concentric contractions (115;540). Moreover, both P_i and ADP increases will reduce the free energy discharge throughout ATP hydrolysis (451) and could therefore be of significance to contractile function.

Finally, as mentioned earlier (section 1.2.15.1), the observed reduction of IEMG during MVC may be an attempt to obtain the optimal force by preventing peripheral transmission failure (42;43;47). This occurrence may be because of a "fail-safe" mechanism to protect the muscle from developing rigour as a result of a depletion of ATP (303;394).

In summary, the effects of ATP and PCr on fatigue during exercise are:

i) ATP levels in the muscular tissue have to be maintained to avoid fatigue.

ii) Type II fibers contain more PCr and ATP than type I fibers.

iii) ATP remains stable and ATP production is accurate for the muscle's requirement.

iv) ATP may be prevented from dropping to critical levels from acidosis, which could disrupt ATP producing mechanisms, which will affect contractile function.

v) Decreased PCr is higher after submaximal exercise than after high intensity exercise, suggesting that energy deficiency is not the major cause of fatigue.

vi) ATP may be prevented from dropping to critical levels by a reduced level of neuromuscular recruitment.

In conclusion, the evidence is clear that ATP never drops to critical levels in normal exercise fatigue conditions. However, what remains uncertain is the mechanism that prevents ATP from dropping to critical levels.

1.3.4. Substrate depletion

It is traditionally believed that endurance exercise performance is limited by a depletion of endogenous carbohydrate stores (118) and that the rate of carbohydrate utilization increases proportionately with work output (172). This theory was based on the finding that the respiratory quotient substantially increased during heavy exercise (173). Early studies showed that carbohydrate supplementation postponed the onset of fatigue during prolonged submaximal endurance exercise (139;207;318). However, the

exact mechanism for this delay was unknown and has been the focus of research for many years. The bulk of evidence supports the hypothesis that carbohydrate ingestion delays fatigue by preserving a high-carbohydrate source in the form of blood glucose (106). Accordingly, it has been hypothesised that carbohydrate oxidation is necessary for the maintenance of prolonged submaximal exercise at moderate to high intensity. Nevertheless, a satisfactory cellular mechanism has not yet been identified to explain the oxidation of carbohydrates as being limiting during fatigue induced by submaximal exercise (173).

Previous studies have shown that reduced levels of muscle glycogen will result in lower levels of glycogenolysis and glucose production during sustained submaximal exercise (237;520). Recently, Balsom et al. (22) studied the effects of high intensity exercise on muscle glycogen utilization. In this study subjects were fed with either a high or low carbohydrate diet and then completed a cycling protocol that consisted of (i) a glycogen depletion ride that involved a 90 minute submaximal ride, four one minute and ten 10 second intense intervals, with the following two days consisting of a random order of either, (ii) a closed loop ride involving 15 bouts of 6 second supramaximal cycling with 30 seconds of rest in between and (iii) a open loop ride involving as many 6 second bouts of supramaximal cycling as possible (still with 30 seconds in between) until exhaustion. This exercise pattern has previously been shown to result in a rapid utilization of muscle glycogen (191). As expected, the performance and glycogen levels were significantly lower in the low carbohydrate group, although muscle glycogen was still not entirely

depleted immediately after exhaustion. Interestingly, the authors did not conclude from this finding the possibility of any central influence on fatigue. Their other main finding was that RQ values, measured at the fixed time points during both cycling and recovery, were significantly higher in the high carbohydrate diet group. This indicated that there was a greater contribution of fat oxidation to the total energy expenditure in the low carbohydrate diet group. Again, the authors did not mention the possibility of a central command acting to coordinate the energy supply to ensure the most economical contraction of skeletal muscle.

Throughout prolonged exercise of submaximal intensity, the cause of fatigue has traditionally been thought of as an inadequate energy supply. This energy supply is controlled either through limitations in the tricarboxylic acid (TCA) action, because of reduced TCA intermediates (450), or through a limited amount of substrate acetylCoA to TCA. A number of studies support the hypothesis that depleted energy supplies are a contributing factor to the cause of fatigue in prolonged exercise. Firstly, exercise duration is significantly correlated to pre-exercise glycogen level (36), secondly, the onset of fatigue is related to very low (~100 mmol/kg dry wt) muscle glycogen concentrations (36;68;119) and hypoglycaemia (36;119). Finally the breakdown of muscle adenine nucleotides to NH_3 and IMP is improved with muscle that contains a reduced level of glycogen (72). It has also been shown that decreased PCr seen during prolonged submaximal exercise is higher than after high intensity exercise. This suggests that energetic deficiency is not the only cause fatigue after prolonged exercise (448).

Blomstrand and Saltin (55), recently studied the effect of pre-exercise muscle glycogen on glucose, lactate and amino acid metabolism during exercise and recovery. Glycogen stores were depleted in one leg by 60% using single leg cycling. The following day subjects performed a steady state submaximal ride (75% of VO_{2MAX}) for 60 minutes. Substrates were measured before, during and after the ride in both legs. The results showed a higher rate of oxygen consumption in the normal leg at rest, but with a higher rate of lactate production. The authors suggested that this was an indication of increased glycolysis at rest and that glycogen breakdown was greater in the normal leg. However, increased lactate levels could be as a response to a greater work output. The uptake of lactate was higher in the low glycogen leg. Similar findings by Gollnick et al. (205) showed that more fat and consequently less carbohydrate were oxidized in the low carbohydrate leg using RQ measurement in their study. The rate of glucose uptake was higher in the low glycogen leg during cycling, which may have been as a result of cycling the previous evening. This could have increased the amount of glucose transporters in the muscle, which would allow a greater rate of glucose uptake the following morning. Accordingly, it was concluded that low pre-exercise muscle glycogen results in a higher rate of lactate and glucose uptake. As both legs received the same arterial supply, these results suggest that the amount of glycogen per se is accountable for these changes.

In conclusion, it appears that glycogen never completely depletes at exercise exhaustion, which would suggest there is some other factor that determines

the termination of exercise. As the rate of glycogen oxygenation increases when glycogen levels are low, it is possible that the presence of a central command, which responds to signals provided by a glycostat (Rauch et al. unpublished data) is likely. This central command would act by first, providing skeletal muscle with more glycogen to delay fatigue and second, when glycogen levels do run critically low, it would act by forcing the body to slow down and stop exercising completely before any serious damage can occur.

1.3.5. Muscle damage

The peripheral mechanisms that are responsible for fatigue occurring during repetitive, large force generating activities seem to have both metabolic and non-metabolic components (128;385). The non-metabolic aspects of fatigue may exist separately from a disruption in the contractile properties of the muscle fiber. This kind of fatigue seems to have manifested because of the large repetitive forces that are produced and cause ultrastructural muscle damage (89;182;396). Concentric contractions may cause a certain element of damage to the muscle cell (183), however it is eccentric contractions such as downhill running (166-168) that are largely responsible for muscle damage. These contractions generate high levels of force and yet have a relatively lower EMG recruitment pattern, resulting in a greater amount of force per muscle fiber (216). Furthermore, Kay et al. (286) examined EMG patterns during 100 seconds of eccentric, concentric and isometric contractions. The results showed that whilst eccentric activity was largely fatigue resistant, isometric and concentric contractions displayed different EMG profiles. The authors suggested that this was probably due to skeletal muscle not being fully activated during eccentric activity, which is probably due to incomplete motor unit activation, which would allow for enhanced rotation of motor units and reduced fatigue. Also, McHugh et al. (355) recorded EMG on damaged muscles during eccentric contraction. The results showed lesser IEMG and greater MPFS, suggesting that there was a selective recruitment of fast twitch motor units to compensate for the incurred muscle damage. Symptoms of muscle damage include Z-band streaming, sarcolemma disruption, leukocyte

and phagocyte infiltration, myofibrillar disorganization, central nuclei and loss of proteins such as desmin and fiber necrosis (12;182;321) (for recent reviews see (299;320;439)).

1.3.6. Effects of fatigue on muscle efficiency

Muscle efficiency is a state of energy transduction, which is defined as the ratio of muscular work to the free energy conversion of the metabolic development driving the contraction (537). Two methods have been identified to measure muscle efficiency. First, Hill (254) measured the rates of energy output as heat and as work during isometric contractions of single frog muscle fiber, whereas Curtin and Woledge (123) provided in vitro measurements on dogfish and examined muscle function in the conditions under which the muscle is used throughout contraction. This latter investigation takes into account the animal's metabolism, which provides a greater understanding into the effects of fatigue. Woledge (537) concluded that the reasons for fatigue lowering efficiency were firstly, a reduction in PCr concentration as a result of PCr splitting together with a concurrent increase in Pi and Cr concentration, characteristic of fatigued muscle. It remains unclear, however, if the initial process is increased to compensate for the fatigue that is occurring. Secondly, there is a conflicting process between power output and efficiency of motor units. During fatiguing exercise the motor units possessing more power are recruited to perform a required task that can no longer be successfully achieved by the less powerful units. Thirdly, Woledge (537) suggests that the time taken for relaxation becomes slower during fatigue, which may mean that

that it is difficult to obtain the brief periods of contraction required for optimal efficiency during quick cyclical and high intensity episodes. Ironically, this can suggests that fatigue could actually increase muscle efficiency due to an improvement in energy conversion during partial activity rather than full activation (87). Woledge (537), concluded that the complete muscular activation is not reached in a muscle that is completely fatigued and that current observations do not yet allow a conclusive interpretation of the exact mechanisms involved.

1.4. FATIGUE CONDITIONS

To gain a clearer understanding of neural fatigue, we decided to study conditions that would either pertubate or stimulate the system into changing the onset of the symptoms for physical processes associated with fatigue. Some of these conditions include environmental temperature, ß-blockade, caffeine ingestion and supramaximal intensity exercise.

1.4.1. Hot and cold environments

The thermoregulatory response of humans exercising in different environmental conditions has been the subject of considerable research. For example previous studies have shown that lowering body temperature by either cooling with ice packs or lowering ambient temperature will improve exercise performance (63;250;316). There have however been no studies that have examined neuromuscular activity in these circumstances (413).

The debilitating effect of heat exposure during performance of submaximal, prolonged exercise is also well recognised (192). Brown et al. (77) observed a decline in exercise duration at 80% of VO_{2MAX} when ambient temperature was increased from 20 to 35°C. They concluded that this was the effect of the increased temperature, which increased the anaerobic contribution to energy production. Prolonged sub-maximal exercise combined with heat exposure will result in dehydration due to high sweat rates. However, the body's inability to control its increase in temperature (thermoregulatory failure), cardiovascular function and changes in metabolic rate may also affect exercise capacity in a hot environment (387;442). It has been suggested that rather than circulatory failure, it is core temperature that is the critical factor limiting exercise capacity in the heat (192;516) (see section 1.2.9.) and that acclimation does not alter the effect of heat strain on fatigue (464).

Petrofsky and Lind (423) showed that the MPFS shifted to the upper part of the spectrum during short duration isometric contractions as temperature increased (see Figure 1.4). Bigland-Richie (42) showed that cooling the muscle and slowing its conduction velocity would produce a general shift to the lower part of MPFS, while heat will increase the conduction velocity, therefore producing a general shift towards the upper part of MPFS.

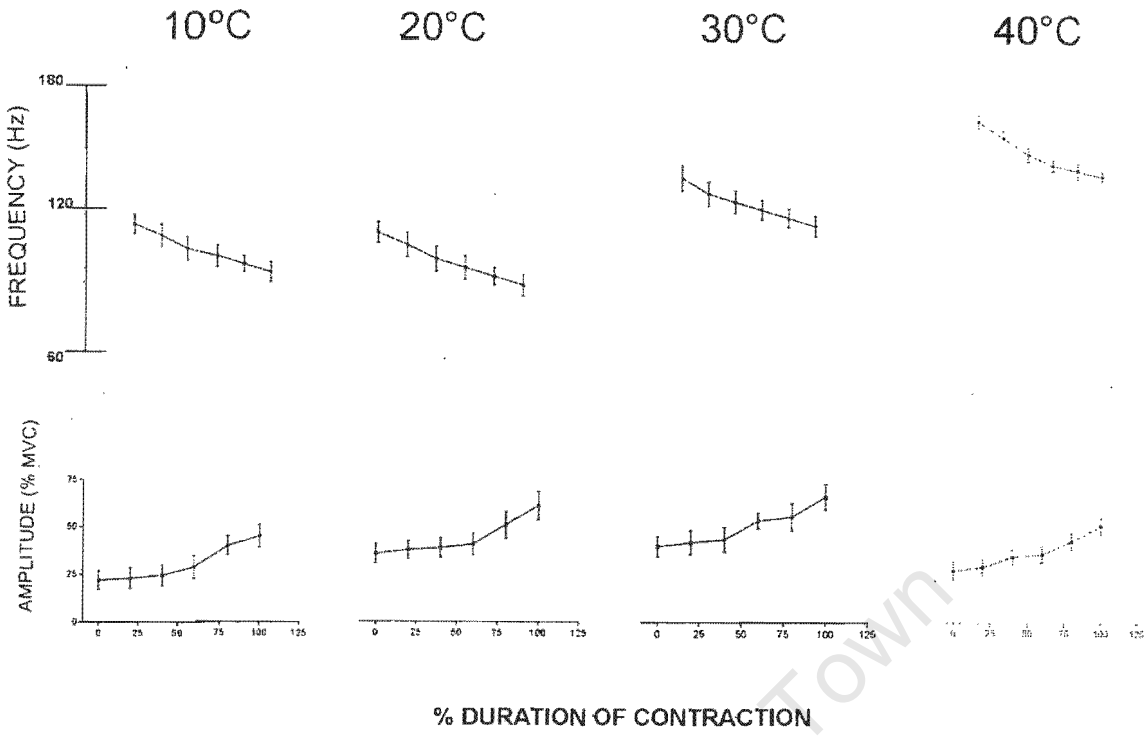


Figure 1.4. The effect of 10, 20, 30 and 40°C temperatures on EMG characteristics during concentric muscle contraction. The top row displays MPFS with the corresponding amplitude on the bottom row (423).

This occurrence could account for premature fatigue during exercise in hot conditions. A contributing mechanism towards this premature fatigue could be from the increase in conduction velocity, which could increase the use of larger fatigueable type II fibers. However Bigland-Ritchie (42) independently cooled and heated the peripheral musculature, which does make it uncertain whether the increased conduction velocity is caused by a change in metabolites in the cell or by hot/cold acting directly on the nerve or CNS properties. Therefore studies to determine the neural recruitment patterns and spectral shifts during submaximal to exhaustive exercise have not been conclusively described.

1.4.2. ß-blockade

A combination of exercise and ß-blockade may be prescribed as treatment against hypertension (243). Patients who ingest ß-blockers for protection against myocardial infarction are advised to exercise regularly (243), however fatigue is a commonly reported adverse effect of ß-blocker ingestion (121;546).

ß-blocker agents reduce exercise endurance performance in healthy subjects as well as in patients with hypertension who are being treated with ß-blocker (500). The main therapeutic effects of ß-blockers are a reduction in myocardial oxygen consumption, decreased resting blood pressure and a decrease in electrocardiographic abnormalities (243). Both non-selective and selective ß-blockers reduce blood pressure and heart rate during both rest and submaximal exercise (317;331). ß-blockers have been shown to reduce exercising heart rate by 20 to 30% and cardiac output by 5 to 23% by some investigators, (273;431) whereupon others have showed no decrease in cardiac output during sub-maximal exercise (185;530). When ingesting ß-blockers, exercise performed at 50 to 60% of VO_{2MAX}, showed a reduced (17) but sufficient maintenance of cardiac output, despite the reduced heart rate, by an increase in stroke volume (159;185;333;530). Skeletal muscle blood flow is unaffected by ß-blockade (277). However, endurance-trained athletes usually have a reduced heart rate and an increased stroke volume. Therefore, when these athletes ingest ß-blocker they are unable to compensate for any heart rate decrease by a concomitant rise in stroke volume, since it is almost

near maximum. Consequently endurance trained athletes exercising at submaximal intensity under ß-blockade may perceive their level of work to be considerably harder than their untrained counterparts (243;273). The reasons for this impaired maximal exercise capacity are not completely understood (500). It has been hypothesised that the effects of ß-blocker agents on energy metabolism are involved (499). However, ß-blockers do not influence muscle power output during high intensity short duration exercise (136). Another possible mechanism responsible for a reduced maximal exercise capacity may be a prevention of the increased rate and force of contraction of the myocardium, which occurs during exercise, resulting in a decrease in heart rate, contractility and cardiac output (6). Another mechanism may be a reduction in adrenaline production, which in turn reduces the level of circulating catecholamines epinephrine and norepinephrine, which reduces the sympathetic exercise response (312). A lowering of plasma renin (81), caused by ß-blocker, may have a central hypotensive effect (519). Lastly ß-blocker may cause an overall reduction in fat oxidation throughout exercise and reductions in free fatty acids and glycerol levels (244-246). The mechanism responsible for adrenergic control of lypolysis is not yet elicited, but adipose tissue lypolysis would appear to be mostly ß1 receptor-mediated (243).

Previous studies of the effect of ß-blockade on MVC found no reduction in achievable power output (209). Other investigators have examined high intensity anaerobic exercise of 30 to 90 seconds duration and shown that power output was markedly reduced with ß-blocker ingestion (279).

Investigators have also found that maximal muscle power during a Wingate test was reduced by ß-blockers (279).

Another mechanism may therefore be the effect of ß-blockers on muscle recruitment activity on central pathways of control. The only experiments that have been conducted to study the influence of ß-blockers on the EMG pattern under exercise conditions was first, Tesch et al. (492) who discovered no significant differences in IEMG or MPFS during cycling, but did find a less pronounced increase in MPFS during ß-Blockade. This could be indicative of muscle fatigue or decreased velocity of the nerve fibers (278). MPFS was calculated by describing data captured at 90 W initial work rate, and all subsequent data was normalised against it. However, Freund (181) showed that the percentage of MVC is the determinant of the recruitment level and firing rate of the active motor unit, therefore it might be a more appropriate normalisation activity. This has to be considered throughout investigation of biological signals in which the electrical and mechanical activities of the recruited motor units are summated (161). Second, Derman (136) examined IEMG patterns of exercising subjects ingesting ß-blockade and found no difference in MVC but significantly higher IEMG activity in the subjects who ingested ß-blockade. Derman (136) concluded that the higher IEMG was due to additional recruitment of non-fatigued skeletal muscle fibers to maintain the same work rate. However, this study was limited as MPFS was not determined.

In summary, the effects of ß-blockade ingestion on exercise are:

i) Reduction of exercise endurance in both healthy and hypertensive subjects

ii) Reduction in myocardial oxygen consumption, decrease in resting blood pressure and electrocardiographic abnormalities.

iii) Reduction in both resting and exercising heart rate.

iv) Maintenance of cardiac output in untrained subjects is achieved by a concomitant rise in stroke volume. However, trained subjects are unable to make this compensation as stroke volume is already near maximum.

v) No influence of muscle power of a short duration

vi) Reduction in catecholamine production from the adrenal gland.

vii) Decrease in plasma rennin will cause hypotension

viii) Could inhibit lypolysis.

ix) Possibly effect central pathways of control by recruiting additional non fatigued muscle fibers.

In conclusion, the effects of ß-blockers on exercise performance are multifaceted and still pose a variety of questions for researchers. The effects of ß-blockers on exercise performance cannot be entirely explained by changes in cardiovascular capacity or metabolism, therefore involvement from the CNS has to be considered.

1.4.2.1. The effects of ß-blockade on plasma potassium

Section 1.3.2.1 discusses potassium and cell depolarisation. A net efflux of K^+ from the working muscle causes an elevation in $[K^+]$ as a result of the contracting muscle (101). The short- term control of the Na^+-K^+ pump is exerted by both impulse-mediated alterations in ion concentration gradients and also via the adrenergic system (101). Previous studies have shown that the Na^+-K^+ pump activity is governed by the β_2 adrenoceptor, as β_2 antagonists increasing plasma $[K^+]$ and β_2 agonists cause a decline in plasma $[K^+]$ (78;276;330). Moreover, it has been postulated that β-adrenergic control of K^+ homeostasis may apply its effect via two main mechanisms, (i) stimulation of K^+ reuptake in non-exercising tissues and (ii) stimulation of the Na^+-K^+ pump in exercising muscle (226).

West et al. (522) examined the influence of exercise-induced hyperkalaemia on muscle excitability and fatigability. This study found no evidence to suggest a reduction in muscle membrane excitability as deduced by measurement of the M-wave, regardless of highly significant increases in plasma $[K^+]$. Nevertheless, a strong association was found between the $[K^+]$ and the recovery force, suggesting an increased extra-cellular $[K^+]$, which could have been exerting its effect by inhibiting in the t-tubules, which are distal to the location of where the membrane action potential is generated. However, most studies have shown that β-blockade increases serum $[K^+]$ even further during exercise (177;210;225;284;305;334;335).

In conclusion, the ingestion of ß-blocker will cause an increased accumulation of K^+ in the transverse tubules of skeletal muscle, where diffusion is limited, which could then slow down action potential conduction and impair recruitment of motor units.

1.4.2.2. The effects of ß-blockade on RPE

Maximal heart rate has been significantly reduced in healthy subjects during graded exercise tests after ß-blocker ingestion while RPE has remained unchanged (127;220;467). It has been proposed that this is from a blocked autonomic system, which suggests that heart rate is not the primary factor for setting RPE during short term exercise (165). Conversely, Davis and Sergeant (467) showed during 60 minutes of submaximal exercise that RPE was higher for a given absolute heart rate, but when it was expressed as a proportion of VO_{2MAX}, the difference was insignificant.

1.4.3. Caffeine

Caffeine is similar in composition to many endogenous metabolites and has also been shown to cross the blood-brain barrier and the placenta, and is dispersed in intracellular fluid (13). These characteristics enable caffeine to affect numerous human tissues, including the cardiovascular system, the CNS and smooth and skeletal muscle (13).

Caffeine is reported to be an antagonist of both adenosine A_1 and A_2 receptors (410). A_1 receptors activate potassium channels, inhibit lipolysis, slow atrial-ventricular nodal conduction and inhibit basal and evoked neuronal firing (410). A_2 receptors inhibit inflammation and dopamine release in the striatum and causes cerebral and peripheral vasodilatation (410).

The current interpretation of caffeine's mechanisms of action still remain unclear, however the physiological effect of caffeine suggests that it has the ability to aid in the improvement of human performance (281). Various studies have reported this finding (see Table 1.2), principally with regard to exercise metabolism and human performance. Studies have also shown an increase in fat oxidation, muscle triglyceride use and a decrease in glycogen use in the presence of caffeine (214;301;505). However, a complete dissociation between caffeine-induced changes in plasma catecholamine concentrations or fat oxidation and exercise performance has been shown (214;301;505). Consequently, it could be suggested that the ergogenic effects of caffeine are possibly mediated via the CNS as opposed to effects on fat oxidation and glycogenolysis (76;203). Indeed, the effects of caffeine could be manifested in numerous locations along the neural pathway from the motor cortex to the contractile mechanisms. Caffeine may play a role in increasing efferent command by blocking the inhibitory effects of adenosine (180), thereby enhancing the capacity to increase motor unit activity. Consequently, it is possible that an increase in synaptic input to the cell body of the α-motoneuron, would increase its activity and thus facilitate maximal activation and force the motoneuron closer to its maximal threshold (281).

Table 1.2. Caffeine ingestion and exercise capacity

Exercise	Effect	References(s)
Progressive exercise to exhaustion in 5-10 min	None	Perkins and Williams (417); Bond et al. (61) Dodd et al. (140)
	Improved	Powers et al. (426)
Peak power < 1min	None	Collomp et al. (109) Greer et al. (218)
	Improved	Anselme et al. (9)
Repeated sprints	None	Paton et al. (415)
Exercise endurance between 30 – 90 min	None	Butts and Crowell (88), Sasaki et al. (456)
	Improved	Ivy et al. (264); Costill et al. (117); Essig et al. 1980 (164); Graham and Spriet (213); Graham and Spriet (214); Pasman et al. (414)
Exercise endurance 3h	None	Wemple et al. (521)
Exercise performance	Improved	Berglund and Hemmingsson (34); Wiles et al. (527) ; Macintosh et al. (338); Kovacs et al. (301); Bruce et al. (79)
Race or time trial	None	Cohen et al. (107)

Note: These studies were conducted on healthy individuals. Caffeine doses ranged from 3-10 mg/kg body mass and were usually ingested 60 min before exercise.

The modern interest in the possible performance-enhancing effects of caffeine were initially raised by the finding of Costill and co-workers (117) Ivy et al. (264) that caffeine ingestion increased the time of cyclists/runners to reach the point of exhaustion.

More recently, evidence supporting a substantial ergogenic effect of caffeine on endurance but not sprint activities (141) has increased. The vast majority of those studies have evaluated performance (465) during open loop cycling (414) or running (141) trials in which subjects maintain the same exercise intensity for as long as possible. Extrapolating the findings from such trials to human sporting performance requires a leap of faith, since most human competitive sport requires that either a specified distance known before the event begins, must be completed as rapidly as possible or, much less commonly, that as much distance as possible must be covered in a given time, also fixed and before the exercise begins.

There have only been two previous studies that examined the effects of caffeine on neuromuscular function (281). Kalmer and Cafarelli (281) studied human subjects under control, placebo and caffeine conditions for electrically stimulated 50% and 100% MVC. Whilst results showed an ergogenic effect on peak force and muscular endurance, intramuscular EMG indicated that subjects who had ingested caffeine were more able to activate their motor pool more efficiently. The authors proposed two explanations for this occurrence; firstly, caffeine is reported to be an antagonist of adenosine. Adenosine has an effect on the cerebral cortex by reducing evoked and spontaneous potentials, diminishing firing rates and suppressing

neurotransmitter release (124;180;424). Because of this antagonistic effect of caffeine, it was suggested that an increase in maximal voluntary activation was produced because of a decrease in inhibition in the motor cortex. The alternative explanation was that there was an increased excitability of the motoneuron pool at the level of the spinal cord as a result of the diminished inhibition. However, the latter explanation was disproved when results showed no change in the Hoffman reflex (62) amplitude from pre to post-test in any trial suggests that caffeine had no effect on excitability at the level of the spinal cord.

Both of these previous studies (281;529) showed no significant alteration in surface EMG from caffeine ingestion. However, Kulmar and Cafarelli (281) suggested that because surface EMG represents a summation of both discharge rate and motor unit recruitment, it may not be the most effective procedure to quantify the effects of caffeine on excitation-contraction efficiency during submaximal contractions. Caffeine could, therefore, via adenosine-receptor antagonism, increase the firing rates of central neurons (124). Kulmer and Cafarelli (281) then concluded that if this were possible, the increase in firing rates might offset the decline in motor unit recruitment, leaving the appearance of the force-EMG relationship unaltered. Conversely, for the firing rates to increase and the motor unit recruitment to decline, surface EMG should display a shift to the upper parts of MPFS and a decline in IEMG amplitude.

In summary, the effects of caffeine on exercise are:

i) Caffeine is an antagonist of the inhibitory receptors adenosine, these receptors will inhibit tachycardia and dopamine release.

ii) Most studies suggest that caffeine has an ergogenic effect that is mediated via the CNS as opposed to fat oxidation and glycogenolysis.

iii) Evidence shows an ergogenic effect during endurance but not sprint activities.

iv) Most studies on caffeine have used open-ended trials, which is not wholly relevant when applied to sporting performance that requires pacing strategies.

v) Studies on the effects of caffeine on neuromuscular function have been inconclusive.

In conclusion, the evidence to suggest that caffeine has an ergogenic effect is clear, however the shortfall of most of these studies is that they are performed using open loop protocols, which do not examine the necessary factor of pacing required in most endurance sporting events. Also, the evidence for the effect of caffeine on neuromuscular function remains limited.

1.4.4. Supramaximal exercise activity

The Wingate Anaerobic Test (WAT) has been used extensively by researchers and sports practitioners alike to examine maximal power output and as a standard exercise task that can help analyse athletes response to supramaximal exercise (25).

WAT was originally based on a 30-second cycling test described by Cumming (122). A 30-second period was considered suitable for stressing the glycolytic pathway (341). The prime consideration in choosing a 30 second duration was based on observations in which 30, 45, and 60 second protocols were compared. Subjects managed all out effort for 30 seconds (Figure 1.5), however in the 45 and 60 second tests, some repeatedly tried to start at less than maximum speeds for fear of not finishing (25).

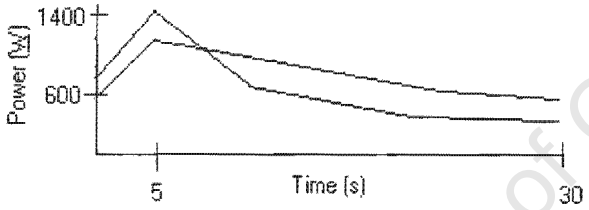


Figure 1.5. Classic Wingate fatigue profiles. The subject that starts off and peaks at a higher power and fatigues at a lesser power, is considered to have a higher proportion of type II fibers.

WAT has been traditionally perceived as being a purely anaerobic, or an oxygen independent test. However, Nioka et al. (403) showed that a maximal 30 second Wingate test caused a desaturation of ~80% of peripheral skeletal muscle compared to that of a maximal aerobic exercise (< 40%), indicating that there is a substantial oxygen consumption requirement from the beginning of the Wingate test. However the described desaturation in the WAT, may also occur due to a relatively restricted blood flow due to increased

intramuscular pressure (403), a similar occurrence was also found in speed skaters (179). Furthermore, Hussain et al. (263) and Bogdanis et al. (58) discovered a significant decrease in pH from baseline levels immediately after the Wingate test. This reduction in pH may have been caused by a restrictive blood flow or an increase in metabolic rate, which may also change the concentration of potassium ion (K+) (73), resulting in changes in the excitability of the muscle fiber membrane, leading to the severe symptoms of fatigue described (10;383).

Maximal power is a function of instantaneous force and velocity (25). During fatiguing maximal cycling, the phasic acceleration and deceleration of the lower limb muscles limits the period for which the muscle can maintain an optimal velocity (352). The only study to have examined neuromuscular fatigue during the WAT was Vandewalle et al. (37), who used a 45 second protocol, it was concluded that a decline in EMG amplitude was observed because of central fatigue occurring. However, this study is limited for two reasons, first because of the WAT protocol used meant that the test was not supramaximal, as mentioned previously it has been shown that subjects will pace themselves to successfully complete a 45 second WAT as opposed to the all out effort seen during a 30 second WAT (25). Second, only EMG amplitude was measured with no EMG spectrum analyses, therefore to make conclusions of whether a task is central or peripheral is speculative.

Other studies have shown a decline in EMG frequency spectrum during maximal running sprint activities (389), however the relevance to WAT can be questioned because of the different muscle groups used, the biomechanical

action and support of body weight. Green (217) proposed that it is only incremental supramaximal motorized treadmill tests and the WAT which enable alactic work capacity to be determined and present more information on performance ability than constant load tests. However, when placing an EMG electrode on a working muscle the technique required to perform the task and the variability between subjects has to be considered. Clearly, the cycling action required to perform the WAT requires far less technique than running, therefore less variability, resulting in a more reliable, comparable EMG signal.

1.5. LITERATURE REVIEW SUMMARY AND CONCLUSIONS

The evidence for both central and peripheral involvement in the development of submaximal and maximal exercise fatigue is clear. CNS involvement in the development of fatigue has been shown by a number of different theories and studies. First, Noakes provides an interesting hypothesis, which involves a "central governor" theory. Second, neurotransmitter research has also shown some appealing conclusions largely from studies involving serotonin. Third, studies of exercise in the heat have shown that severe fatigue commences once a maximal critical internal temperature exists. Fourth, strength training has shown CNS involvement from strength gains in rested contralateral limbs and imagined contractions. Fifth, studies of diaphragm fatigue have shown neural derecruitment. Finally, Vissing (512) suggests that there is a feedback regulatory control mechanism for glucose homeostasis.

Furthermore, there have been studies of CNS recruitment of motor units to identify the most economical force production in any given environment. These studies have produced theories such as the Henneman's size principal, muscle wisdom, rate coding, inhibitory reflex and motor unit substitution. One of the most effective methods to measure motor unit recruitment is with the use of EMG, which is considered to be the most controllable non-invasive method to capture the whole electrical signal sent to the muscle via the CNS. This EMG methodology will measure both the amplitude of the myoelctrical signal (IEMG) and mean power frequency spectrum (MPFS), which is considered to be a reliable indicator of conduction velocity, which will decline in most instances of fatiguing contractions. As well as voluntary contractions, electrical stimulation is widely used as a method of inducing fatigue to determine the level of CNS involvement. Electrical stimulation is administered at varying frequencies (low frequency < 30Hz and high frequency > 30Hz) to obtain different effects. Finally, protocol design for human submaximal endurance will inevitably tease out different factors when testing in a laboratory environment. For example, closed loop protocols will test out factors such as "teleoanticipation", because of the pacing strategy required to complete the protocol in the fastest time possible.

Peripheral fatigue was described in the second section of this literature review and has been traditionally thought to be one of the main causes of both maximal and submaximal exercise fatigue, these causes are summarised by the following: i) EC failure is considered to be caused by a number of factors involving failure of mechanism between calcium release and action potential, failure of action potential transmission or a failure of calcium regulation, ii) The

K^+ induced depolarisation of muscle membrane and ultimately excitability of the muscle membrane causes EC breakdown. iii) The Na^+K^+ pump is unable to sustain the necessary ionic incline required for cell excitability and the V_m. iv) Sarcolemma excitability is affected by sarcolemma and t-tubular depolarisation, which may cause a rise in excitation threshold, decrease the conduction velocity and action potential spike height thus resulting in possible inactivation. v) Evidence of the role of K^+ in t-tubules and consequently in fatigue is conflicting, therefore further research is required before a satisfactory explanation can be provided. vi) Rather than an accumulation of lactic acid contributing towards fatigue, it is an accumulation of H^+ in the skeletal muscle that causes a reduction in pH and a consequent rise in acidosis that causes fatigue. vii) ATP never drops to critical levels during normal exercise conditions and the mechanism preventing this from occurring remains unclear. vii) Glycogen levels never completely deplete during fatigue, again suggesting the possible involvement of a mechanism preventing total depletion from occurring. viii) Muscle damage will inevitably contribute towards peripheral fatigue, by impairment of contractile function. From this review we can conclude that peripheral fatigue has many possible mechanisms contributing to both maximal and submaximal exercise fatigue. Some of the described mechanisms have been extensively researched and are still unable to provide a satisfactory explanation, whilst others are clearly in need of further study with a fresh adopted paradigm that includes the possibility of CNS involvement.

To elucidate neural contributions to fatigue, investigators have designed experiments within differing conditions to either enhance or delay fatigue, to

help gain a greater understanding of mechanisms involved. Some of these conditions have involved: i) ß-blocker ingestion which will predominantly inhibit cardiovascular capacity, however the question of neural involvement remains largely unanswered. ii) Hot and cold environments will speed up and slow down conduction velocity respectively. However, it remains uncertain whether the change in regulation is caused by an alteration in metabolism in the cell or by the hot/cold acting directly on CNS properties. iii) The majority of evidence suggests that caffeine has an ergogenic effect, however most of these studies ere performed using open loop protocol, which asks the question of relevance to sporting performance, when there is no required pacing strategy employed. Also, studies on the effects of caffeine on neuromuscular function have shown an increased activation of motor pool efficiency, however these studies remain limited. iv) It has been shown by >80% oxygen desaturation that the Wingate protocol is not completely oxygen independent. The evidence suggests that the fatigue response to the Wingate protocol is predominantly humoral. However, few studies have examined neuromuscular recruitment patterns during this type of exercise.

The proportion of involvement and the exact mechanisms responsible for fatigue still remain as challenging questions for investigators. Most research on neural fatigue and its different conditions have concentrated on examining skeletal muscle in isolation which has its limitations when attempting to elucidate the contributing neural mechanisms in both submaximal and maximal exercise fatigue.

Accordingly, in an attempt to gain further insight into neural mechanisms responsible in both maximal and submaximal fatigue, we considered it necessary to develop and apply existing electromyographic methodology of skeletal muscle to the exercising body. Once this methodology has been developed, we will then be able to examine neural fatigue by manipulating the conditions of exercise.

QUESTIONS

The principal research questions in this thesis are:

1. To gain further understanding of the neural involvement in exercise fatigue, what are the effects of different exercising fatiguing conditions on the neuromuscular recruitment pattern?

- What is the effect of both hot and cold conditions on the neuromuscular recruitment pattern during submaximal fatigue?
- What is the effect of ß-blocker ingestion on the neuromuscular recruitment pattern during both submaximal fatigue and MVC?
- What is the effect of caffeine ingestion on 100 km cycling performance, pacing strategy and the neuromuscular recruitment pattern?
- What is the effect of performing the Wingate supramaximal cycling protocol on the neuromuscular recruitment pattern?

However, in order that these questions be addressed it is necessary to first answer the following questions:

2. What is the most reliable EMG capture rate to be used for both MVC and submaximal to fatigue cycling?

3. What is the most effective method for normalising the EMG signal for submaximal cycling to enable intersubject comparison?

CHAPTER TWO

EMG AMPLITUDE IN MAXIMAL AND SUBMAXIMAL EXERCISE IS DEPENDENT ON SIGNAL CAPTURE RATE

2.1 INTRODUCTION

Electromyography (EMG) is often used as a research tool by muscle physiologists, neuroscientists and clinicians (194) in clinical (82;100) and research fields (195;200;361). The surface EMG signal provides an expedient non-invasive approach to study the complexity of muscle neurophysiology throughout various types of contractions (351). The EMG signal is the electrical revelation of the neuromuscular activation affiliated with a contracting muscle. The signal is influenced by the anatomical and physiological properties of muscles and the type of instrumentation that is used to detect and observe it (29).

An important feature of EMG analyses is the rate at which the signal is captured. A number of commercially available EMG signal acquisition units used by researchers and physiotherapists will capture EMG at a rate ranging from 32 – 500 Hz (29;156). Most muscles in the human generate an EMG trace, which has a bandwidth of 0 to 500 Hz. The Nyquist theorem states that the sampling rate should be twice the highest frequency generated by the muscle being sampled. If the Nyquist criterion is not met, a condition known as aliasing will occur due to under sampling (259). This occurs when upper frequencies get reflected into the lower frequencies, where in effect a high-frequency component takes on the identity of a lower frequency (411). Therefore the minimal frequency required to capture all the frequency content of the signal is suggested to be 1000 Hz (29).

Other researchers have found the range of signal frequencies for surface EMG's in certain instances to be between 1 to 3000 Hz (536). Applying the Nyquist sampling theorem, would advocate that in such cases a sampling frequency of 6000 Hz is necessary. Conversely, most of the power of the signal is in the range 50 to 150 Hz (509). For this reason, it has been suggested that a sampling frequency of 500 Hz would be more than sufficient for surface EMG (509).

There is, however still some confusion about what constitutes adequate capture rates for a functional, rehabilitative or sporting activity. Accordingly, the aim of this study was to assess the reliability of EMG data captured at different frequency capture rates by sampling surface EMG during fatiguing activities at a number of differing rates, and assessing the relationships between amplitudes recorded in each case.

2.1.1 Question

The question addressed by this study is:

1. What is a reliable EMG capture rate to be used for both MVC and submaximal to fatigue cycling?

2.2 METHODS

2.2.1 Subject selection and sampling

Ten healthy males volunteered for this study. The mean age, height and body mass of the subjects were 25.5 ± 3.5 yr, 180 ± 13 cm and 78.8 kg ± 16 kg. All subjects were physically active and each signed an informed consent before the study. The Research and Ethics Committee of the University of Cape Town Medical School approved the study.

2.2.2 Preliminary testing

Peak power output (PPO) was measured as described by Hawley and Noakes (240). Subjects began riding on an electrically braked cycle ergometer (Lode, Groningen, Netherlands) at a starting work rate of 2.5 $W.kg^{-1}$ body weight (BW) for 150 s, after which the power output was increased by 25 W every 150 s until the subject became exhausted. Exhaustion was defined as a drop in the subject's pedalling frequency from ~90 to <50 revolutions/minute. PPO was defined as the last completed work rate in W plus the fraction of time spent in the final non-completed work rate multiplied by 25 W. Mean PPO value for all the subjects were 347 $\pm$ 33 W

2.2.2.1 Anthropometry

Mass and stature were recorded, and an anthropometric assessment was conducted on each subject. Body fat was calculated using the equation of Durnim and Wormersley (146) from the sum of seven skinfolds (438). Lean thigh volume for each subject was calculated according to the method adopted from Katch and Katch (283), based on the assumption that the upper lower limb is a truncated cone.

2.2.3 Experimental trial

2.2.3.1 *Force/ voltage*

In order to compare cycling and maximal isometric voluntary contraction data, The following simplified relationship was used;

$P = FV$

Where P is the power output (Watts), F is the force applied to the pedal (N), and V is the velocity (W/S) of the foot on the pedal. Rearranging this equation,

$$F = \frac{P}{V}$$

and since the length of the crank was 0.173 m and the pedal rate were 1.5 m revolutions per second (which is 3π radians per second),

$V = 0.173 \text{ m} \times 3\pi \text{ radians/s}$

$= 1.626 \text{ m/s}$

so $F = 0.615.P$

2.2.3.2 *MVC testing*

In the week after PPO testing, each subjects' right knee extensor strength capacity was measured on a dynamometer (Kin - Com, Chattanooga Group Inc., USA). Subjects sat on the dynamometer with their arms folded across their chest and their hips, thighs and upper bodies were firmly strapped to the seat. In this position their hip angle was at 100° flexion. The right lower leg was then attached to the arm of the dynamometer at a level slightly above the lateral malleolus and the axis of rotation of the arm was aligned with the lateral femoral condyle. The arm was then set so that the knee was at a 60° angle from full leg extension (Figure 2.1). Each subject performed four sub-maximal familiarisation contractions prior to performing two maximal MVC's, the latter of which were used for subsequent analyses. All subjects were encouraged verbally to exert maximal effort during both MVC's and were provided with Kin Com visual feedback.

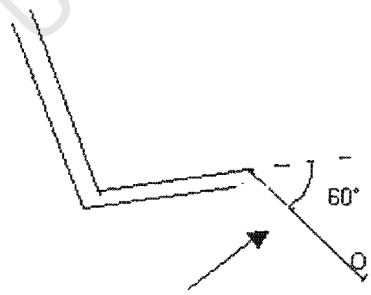


Figure 2.1. Isometric maximal voluntary contractions for leg extension (MVC) at a knee joint angles of 60°. The arrow indicates direction of force.

2.2.3.3 *Progressive Exercise Test*

Following the MVC's, subjects performed a 45 minute ride on a cycle ergometer (Lode, Groningen, Netherlands) at increasing work rates. Work rate was started at 30% of PPO and increased to 50% (SUB) and then to 70% of PPO for 15 minute periods. Immediately after these 15 minute rides at constant work rates, the work rate was progressively increased by 15 $W.min^{-1}$ until the subject reached the point of exhaustion and could no longer continue exercise (PWATT) (Figure 2.2). PWATT, time taken to reach exhaustion (TIME) and peak VO_2 achieved (VO_{2MAX}) were 330.3 $\pm$ 44.7 W, 49.5 $\pm$ 1.4 min and 50.7 $\pm$ 6.1. $ml.kg^{-1}.min^{-1}$ Individual physical performance values of the subjects are described in Table 2.1.

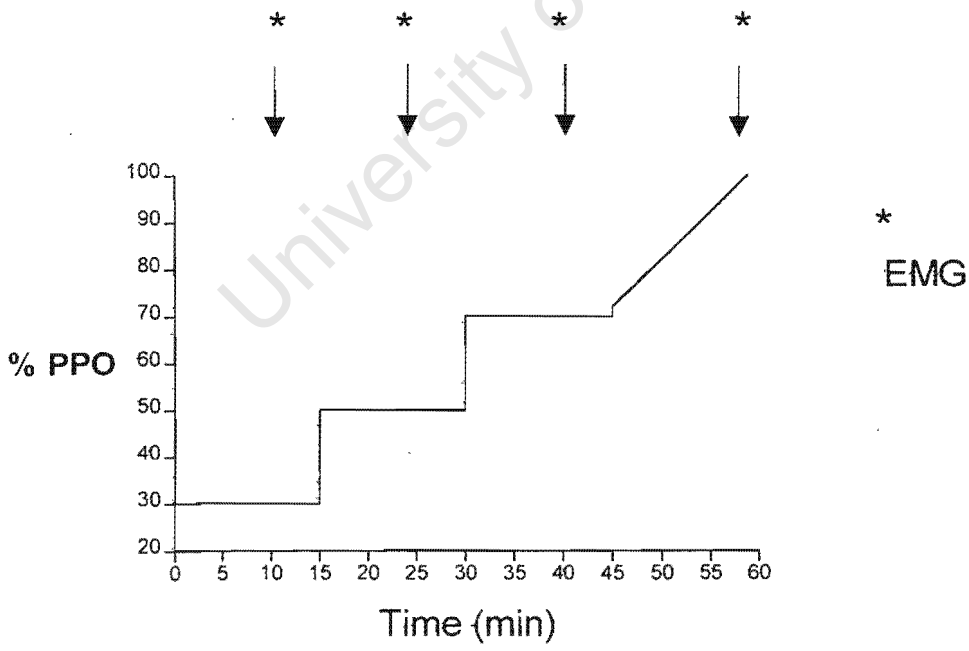


Figure 2.2. Progressive cycle exercise test protocol. Electromyographic (EMG) data were all recorded at 10, 25, 40 minutes and at exhaustion during the ride.

Table 2.1. Subject information. Peak power output (PPO), peak watts reached at cycling exhaustion (PWATT), time taken to reach exhaustion (TIME), maximal volume of oxygen uptake (VO_{2MAX}).

SUBJECT	PPO	P WATT	TIME	VO_{2MAX}
1	346	347	51.1	43.7
2	311	329	50.3	57.5
3	356	359	49	51
4	373	347	49	52
5	343	346	51	46
6	364	377	50.3	51
7	357	340	50	53
8	312	248	46	40.5
9	298	250	49.2	52
10	408	360	49.4	61.2
Mean	346.8±33	330.3±44.7	49.5±1.4	50.7±6.1

All values are mean ± SD

2.2.3.4 *EMG testing*

During the MVC's and progressive cycling tests, the EMG activity of the rectus femoris muscle was recorded. The EMG electrode was placed over the 'belly' of the rectus femoris muscle approximately midway between the superior surface of the patella and the anterior superior iliac crest. Before placement of the electrode, the skin was shaved, abraded with sandpaper and cleaned with ethanol. A triode electrode was then attached to the leg, covered with cotton swabs to minimise interference from sweat and connected to a pre-amplifier. Outputs from the pre-amplifier were relayed to a Flexcomp/DSP EMG apparatus (Thought Technology USA) via a fiber optic cable and stored by an

on line computer. EMG was recorded for 5 seconds during MVC, after 25 minutes during SUB and 30 seconds before reaching PWATT during the cycle ride. The same EMG signal was captured simultaneously on 7 channels programmed to record at frequencies of 32, 64, 128, 256, 512, 1024 and 1984 Hz. All the data for each subject was captured throughout one session with no change in electrode movement.

During recordings, EMG signals were notch filtered at 50 Hz to limit electrical interference and anti-aliasing filters were used for all 7 different channels.

Although Figure 2.3 shows raw signals, the EMG signals were subsequently converted to positive values by full-wave rectification. Post-hoc filtering was not possible on the data at low frequency capture rates and statistical differences in raw EMG amplitude for all capture rates were therefore assessed.

The spectra in Figure 2.6 were produced by fast fourier transform in MATLAB of the signal sampled at 1984 Hz. The frequencies up to 512 Hz are shown in A and C, and up to 32 Hz in B and D.

2.2.4 Statistical analyses

Data statistics are presented as means and standard deviations. Significant differences between EMG amplitudes of MVC, PWATT and SUB were assessed by using analyses of variance with repeated measures. Where significant differences occurred, Scheffe's post hoc test was used to locate the

differences between groups. Single comparisons between PWATT and MVC for N/V were analysed with a paired Students t-test.

Raw data for the 5s sample period for each subject was fitted to the optimal rectangular hyperbola for each capture rate. Using the Inplot GraphPad Programme, asymptote hyperbola values were determined by differentiating the hyperbola and determining where dEMG/dHz became 0/dkHz. The force output during the isometric test and cycle ride was calculated and related to the mean EMG activity during the 5 second time period when the data was captured. Pearson correlation coefficient was used to assess the relationships between the parametric data.

2.3 RESULTS

2.3.1 *Individual raw data*

Figure 2.3 shows raw data for an individual subject for PWATT and MVC captured both at 32 Hz and 1984 Hz. This data demonstrates that the amplitude captured for both activities is considerably higher at 1984 Hz than 32 Hz. When comparing 32 Hz MVC (Figure 2.3 a) with PWATT (Figure 2.3 c) the difference in amplitude appears to be marginal. However, visually there are marked differences at 1984 Hz between MVC (Figure 2.3 b) and PWATT (Figure 2.3 d) in this representative individual's data.

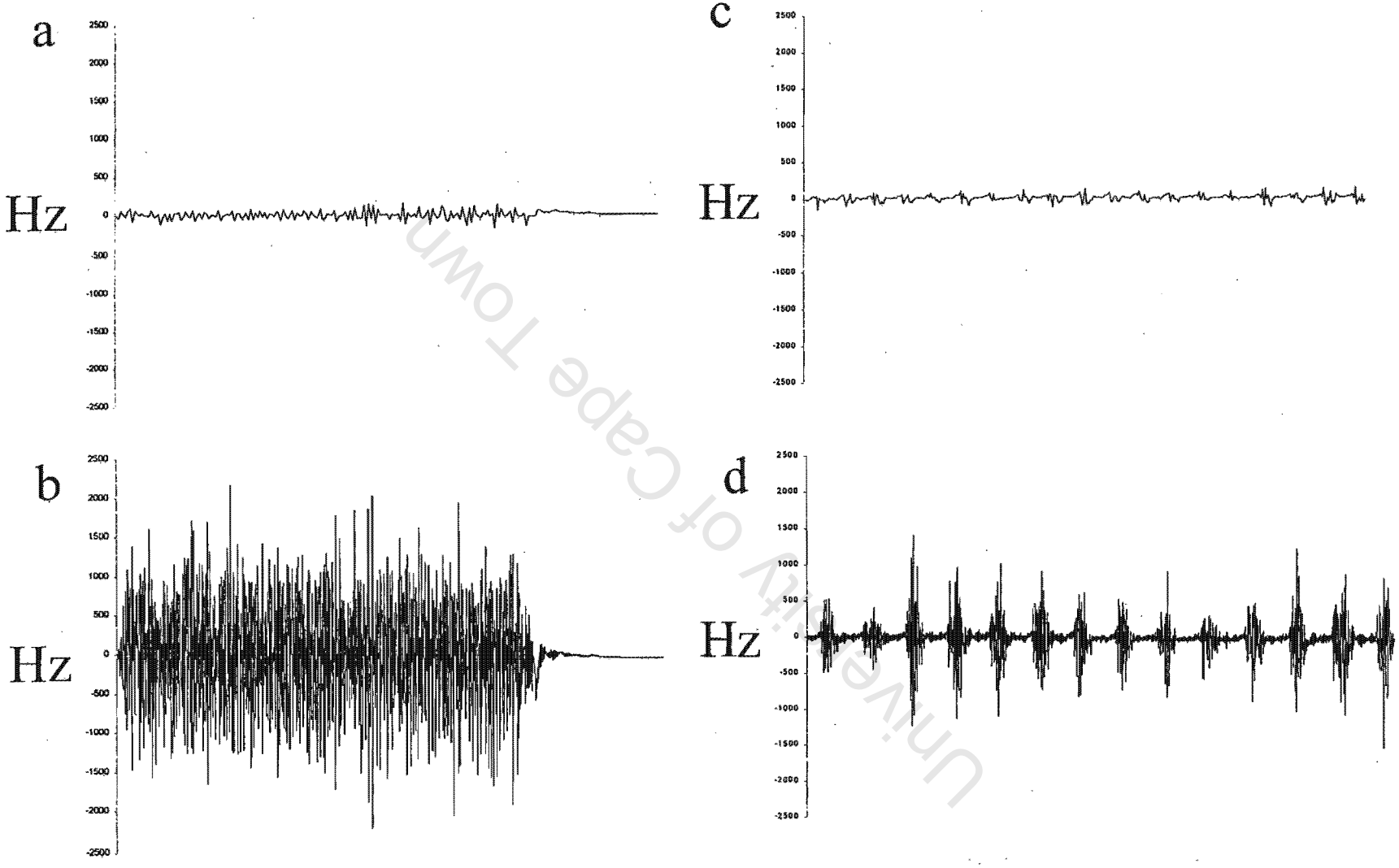


Figure.2.3. Individual raw data captured over a period of 5 seconds for MVC at (a) 32Hz and at (b) 1984Hz and cycling exhaustion at (c) 32Hz and (d) at 1984Hz.

2.3.2 The effect of different EMG capture rates

Table 2.2 and Figure 2.4 shows the effect of different EMG capture rates during MVC, PWATT and SUB. Highly significant differences ($P < 0.01$) were found for all frequencies during each activity. Highly significant differences ($P < 0.01$) are shown (Figure 2.4) with asymptote values of 326.2 ± 105.4 Hz for SUB, 503.1 ± 236.2 Hz for PWATT and 1604 ± 235.6 Hz for MVC. The linearity above the asymptote value for MVC, PWATT and SUB indicates consistent and reliable recordings, whereas below shows differing profiles and wider variability, therefore unreliable recordings.

Table 2.2. Sampling frequency of mean EMG (Mv) during maximal voluntary contraction (MVC),cycling exhaustion (PWATT), and 50% of peak power output (SUB).

	32	64	128	256	512	1024	1984
MVC	41.4 ± 21.8 *	75.9 ± 25.8 *	121.6 ± 46.4 *	195.3 ± 65.5 *	229.1 ± 75.1 *	241.5 ± 78.6 *	244.1 ± 78.7
PWATT	65.8 ± 75.5 *	70.6 ± 73.9 *	76.8 ± 72 *	84 ± 75.1 *	84.8 ± 71.9 *	85.7 ± 71.9	83.6 ± 72.9
SUB	25.6 ±16 *	28.2 ±16.3 *	25.6 ± 16.1 *	32.4 ± 15.1 *	37.3 ± 17.1 *	36.9 ±17.1	37.3 ± 16.4

Values are means ± SD

* - $P < 0.01$ MVC, 32 – 1984 Hz. PWATT, 32 – 512 Hz. SUB, 32 – 512 Hz.

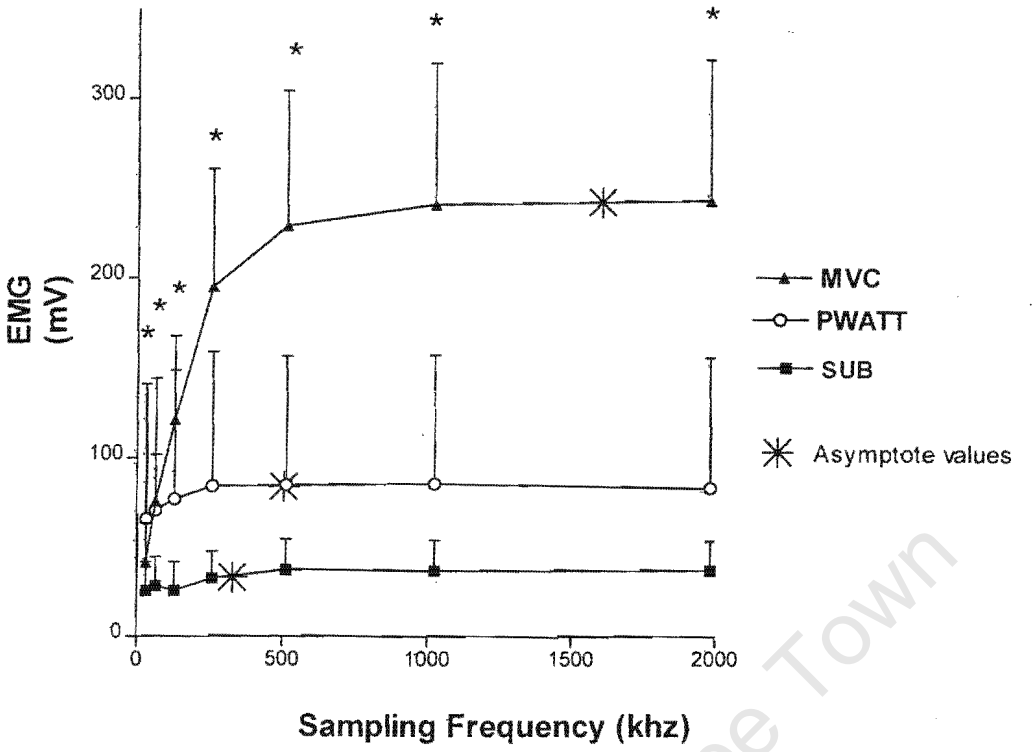


Figure 2.4. The effects of sampling frequency of mean EMG during maximal voluntary contraction (MVC), cycling exhaustion (PWATT), and 50% of peak power output (SUB) (* - $P < 0.01$ main effect). Highly significant differences ($P < 0.01$) are shown by the marked asymptote values of 326.2 $\pm$ 105.4 Hz for Sub, 503.1 $\pm$ 236.2 Hz for PWATT and 1604 $\pm$ 235.6 Hz for MVC.

2.3.3 *The difference between activities*

Table 2.3 and Figure 2.5 shows the comparison between PWATT and MVC of all the capture rates of each activity as a percentage of its' capture rate of 1984 Hz. The largest percentage difference at MVC, between 32Hz and 1984 Hz capture rate, was approximately 68%, as compared to the PWATT difference, which was less than 20%.

Table 2.3. Percentage of maximal data (%) captured at 1984 Hz for both cycling at exhaustion (PWATT) and maximal voluntary contraction (MVC) at 32 – 1024 Hz.

	32 Hz	64 Hz	128 Hz	258 Hz	512 Hz	1024 Hz
PWATT	69 ± 16	77.0 ± 16.3	68.0 ± 16.1	87.0 ± 15.1	100 ± 17	99 ± 17.4
MVC	17.0 ± 21.8	31.0 ± 25.9	49.8 ± 46.5	80.0 ± 66.6	93.0 ± 75.1	98.0 ± 78.6

Values are means ± SD

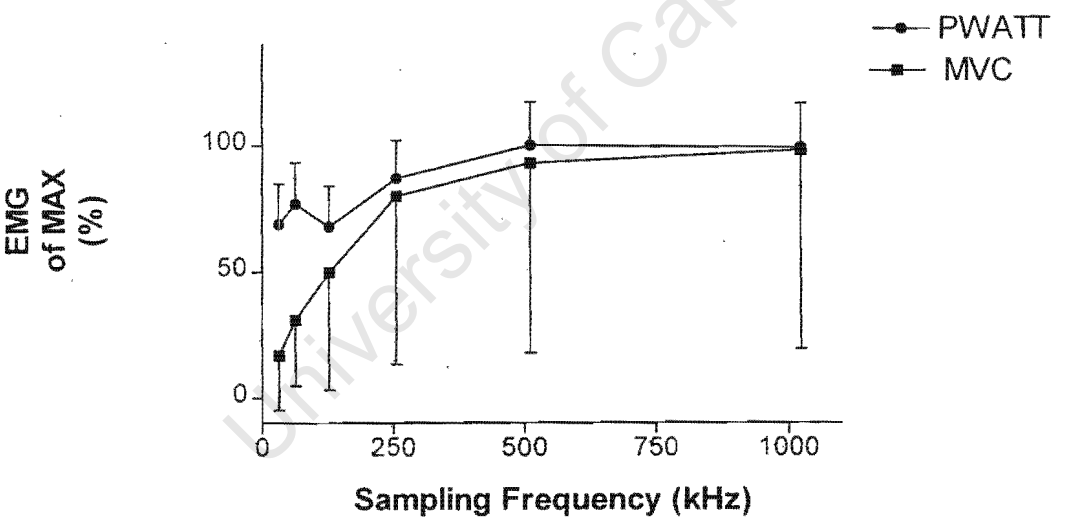


Figure 2.5. Capture rates (32, 64, 128, 256, 512 and 1024 Hz) of cycling at exhaustion (PWATT) and maximal voluntary contraction (MVC) displayed as a percentage of their respective highest capture rate of 1984 Hz.

2.3.4 Individual frequency spectrum data

Figure 2.6 shows the individual frequency spectrum data sampled at 1984 Hz that is normalised to the maximum peak amplitude achieved within that activity, to enable comparison between MVC and PWATT. Figure 4 a and b describes an individual's data which was captured during MVC whilst Figure 4 c and d is captured throughout PWATT. Figure 2.6 b and d is a magnified version of a and c respectively and shows data up to 30 Hz. According to the Nyquist theorem (259), 16 Hz and below are the frequencies of data that are collected when using a capture rate of 32 Hz.

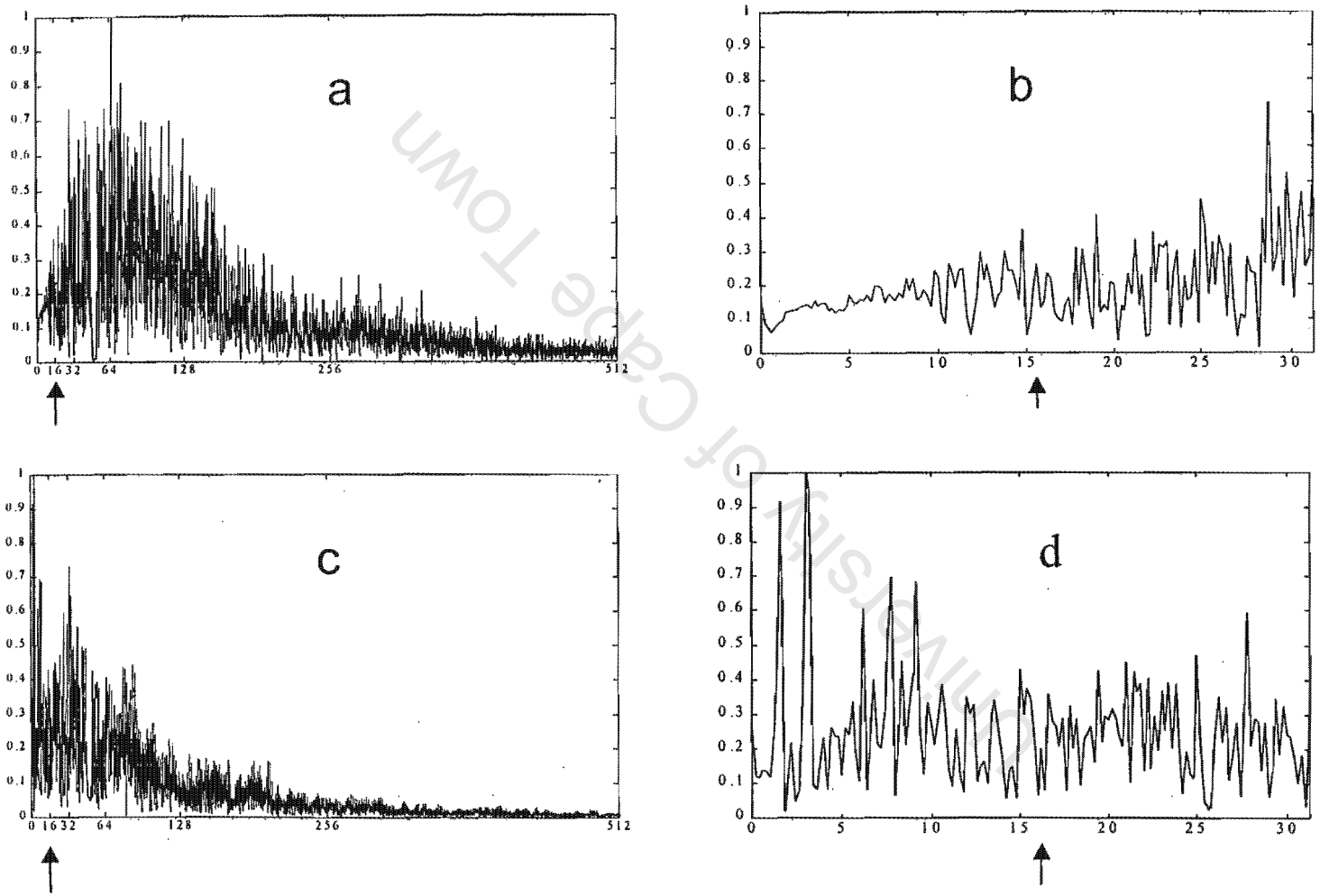


Figure 2.6. Individual data displaying frequency spectra normalized to the maximum. a and b is data captured during MVC, whilst c and d is data captured throughout PWATT. According to the Nyquist theorem, the arrow shows that while using a capture rate of 32Hz, data will only be collected at <16 Hz (data to the left of the arrow).

2.3.5 Force/voltage

Table 2.4 shows power expressed as force (N) to enable calculation of the force per voltage EMG (N/V) relationship from the varying rates. Subject 6 was omitted from the data because of unusually high amounts of EMG artifact recorded. There were no significant differences in the values captured at 1984 Hz (3.8 ± 1.7 N/V vs 2. ± 0.9 5N/V). However, significant differences found ($P < 0.01$) at 32Hz capture rate (6.2 ± 3.8 N/V vs 16 ± 8 N/V).

Table 2.4. Newtons/volt (N/V) data of cycling at the point of exhaustion (PWATT) and maximal voluntary contraction (MVC) whilst captured at rates of 1984 Hz and 32 Hz.

Subject	PWATT 1984 Hz	MVC 1984 Hz	PWATT 32 Hz	MVC 32 Hz
1	2.4	3.4	3.1	31
2	2.7	1.1	3	3.7
3	4.5	2.5	3.2	11.2
4	6.5	3.8	14	24.5
5	2.5	3.1	2.8	18.4
6				
7	3.5	2.6	8.2	13.9
8	6	2.7	9.3	15.7
9	1.6	1.4	6	11.5
10	4.4	1.7	6.4	14.4
Mean	3.8 ± 1.7	2.5 ± 0.9	6.2 ± 3.8**	16.0 ± 8.0

All values are mean ± SD

** - $P < 0.01$ Ex N/V at 32 Hz vs MVC N/V at 32 Hz

2.3.6 *Correlations*

Low correlations were found between EMG activity and lean thigh volume, and MVC, whilst a non significant negative correlation was seen for body fat percent (Table 2.5). Similarly, no correlations were found between EMG activity and PPO and PWATT.

Table 2.5. Mean lean thigh volume cc (LTV), body fat percentage (%), cycling exhaustion power output (PWATT), maximal voluntary contraction (MVC) and peak power output (PPO) correlated with IEMG amplitude captured at 1984Hz during cycling exhaustion (PWATT).

	r	P
LTV	-0.36	0.31
Body fat %	-0.63	0.68
PPO	-0.04	0.19
PWATT	-0.5	0.14
MVC	0.28	0.48

2.4 DISCUSSION

The main findings of this study were, i) that significant differences in EMG amplitude were found for the different EMG capture rates, with varying EMG amplitudes from 32 Hz to 1984 Hz capture rates; ii) that asymptote values for MVC (1604 $\pm$ 235.6 Hz), PWATT (503.1 $\pm$ 236.2 Hz) and SUB (326.2 $\pm$ 105.4 Hz) were significantly different; iii) the force/EMG relationship for PWATT and MVC was significantly different ($P < 0.01$) at 32 Hz capture rate but were similar at 1984 Hz capture rate.

For MVC, PWATT and SUB differences in EMG amplitudes were observed for all the capture rates. The absolute difference in amplitude between MVC, PWATT and SUB at all the capture rates is to be expected, as different activities and exercise intensities will result in different neuromuscular recruitment patterns. However, at the lower capture rates it is clear that significantly less amplitude is recorded. It is logical to conclude that when, for example, using a low capture rate of 32 Hz on an activity that is generating approximately 500 mV of amplitude, that only a small proportion of that signal will be recorded (Figure 2.5). However, this may not be a limitation. In Figure 2.3, if the gradient of increase in generated amplitude with capture rates for MVC, PWATT and SUB were the same, it may be acceptable to use low capture rates for all activities and exercise intensities as the data would be comparable. Moreover, it would be possible to capture data at 32 Hz and by applying a formula based on the exponential rise in amplitude with capture rates, accurately predict what the amplitude would be if captured at 1984 Hz. However, because asymptote values are significantly different between MVC,

PWATT and SUB, means that the gradient rise in amplitude with capture rates is different between activities. This difference in gradient is described in Figure 2.4, which shows the percentage difference between 32 Hz and 1984 Hz capture rate was approximately 68% for MVC, while PWATT was less than 20% difference. This would therefore suggest that to use a capture rate of less than 1604 Hz for MVC, 503.1 Hz for PWATT and 326.2 Hz for SUB is unreliable because a different proportion of the signal is being captured. The cause of this difference at low capture rates could be that not sampling at rates that are twice that of the highest frequency generated will cause aliasing (259). For example, Figure 2.5 shows that if a capture rate of only 32 Hz is used, only 16 Hz will be captured and when comparing MVC with PWATT, the majority of the MVC data will not be captured at this low capture rate. However, in this study the EMG equipment used, employed the use of anti-aliasing filters, which will prevent aliasing from occurring. Therefore, some other condition is occurring which is causing such large variability at low capture rates between activities.

A possible cause of this large variability could be first, from motion artifact generated during cycling which is shown by the large spikes in Figure 2.5 d. Motion artifact could be caused from any relative movement of the electrode and tissue, which would occur during the continual action of cycling, unlike the minimal movement during MVC. As the electrode and skin tissue have different electrical properties, contact between the two will cause general polarization potential, which occurs through a lack of chemical equilibrium (29). When using high capture rates, it is possible to use a high pass filter, which will smooth and rectify the signal to take out the effects of motion

artifact. However, when using low capture rates it is not possible to use a high pass filter, as there will be minimal signal left after the signal has been smoothed and rectified. For example, motion artifact is considered to occur at EMG signals of approximate frequencies < 10 Hz, therefore, if according to Nyquist a capture rate of 32 Hz is used for SUB and PWATT, only 16 Hz of the signal is captured, 10 Hz of it will be motion artifact (Figure 2.5) (29), therefore resulting in a unreliable signal. However, this only explains the different asymptotes between MVC and cycling, but not between SUB and PWATT. If the difference in asymptote values were due to motion artifact, a lower asymptote value would be expected in PWATT than SUB because of a more aggressive motion producing extra motion artifact. A possible explanation for this occurrence is that in PWATT there is more amplitude generated than SUB, which is probably due to additional motor unit recruitment from the increase in force production (381). From this increase in motor unit recruitment, it can then be assumed that there is an accompanying rise in median frequency (37), which means higher frequencies in the spectrum therefore resulting in higher asymptote values in PWATT.

Second, the action of prolonged cycling during PWATT will increase the temperature of the muscle, resulting in decreased EMG amplitude (421) in comparison to MVC. This decrease could be as a result of altered tissue characteristics acting as a low pass filter (535). It is therefore possible that this "low pass filter" may filter out different portions of the PWATT signal received at low capture rates in comparison to the signal received at high capture rates.

Also, the force/EMG relationship displayed highly significant differences between PWATT and MVC at 32 Hz and no significant differences at 1984 Hz. The force/EMG signal should be similar for any given activity or intensity. Again, this suggests that to use a capture rate as low as 32 Hz is unreliable when comparing different activities and/or intensities for quantitative data assessment. Possible causes are by sampling at 32 Hz, which is below the 1604 Hz asymptote for MVC and 503.1 Hz asymptote for PWATT, results in capturing an unreliable portion of the signal, possibly due to motion artifact and increase in muscle temperature generated in PWATT or other reasons not elucidated in this study.

Finally, low correlations found between EMG activity at 1984 Hz during PWATT and lean thigh volume, MVC, PPO and PWATT, suggests that no regression formula can be applied to predict EMG activity at differing amounts of force output. This suggests that EMG amplitude amounts during cycling activity will represent the individual's distinct neural recruitment pattern, which will only be proportionate to their own maximal amount of recruitment. The possible causes for these low correlations are first, lean thigh volume showed little correlation with total EMG activity because of varying quantities of subcutaneous fat deposits at the electrode placement site, which will invariably interfere with the level of EMG activity. Second, PWATT and PPO showed poor correlation with EMG activity, perhaps due to variables such as individual cardiorespiratory efficiency influencing the output at fatigue. Finally, MVC is affected by the position of the electrode, the fiber typing (proportion of fast twitch to slow twitch muscle fibers) and the inclusion of synergist muscle groups, which may be different to those used during cycling activity (219).

Although not significant, body fat percentage showed a negative correlation, meaning that the higher the individual's body fat the lower the amplitude recorded. This would indicate that increasing body fat acts as filter which impedes the signal. However, body fat percentage was predicted by means of skinfold caliper, which often serves as a poor calculation due to the variability of fat deposited in the skinfold site. Consequently a correlation was *computed* with the thigh skinfold measurement, but this also showed to be low due to the wide variability in fat deposits within each subject.

In summary, MVC, PWATT and SUB showed differences in all the EMG capture rates tested, differences in asymptote values and highly significant differences between PWATT and MVC at 32 Hz and no significant differences at 1984 Hz in the force/EMG relationship. This suggests that data captured at low frequencies is unreliable because the possible consequences of motion artifact will be exaggerated at low capture rates during cycling resulting in proportionately varied amplitude and a different proportion of the frequency spectrum profile captured. Also, low correlations between EMG activity at 1984 Hz and lean thigh volume, MVC, PPO and PWATT and an insignificant negative correlation for body fat percentage, suggests that no regression formula can be applied to assume EMG activity at differing amounts of force output.

In conclusion this study shows that EMG data captured below the asymptote values 1604 Hz for MVC, 503.1 Hz for PWATT and 326.2 Hz for SUB at 32 Hz is unreliable. It is therefore suggested that EMG data for MVC, PWATT and SUB activity is captured above the aforementioned asymptote values for

qualitative data analyses. Therefore, we decided that the highest capture rate on our EMG equipment of 1984 Hz would be used for all data collection in this thesis. This would enable inter activity comparison and ensure that it is at least twice as high as the maximum amplitude generated.

CHAPTER THREE

EMG NORMALISATION FOR CYCLE FATIGUE PROTOCOLS

3.1 INTRODUCTION

Electromyography (EMG) is used to display muscle activation patterns and has been extensively employed to interpret both dysfunctional and functional muscle recruitment patterns related to cycling activity (219).

Absolute EMG signals only provide the investigator with a subjective measurement of muscle activation. This subjectivity is caused by first, high levels of neural recruitment inter-subject variability and second, the inaccuracy associated with using different surface electrode placement sites when conducting repeated trials. To overcome these problems of quantifying an EMG signal so that the muscle's relative activity can be assessed it is necessary to use a normalisation technique. Comparing a specific EMG muscle activity with a reference EMG value and expressing the activity of the muscle as a percentage of this reference value can establish relative muscle activity. This enables the conversion of subjective EMG values into data points that have a significant interpretative meaning (376).

EMG normalisation allows for comparisons obtained over a variety of conditions. Between subject comparisons can be made after normalising the EMG activity, because the reference activity for a given subject is compared with the relative amount of activity for that subject and is therefore dependent on each individuals' own proportion of maximal activity. The investigator then has the ability to compare the relative EMG for a given workload across subjects. Furthermore, EMG normalisation also allows for slight changes in variables such as electrode placement and skin impedance (376).

For EMG normalisation to be effective the reference activity has to be reliable and repeatable. Knuston et al. (297) compared dynamic with isometric leg extension. Their results showed that the isometric leg extension tests had a higher intra-class correlation coefficient between trials compared to dynamic leg extension, therefore improving the reproducibility of the data.

As well as being a reliable reference point, the EMG normalisation method should also have relevance to the given task. Yang et al. (542) concluded that the isometric leg extension maximal voluntary contraction (MVC) is the only method that aims to reveal the percentage of the maximum activity of the muscle performance requirement to a specific task. However, when normalising EMG data to a dynamic task such as cycling there are a number of differences in the actions of the muscles involved that make the relevance of isometric MVC questionable.

Previous researchers have documented that a maximum contraction implies that all motor units are firing at their maximum rate (365). To obtain a maximum contraction during static activity it is essential to determine the optimal knee angle. Previous studies have shown (59;479) that during isokinetic activity, the optimal knee angle (reference 0° with the leg in full extension) for peak force during concentric activity was between 60° and 65°. In support of this, it has been widely recognized that the EMG recorded during isokinetic MVC's of knee extensor muscles is largest in mid range of motion (56;154), and is greater for concentric in comparison to eccentric activity (7;154;525). However, Bolourchi and Hull (60) reported that during cycling, the

peak pedal load was recorded between 90 and 100° out of the 360° cycle (0° being with the pedal at 12 o'clock), which means that the knee angle is greater than 60° at this pedal position. We therefore estimated a knee angle of 108° for peak force by seating a subject on the bike, setting the pedal at 95° and measuring the angle of that knee.

To the authors' knowledge there are no data on the most effective EMG normalisation protocol for cycle fatigue protocols. Accordingly, with the aim of determining the most effective normalisation procedure for cycling protocols, we designed four contractions for EMG measurement: i) isometric MVC; ii) isometric cycle pedal contractions of 60° (60°A) and; iii) 108° (108°A), and; iv) a one dynamic maximal pedal contraction (1REV).

3.1.1 Question

The question addressed by this study is:

2. What is the most effective method for normalising the EMG signal for submaximal cycling to enable intersubject comparison?

3.2 METHODS

3.2.1 Subject selection and sampling

Ten healthy males volunteered for this study. The mean age and body mass of the subjects were 21.4 ± 2.6 years and 72.3 ± 8 kg respectively. All subjects were physically active and each signed an informed consent before the study. The Research and Ethics Committee of the University of Cape Town Medical School approved the study.

3.2.2 Experimental trial

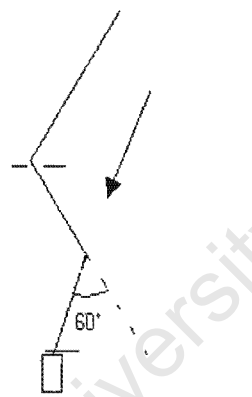
3.2.2.1 *MVC testing*

The MVC methods are as described in chapter 2.

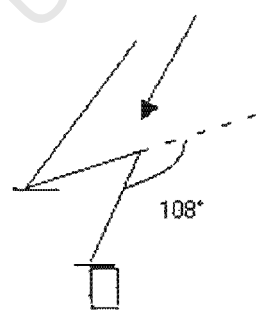
3.2.2.2 *Fixed pedal isometric maximal voluntary contraction*

The subject was positioned on to an electronically braked cycle ergometer (Watsoft, Watsystem, ITC Corp, Bloomington, USA). The saddle height was adjusted to a position chosen by the subjects to be the most economical for their own cycling performance. With the use of a goniometer a knee joint angle of either 60° (60°A) or 108° (108°A) (from full leg extension) (Figure 3.1 a and b) was determined by placing the axis of rotation on the lateral femoral condyle and lining up each of the two arms on the centre plane of the femur and fibula. A wooden block was built to a dimension of 16 x 8 x 8 cm with a

deep groove inserted across the 8 x 8cm section to accommodate the bottom of the peddle. The block was then positioned underneath the pedal and its height was adjusted to fit the required knee joint angle by placing the appropriate number of wooden tiles between the floor and block. Each subject performed four sub-maximal familiarisation contractions prior to performing two maximal isometric contractions, the latter of which were used for subsequent analyses. All subjects were encouraged verbally to exert maximal effort during both contractions.



(a)



(b)

Figure 3.1 a and 3.1 b. Isometric maximal voluntary contractions fixed cycle pedal contractions at knee joint angles of 60° (60°A) (a) and 108° (108° A) (b). The arrow indicates direction of force.

3.2.2.3 *One revolution maximum (1REV)*

The feet of the subjects were firmly strapped into the pedals and the right knee joint set at a starting angle of 108° using a goniometer as described above. With a starting load of 0.7 $kg.kg^{-1}$ BW the subject was instructed to pedal as many revolutions as possible, if two or more revolutions were completed the subject was instructed to stop. The protocol was continually repeated by adding 0.05 $kg.kg^{-1}$ BW on the starting load each time until the subject could no longer complete a full revolution of the pedal. The data from the last single full revolution was subsequently used for analyses.

3.2.2.4 *EMG testing*

Prior to maximal isometric strength testing on the Kin-Com isokinetic dynamometer, the same EMG methodology was applied as described in chapter 2. In this instance however, EMG was recorded for 5 seconds during MVC, 60°A, 108°A and for the time taken to complete 1REV. The EMG signal was captured at 1984 Hz, notch filtered at 50 Hz to limit electrical interference and antialiased by hardware.

The raw EMG signals were full wave rectified, movement artefact removed using a high-pass second order Butterworth filter with a cut off frequency of 15 Hz, then smoothed with a low-pass second order Butterworth filter with a cut-off frequency of 5 Hz. This was performed using MATLAB™ gait analysis software. This IEMG was used for subsequent analysis.

The subject variability for the different contractions was expressed as a proportion of their means by the coefficient of variation (CV):

CV = (SD/mean) x 100%

Joules (work) was instantaneously calculated by both the electronically braked cycle ergometer and isokinetic dynamometer upon completion of the task.

3.2.3 Statistical analyses

A one way ANOVA (with repeated measures) was used to evaluate statistical significance of the variables measured. A Scheffe's post hoc test was used to detect differences of the activities measured. Single comparisons between activities for work/EMG were analysed with a paired Students t-test using two-tailed values of P. Significance was accepted as $P \leq 0.05$, and all data expressed as mean $\pm$ SD.

3.3 RESULTS

3.3.1 *IEMG*

Integrated EMG (IEMG) for MVC was significantly ($P < 0.01$) greater than 60°A, 108°A and 1REV (Table 3.1, Figure 3.2). Table 3.1 shows that 60°A, 108°A and 1REV produced 93, 86 and 75% respectively, less IEMG than MVC. 108°A produced 50% more IEMG than 60°A. Inter subject coefficient of

variation for IEMG during MVC, 60°A, 108°A and 1REV was 57, 71, 82 and 45 % respectively.

Table 3.1. Mean amplitude values (IEMG)(mV) captured for maximal voluntary contraction on dynamometer (MVC), fixed pedal with knee angle at 60° (60°A) and 108° (108°A) and one maximal revolution of the pedal crank (1REV).

Subject	MVC	60°	108°	1REV
1	43.8	5.8	21.4	8.2
2	71.6	29.3	14.9	57.6
3	137.4	8.8	21.8	74.5
4	397.2	6.1	23.3	32.8
5	198.9	5.2	14	102.7
6	138.1	18.5	48.6	45.9
7	281.3	24.7	101.4	72.2
8	318.9	5.2	27.8	50.3
9	401.8	18.9	27.7	69.1
10	316.6	35.1	18	52.4
Mean	230.6	15.8	31.9	56.6
	± 130.5 **	± 11.2	± 26.3	± 25.7

All values are mean ± SD

** - P < 0.01 MVC vs 60°A, 108°A, and 1REV

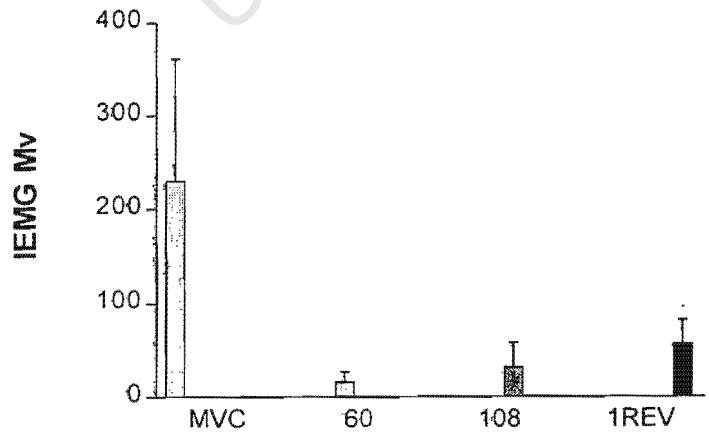


Figure 3.2. Mean IEMG captured MVC, 60°A, 108°A and one maximal revolution of the pedal (1REV).

3.3.2 *Work/EMG relationship*

Although there were no significant differences between MVC and 1REV work/EMG relationship, MVC produced 37% more work/EMG than 1REV (Table 3.2).

Table 3.2. Work/IEMG ($J.Mv^{-1}$) for both maximal voluntary contraction (MVC) and one maximal revolution of the crank (1REV).

Subject	MVC	1REV
1	6.3	1.1
2	8.3	15.2
3	36	7.3
4	12.3	4
5	11.9	13.5
6	22.1	9.4
7	44.3	11.4
Mean	20.2 ± 8.8	14.7 ± 5.1

All values are mean ± SD

3.3 DISCUSSION

The findings of this study were that MVC produced greater IEMG activity than 60°A and 108°A, which would suggest a higher rate of motor units firing, or recruitment of a greater number of motor units. This could be caused by first, a greater force output in the MVC group. Although force was not measured in 60°A and 108°A, previous studies have shown that during isometric contractions the EMG signal is proportional to the amount of force generated by the muscle (351).

Second, MVC involves just the knee joint whereas 60°A and 108°A involves the hip, knee and ankle joint. During MVC the subject is tightly strapped into the chair to minimize movement and isolate contraction of the quadriceps femoris muscle group. Throughout 60°A and 108°A, twelve superficial muscles that represent the prime movers involved in imparting energy to a bicycle are likely to be used (219). Subsequently, it is possible that because of the number of synergists involved, they will not all be contracting maximally and simultaneously, which results in a lesser IEMG recording from the rectus femoris. Moreover, if this is the reason for the differences found in this study, reduced IEMG may not only be a consequence of reduced force output during 108°A and 60°A but also a reduced force/EMG relationship. Thus, 60°A and 108°A actions could result in trade off synergism, which is an attempt to delay fatigue by adapting muscle activities so that the load can be sustained (466).

Third, it has been suggested that during leg press activity the rectus femoris muscle may act as an antagonist by flexing about the hip joint therefore

reducing its activity in comparison to the knee extensors (541). A similar situation could occur with 60°A and 108°A in comparison to MVC. The EMG activity was assessed in the rectus femoris, which could result in reduced IEMG activity. However, it has been suggested that there is antagonistic activity of the bicep femoris, which will progressively increase to stabilize the knee and reduce the force output during leg extension (29). This being the case, a reduced IEMG activity would also be observed during MVC. Interestingly, Alkner (4) showed no variance in IEMG results between both leg extension and leg press and concluded that isometric actions performed at a high degree of flexion about the knee joint is very similar in the multi-joint leg press and single joint leg extension. However, in comparison to the leg press 60°A and 108°A actions are likely to involve a far greater number of synergist muscles and because the subject is sitting on a cycle saddle will allow the body weight to transfer over to assist the contracting leg, which would also account for the larger variations for these contractions between subjects.

There was a higher IEMG for MVC than 1REV and higher, though not significant greater work/EMG relationship for MVC. The increase in IEMG is probably caused by a smaller force output for 1REV. However, it is surprising that 1REV did not produce more IEMG, when the action had increasing angular velocity. IEMG increases with increasing angular velocity during concentric contractions (7;154;525). Hunter et al. (Unpublished data) recorded higher peak IEMG throughout a 30 second Wingate cycle protocol than for a 5 second MVC, which was due to a very high angular velocity from pedalling speeds in excess of 120 rpm. Nevertheless, because there was insignificantly more work/EMG during MVC, it can be assumed that the angular velocity

achieved during 1REV was insignificant. Furthermore, as with 60°A and 108°A, 1REV involves more synergist muscles, which will inevitably produce a lower work/EMG relationship than MVC.

Finally, there was a tendency for IEMG during 108°A to be higher than IEMG for 60°A. Assuming that this was different then it can be explained from a more efficient knee angle, which allows for a greater generation of force production when the knee is fixed at 108°. However, as described earlier because there are so many synergist muscle groups and the ability to transfer the body weight, any efficiency gained from a different knee angle will be far outweighed by other factors and result in minimal IEMG differences.

In summary IEMG was significantly greater in MVC than 60°A and 108°A due to either greater force output in MVC, more synergist muscle involvement in 60° and 108° causing a decrease in force/EMG relationship and flexing of the hip joint throughout 60° and 108° by the rectus femoris acting as an antagonist resulting in a reduced IEMG. Second, MVC produced more IEMG than 1REV, however there was no significant difference in the work/EMG relationship which could have been caused by 1REV producing less force, greater inclusion of synergist muscles and minimal angular velocity. Finally, there was a tendency for IEMG for 108°A to be higher than IEMG for 60°A, possibly due to more efficiency gained from a different knee angle, however, other factors such as shifting of body weight and inclusion of synergist muscle groups in 60°A to compensate for a comparatively inefficient knee angle will effect the IEMG.

In conclusion, this study has shown that MVC will record a higher IEMG than 60°A, 108°A and 1REV and that there is no significant difference in the force/EMG relationship between MVC and 1 REV. It would appear that MVC has fewer variables to affect both IEMG and force output. However, further investigation is required to investigate the force output/EMG relationship and reliability for 60°A and 108°A. This work supports previous findings that MVC produces highest levels of EMG, and therefore will be more effective in describing maximal muscle recruitment activity and suggests that MVC can be used as a normalisation procedure for dynamic cycling activity.

CHAPTER FOUR

THE EFFECTS OF HOT AND COLD ENVIROMENTS ON ELECTRICAL ACTIVITY OF SKELETAL MUSCLE IN ENDURANCE EXERCISE.

4.1 INTRODUCTION

As described in the previous two chapters a repeatable, reliable methodology has been developed for measuring EMG activity during successive, incremental, submaximal cycling to fatigue.

To gain a better understanding of neuromuscular recruitment in fatigue we first decided to study the effects of different environmental temperatures on subjects' neuromuscular recruitment patterns during submaximal exercise to fatigue. The onset of fatigue is either prolonged by cold environments (63;250;316) or shortened by hot environments (192). Traditionally, it is believed that this occurrence is mainly governed by metabolic mechanisms (77). However, recent studies (516) have indicated an additional central involvement. As discussed in section 1.4.2, all studies of the effect of different temperatures on the recruitment patterns of skeletal muscle have been studied in isolation, without the involvement of other mechanisms caused by submaximal fatigue. Therefore studies to determine the neural recruitment patterns and spectral shifts during submaximal exercise to exhaustion require further investigation. Accordingly, we examined the effects of hot (35 °C) and cold (15 °C) environments on IEMG and MPFS during successive, submaximal, incremental cycling to fatigue.

4.1.1 Question

The questions addressed by this study is:

3. What is the effect of both hot and cold conditions on neuromuscular recruitment patterns during submaximal fatigue?

4.2 METHODS

4.2.1 Subject selection and sampling

Eight healthy males volunteered for this study. All subjects performed recreational sporting activities three or more times per week. The mean (± SD), age, height, body mass and peak power output (PPO) of the subjects were, 25.5 ± 4.4 yr, 179.5 ± 7.9 cm 73.4 ± 11.9 kg and 298.5 ± 47.9 W. All subjects were physically active and each signed an informed consent before the study. The Research and Ethics Committee of the University of Cape Town Medical School approved the study.

4.2.2 Preliminary testing

4.2.2.3 *PPO testing*

PPO was determined as described in chapter 2.

4.2.3 Experimental trial

4.2.3.1 *MVC testing*

In the week after PPO testing, MVC testing was performed to normalise EMG recordings during cycling and were determined as described in chapter 2.

4.2.3.2 *Environment and temperature recordings*

Following the MVC, the subjects reported to the heat chamber and inserted a rectal thermometer (Mon-a-therm, Mallinckrodt, OH, USA) 10cm beyond their anal sphincter. Four surface thermometer (YSI 400, Yellow Springs, OH, USA) were then taped to the sternum region, left bicep, left thigh and left calf. The subjects then completed one of the two progressive cycle tests in an environmental chamber (Scientific Technology Corporation, Cape Town, South Africa) at an ambient temperature setting of 15° or 35°C, a relative humidity of 50 $\pm$ 0.9 % and a wind velocity of 15 $\pm$ 0.4 $km.h^{-1}$.

4.2.3.3 *Progressive exercise test*

As with the preliminary PPO testing, the Lode ergometers were used for the endurance testing, the subjects performed the same progressive exercise test as described in chapter 2. Rating of perceived exertion (RPE) and thermal comfort were determined as described by Borg (67) and Bedford (31) respectively. At rest, 10, 25, 40 minutes and at exhaustion, skin and rectal temperatures, heart rate, RPE and thermal comfort were recorded (Figure

4.1). The ride was repeated again with the same methods but during a different temperature.

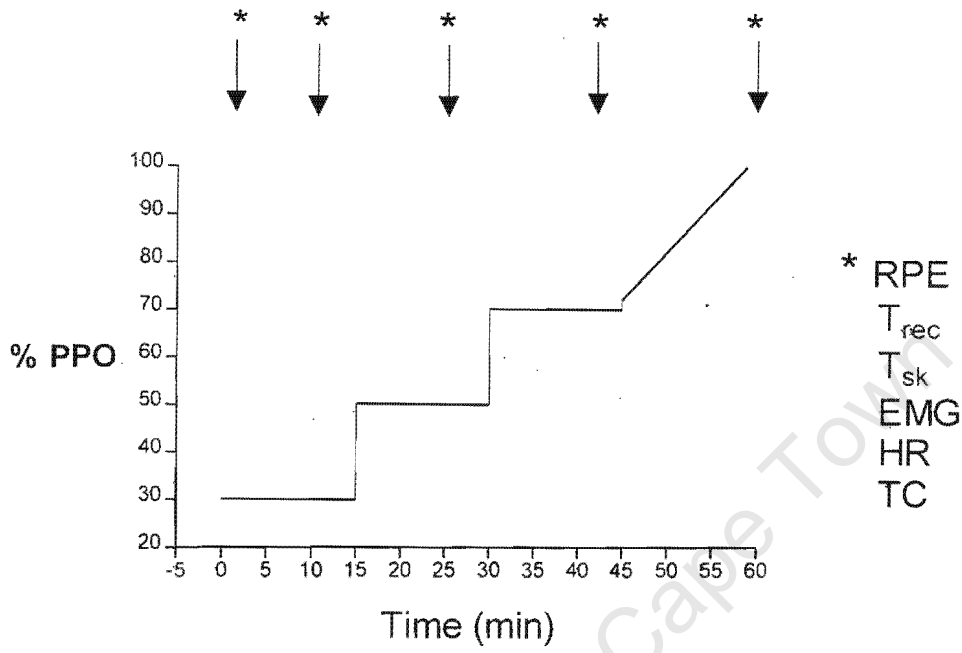


Figure 4.1. Progressive cycle exercise test protocol. Rate of perceived - exertion (RPE), rectal temperature (T_{rec}), skin temperature (T_{sk}), EMG, heart rate (HR) and thermal comfort were all recorded at rest, 10, 25, 40 minutes and at exhaustion during the ride.

4.2.3.4 *EMG testing*

Before MVC, the same EMG methodology was applied as described in chapter 3. The raw signals were processed as described in chapter 3. Each activity was sampled at a 1984 Hz capture rate for 5-second bouts. Recordings were taken on the second maximal isometric trial and during the cycling trial at 10, 25, 40 minutes and at exhaustion (Figure 4.1) thus yielding a raw signal. MVC EMG data was recorded before both cycle rides to ensure

similar normalisation of EMG in both trials. The raw data were divided into 5 x five second epochs. The first epoch included all data collected during the second MVC trial, and the remaining four epochs included data collected on the ride at 10, 25, 40 minutes and at exhaustion.

The EMG signals were full wave rectified, movement artifact removed using a high-pass second order Butterworth filter with a cut off frequency of 15 Hz, then smoothed with a low-pass second order filter with a cut off frequency of 5 Hz. This was performed using MATLAB™ gait analyses software. This integrated data (IEMG) was used for subsequent analyses.

The spectrum of the frequency for each epoch of data collected during the cycle ride was assessed using the raw EMG data by using a fast Fourier transformation algorithm. The analyses for frequency spectrum were restricted to frequencies of the 5-500 Hz range, due to the EMG signal content consisting mostly of noise when it is outside of this bandwidth. The frequency spectrum from each epoch of data was compared with that derived from the MVC, and the amount of spectral compression was estimated. This technique was performed as described by Lowery et al. (332), which is a modification of the work of Lo Conte and Merletti (329) and Merletti and Lo Conte (362). The spectrum of the raw signal of each epoch was obtained and the normalised cumulative power at each frequency was calculated for each epoch. The shift in percentile frequency was then examined (i.e. at 0%...50%...100% of the total cumulative). The percentile shift was then estimated by calculating the mean shift in all percentile frequencies throughout the mid-frequency range (ie. 5-500 Hz). This method has been suggested as a more accurate estimate

of spectral compression than median frequency analyses, which uses single value of (50th) percentile frequency (332). This change in mean percentile frequency (MPFS) data was used for subsequent analyses.

4.2.3 Statistical analyses

All data are expressed as means $\pm$ SD. A two-way ANOVA for repeated measures was used to evaluate statistical significance of all the variables measured. Post hoc analyses of the main effect of time were done using a Scheffe's test. Single comparisons between temperatures were analysed with a paired Students t-test using two-tailed values of P. Significance was accepted at $P \leq 0.05$.

4.3 RESULTS

4.3.1 *Performance*

There were no significant differences for maximum force output at exhaustion (PWATT) and time to exhaustion (TIME) between the 35°C ride (HOT) than the 15 °C ride (COLD) (Table 4.1).

Table 4.1. Peak watts (PWATT) coinciding with volitional exhaustion: and time taken to reach exhaustion (TIME) during 15 °C (COLD) and 35 °C (HOT) trials.

	COLD	HOT
PWATT (W)	267.4 ± 67.7	250.1 ± 61.5
TIME (min)	55.7 ± 16.7	54.5 ± 17.1

All values are mean ± SD

4.3.2 *Skin temperature*

All skin temperature values taken throughout the cycle ride were significantly higher for HOT than COLD ($P < 0.01$) (Table 4.2, Figure 4.2 a-d). Calf skin temperature increased from 40 min to exhaustion in both groups ($P < 0.01$) (F: group – 56.05, time – 83.89, interaction – 25.34) (Figure 4.2 d).

Table 4.2. Skin temperature (°C) was taken from the sternum, bicep, thigh and calf region and captured at rest, 10, 25, 40 mins and exhaustion during the cycle ride during COLD and HOT.

	Sternum		Bicep		Thigh		Calf	
	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot
Rest	24.9 ± 2.3 **	33.5 ± 1.1	22.06 ± 3.3**	33.56 ± 0.47	23.5 ± 2.6 **	32.6 ± 0.7	23.8 ± 0.8 **	32.2 ± 1.1
10 min	23.9 ± 1.5 **	33.3 ± 0.9	22.04 ± 1.7**	33.4 ± 0.55	23.7 ± 1.3 **	33.6 ± 0.7	23.8 ± 2.2 **	33.4 ± 0.9
25 min	23.2 ± 2.2 **	33.4 ± 1.7	21.2 ± 3.45 **	33.08 ± 0.84	24.2 ± 1.8 **	33.7 ± 1.1	23.6 ± 0.5 **	33.4 ± 1.2
40 min	24.1 ± 2.5 **	32.9 ± 1.8	20.62 ± 4 **	33.27 ± 0.7	24.4 ± 1.7 **	33.8 ± 0.9	23.1 ± 2.8 **	33.5 ± 1.2
Exh	25 ± 3.1 **	33.7 ± 1.5	21.03 ± 3.7**	33.8 ± 1.14	25 ± 2.3 **	33.9 ± 0.7	33.6 ± 1.1 **	36.3 ± 1.3

All values are mean ± SD

** - $P < 0.01$ COLD vs HOT for all regions

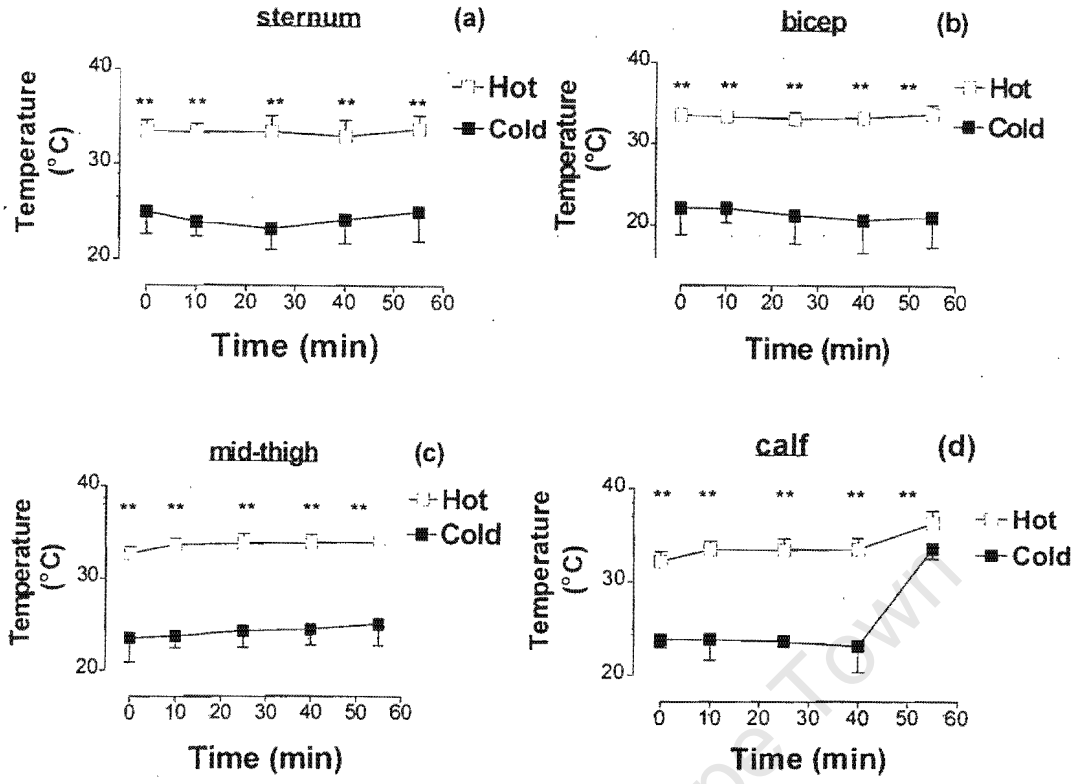


Figure 4.2. Skin temperature values were taken from the (a) sternum, (b) bicep, (c) mid-thigh and (d) calf regions at rest, 10, 25, 40 mins and exhaustion during the cycle ride during 15°C (COLD) and 35°C (HOT). (** - P < 0.01)

4.3.3 *Rectal temperature*

Rectal temperatures increased similarly in both groups during the trial (P < 0.05) (Table 4.3, Figure 4.3). Although there was a tendency for rectal temperature to be higher throughout the HOT trial, this difference was not significant.

Table 4.3 Rectal temperature (°C) captured at rest, 10, 25, 40 mins and at exhaustion during the cycle ride during COLD and HOT.

	COLD	HOT
Rest	36.7 ± 1.2	37 ± 1.2
10mins	36.9 ± 1	37.3 ± 0.4
25mins	37.2 ± 1.1	37.6 ± 0.9
40mins	37.1 ± 1.2	38 ± 0.3
Exhaustion	37.3 ± 1.3	38.1 ± 0.5

All values are mean ± SD

P < 0.05 increase over time for both groups

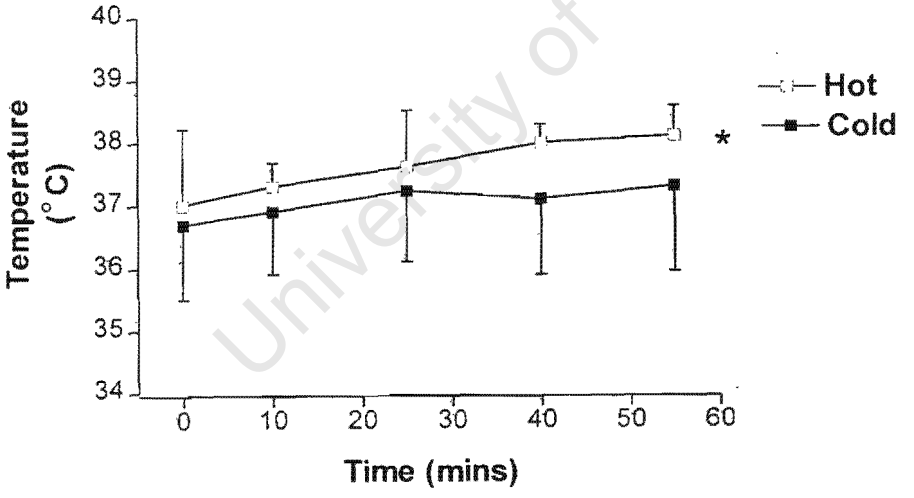


Figure 4.3. Rectal temperature values captured at rest, 10, 25, 40 mins and exhaustion during the cycle ride during 15°C (COLD) and 35°C (HOT) (* - P < 0.05 increase over time for both groups).

4.3.4 *Heart rate*

Heart rate values were higher in HOT (group main effect $P < 0.05$). Heart rate in both groups increased similarly over time (time main effect $P < 0.05$) (Table 4.4 Figure 4.4).

Table 4.4. Heart rate ($b.min^{-1}$) captured at rest, 10, 25, 40 mins and exhaustion during the cycle ride during COLD and HOT.

	COLD	HOT
Rest	63.4 ± 14.9	82.9 ± 19.5
10	102.9 ± 11.5	109.1 ± 8.4
25	124.4 ± 13.4	137.1 ± 8.6
40	150.4 ± 12.1	158.4 ±
Exhaustion	172.62 ± 9.1	175.8± 8.6

All values are mean ± SD

$P < 0.05$ Heart values COLD vs HOT (between groups and over time)

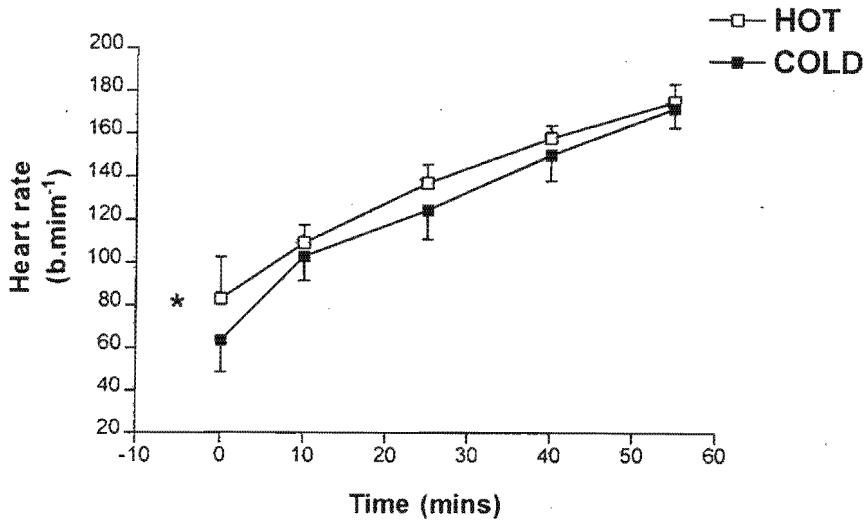


Figure 4.4. Heart rate values captured at rest, 10, 25, 40 mins and exhaustion during the cycle ride during 15°C (COLD) and 35°C (HOT) (* - $P < 0.05$ differences between groups and over time).

4.3.5 *IEMG*

IEMG measured throughout the cycle ride and normalised as a percentage of MVC increased ($P < 0.01$) during the ride for both COLD and HOT (Table 4.5, Figure 4.5). Although there was a tendency for IEMG to be higher in HOT vs COLD, this was not significant.

Table 4.5. IEMG (%) captured at rest, 10, 25, 40 mins and exhaustion during the cycle ride during COLD and HOT.

	COLD	HOT
10	5.4 ± 3.9	8.3 ± 8
25	7.3 ± 4.3	7.4 ±4.7
40	9.9 ± 5.6	18.2 ± 14.8
Exhaustion	12.5 ± 8.4	20.7 ± 15.8

All values are mean ± SD

P < 0.01 increase over time for both groups

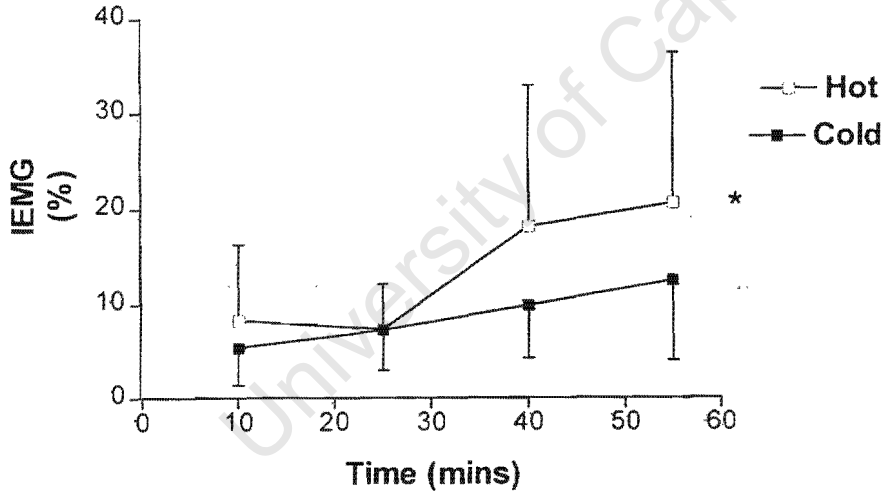


Figure 4.5. Mean amplitude values (IEMG) captured at 10, 25, 40 mins and exhaustion during the cycle ride have been normalised as a percentage of Maximal voluntary contraction (MVC) for both 15°C (COLD) and 35°C (HOT) (* - P < 0.01 increase over time for both groups).

4.3.6 MPFS

There were no differences in MPFS between groups or over time.

(Table 4.6, Figure 4.6).

Table 4.6. MPFS (%) captured at rest, 10, 25, 40 mins and exhaustion during the cycle ride during COLD and HOT

	COLD	HOT
10	0.9 ± 0.26	0.97 ± 0.28
25	0.92 ±0.27	0.98 ± 0.28
40	0.95 ± 0.25	0.99 ± 0.21
Exhaustion	0.94 ± 0.25	0.96 ± 0.12

All values are mean ± SD

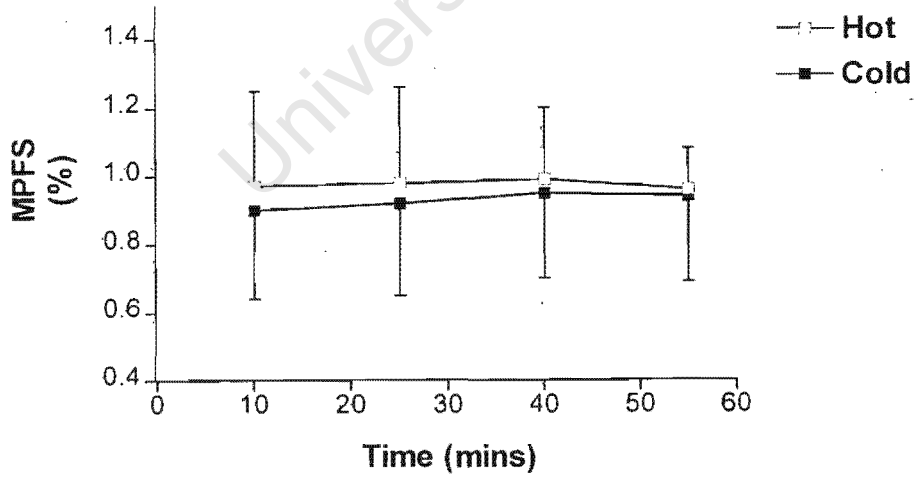


Figure 4.6. Normalised EMG mean power frequency spectrum (MPFS) data values for both 15°C (COLD) and 35°C (HOT).

4.3.7 *RPE*

RPE for HOT and COLD increased significantly during the ride ($P < 0.01$) (Table 4.7, Figure 4.7). There were no significant differences for RPE between groups. The RPE for HOT at 10 minutes (12.2 ± 8.6) tended to be higher than COLD (7.2 ± 1.2) although this was not significant.

Table 4.7 RPE (units) captured at 10, 25, 40 mins and exhaustion during the cycle ride during COLD and HOT.

	COLD	HOT
10	7.2 ± 1.2	12.2 ± 8.6
25	11.7 ± 2.1	12.4 ± 2.9
40	14.2 ± 1.9	15.2 ±1.9
Exhaustion	16.7 ± 2.1	18.5 ±1

All values are mean ± SD

$P < 0.01$ increase over time for both groups.

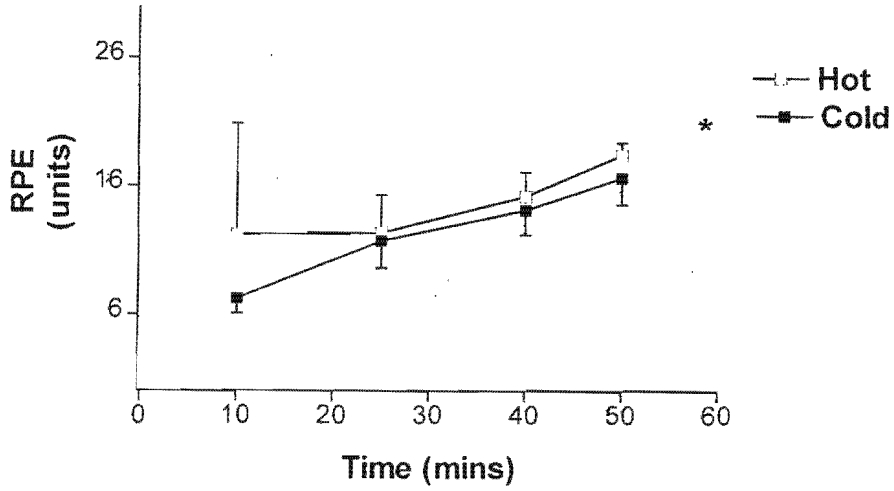


Figure 4.7. Rating of perceived exertion values captured at 10, 25, 40 mins and exhaustion during the cycle ride during 15°C (COLD) and 35°C (HOT) (* - $P < 0.01$ increase over time).

4.3.8 *Thermal comfort*

Thermal comfort was significantly greater for HOT throughout the ride ($P < 0.01$) (F: group – 52.5, time – 19.4, interaction – 5.5) (Table 4.8, Figure 4.8). The differences for thermal between HOT and COLD become less as the ride progressed (Figure 8) with the interaction due to the greater relative increase in thermal comfort ratings during COLD. For example, at the start of the ride the difference was 67%, and this decreased to 30% at the end of the ride.

Table 4.8 Thermal comfort (units) captured at rest, 10, 25, 40 mins and exhaustion during the cycle ride during COLD and HOT.

	COLD	HOT
Rest	1.7 ± 0.7 **	5.4 ±1.1
10	2.2 ± 0.7 **	5.6 ± 0.7
25	3.2 ±1.1 **	5.7 ±0.7
40	3.9 ± 0.8 **	6.2 ± 0.7
Exhaustion	4.4 ± 1 **	6.3 ± 0.7

All values are mean ± SD

** - P < 0.01 COLD vs TIME interaction between groups and over time

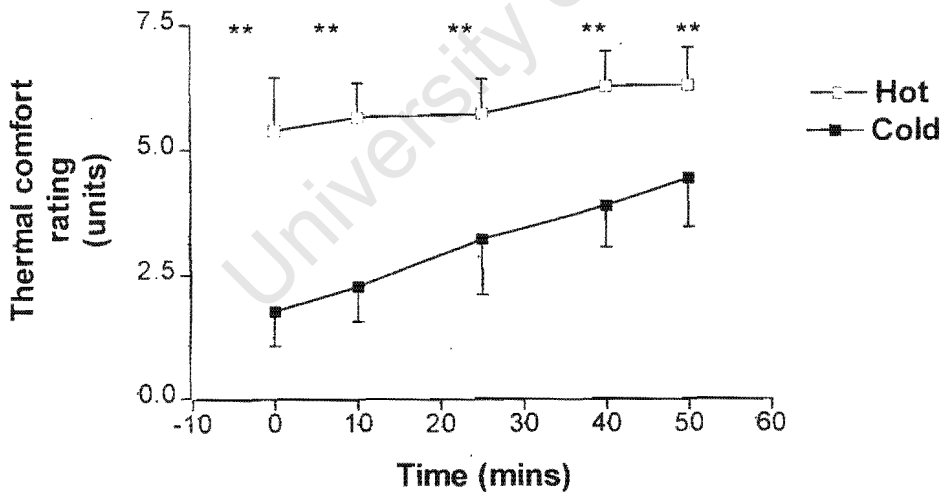


Figure 4.8. Thermal comfort values captured at rest, 10, 25, 40 mins and exhaustion during the cycle ride during 15°C (COLD) and 35°C (HOT) (** - P < 0.01).

4.4 DISCUSSION

Previous studies of exercise in hot environments have shown an earlier onset of fatigue. It is considered that this premature fatigue is caused by a reduced cardiac output from thermoregulatory mechanisms, which will limit the quantity of oxygen to the working muscles (441). In our study however, no differences in performance were seen, possibly because of effective thermoregulation mechanisms. The lack of early fatigue in the HOT group data indicates the successful involvement of thermoregulatory response mechanisms, which is indicated by a significant increase in skin temperature and a higher heart rate and similar rectal temperature values in the HOT group. These results suggest that the increase in skin temperature is as a result of increased skin blood flow (400), which is caused by a reduction in cardiac return in an attempt to move more blood to the periphery to transfer heat out of the body by conduction from an increase in sweat rate (206;400). As a consequence of reduced cardiac return the stroke volume decreases (443), therefore to compensate, the heart rate becomes elevated (206), which is shown in this study. This heart rate response could be partly due to both central stimuli from the brain and peripheral mechanoreceptors situated in the tendon or its active muscle (116;354). Therefore, it would appear that because heat is effectively being conducted out of the body, core temperature in our study was controlled without compromising performance from an elevation in heart rate. Also, pre-cooling the COLD group would have caused lower skin blood flow and increased venous tone. This would have resulted in increased venous return and stroke volume and lower heart rate (442).

Further evidence for an effective thermoregulatory mechanism comes from the subjects' perception data. Although not significant there is a higher RPE value for the HOT group during the first 10 minutes and no difference thereafter. This could be as a result of delayed thermoregulatory response, which takes more than 10 minutes to come into effect, after which the afferent signals relay a similar RPE for both HOT and COLD groups. Maw et al. (350) showed higher RPE scores in subjects exercising in the heat (40°C) in comparison to the cold (8°C). Furthermore, González-Alonso et al. (206) showed that exhaustion during moderate exercise occurred at the same high level of internal body temperature and RPE. However, thermal comfort values were significantly greater in HOT, which could result in circulatory, thermal and muscular discomfort, which would counteract motivation and gradually reduce the drive to exercise (80). However, this being the case a reduced MPFS and exercise performance would be expected in this study. Therefore, in this study even though the subjects' report differences in thermal comfort, it is likely that effective thermoregulation resulted in similar RPE values.

The second reason for no significant differences in performance could be because: i) The ramp protocol used meant the subjects were only working at 70% of PPO in the latter 15 minutes, after which the load increased to 15 $W.min^{-1}$ to exhaustion every minute thereafter (Figure 4.1). Therefore, it could be argued that the time spent at a high intensity during the protocol was too short for any significant changes in core temperature and consequently performance; ii) A cycling as opposed to a running protocol was used in this study. Runners will operate at a higher heart rate than cyclists at any given RPE (239) because runners use larger muscle groups than cyclists and unlike

cyclists have to support their own body weight. In support of this it has been shown that during hot humid conditions, runners with a smaller body mass produce and store less heat than their heavier counterparts at the same running speed and can therefore run faster or further before reaching a limiting rectal temperature (342). Therefore it is logical to assume that core temperature will rise earlier and higher during submaximal exercise in runners than cyclists; iii) The environmental temperature of 35°C may not have been hot enough, as previous studies have shown reduced performance in cyclists at temperatures of 40°C (206;413); iv) Finally, the environmental humidity of 50% may not have been high enough, Nielson (399) showed that at a temperature of 35°C, the humidity has to be 60% before a reduction in performance can be expected.

Consequently, because core temperature remained unchanged, possibly from an effective thermoregulation mechanism, or from low heat storage in both conditions (400-402) the afferent command to the CNS from the periphery resulted in similar efferent neural recruitment strategies from CNS to the working muscle in both groups. This was shown by no significant differences in EMG recordings between groups. First, there was no significant change in IEMG between groups, although there was a slight increase in IEMG for the HOT group, which does suggest a tendency for efferent command changes to recruit more muscle fibers to compensate for a possible decline in cardiac output. The increase in peripheral temperature may "confuse" the afferent input and the central nervous system, which perceives the same work rate as more difficult, thus recruits more fibres for the same work rate in the HOT group.

Second, there were no changes in MPFS, which indicates that there were no reductions in central drive in HOT or COLD group. Bigland-Ritchie (42) concluded that conduction velocity slows down when the muscle is cooled resulting in lower MPFS, conversely by heating the muscle the conduction velocity increased causing an increase in MPFS. However, this was found in an in vitro study and has not been confirmed in an exercising human model, which will involve other variables such as changes in intramuscular pressure, haemodynamics and resulting metabolite accumulation. Also, the highly significant differences in skin temperature in COLD and HOT trials are not necessarily representative of the muscle temperature. There were no differences in core temperature, suggesting that the thermoregulatory mechanisms were keeping the working musculature at optimal temperature. Accordingly, because there was no difference between groups in MPFS, suggests that the muscle temperature did not increase sufficiently in the HOT group to change conduction velocity. Furthermore, the effect of pre cooling in the COLD group could soon be lost by the time the subject commenced cycling and circulated blood to the working musculature, therefore resulting in minimal changes in conduction velocity and MPFS from pre cooling. These findings indicate that peripheral temperature changes and increased thermal comfort values do not result in significant efferent neural command changes.

Finally, there was a sudden increase in skin temperature in the calf of the COLD group at exhaustion. This suggests that because of the final large power output at exhaustion, there will inevitably be an increase in blood flow to the working muscle with increased blood volume to the vascular beds

(441). These changes may cause a rapid increase in temperature to the calf region in the COLD group. The HOT group did not show the same increase at exhaustion as COLD, probably because the skin had almost reached its maximal temperature in these conditions. The final skin temperature in HOT was 36.3° C and to our knowledge no study has recorded exercising subjects in hot environments with skin temperatures greater than approximately 37° C. This increase seen only in the COLD calf at exhaustion would also be expected in the thigh as it is the main assisting contracting muscle group to generate force for cycling. However, the musculature of the thigh region is much larger than the calf, which may result in a longer period of time before the skin temperature rises after blood volume has increased.

In conclusion, this study and other studies indicate a number of factors that will affect exercising under hot and cold conditions. However, in this study the HOT protocol caused changes in skin temperature and heart rate, but not in rectal temperature. EMG was not altered in the presence of elevated skin temperature and thermal comfort. RPE was the same for both HOT and COLD, suggesting that peripheral mechanisms and/or low heat storage resulted in unchanged central drive. Therefore, it appears that during HOT, effective peripheral thermoregulation mechanisms control core temperature, resulting in an unchanged neuromuscular recruitment strategy. Further work is needed to assess changes in core temperature as opposed to peripheral temperature changes or neural recruitment changes during fatiguing activity.

CHAPTER FIVE

THE EFFECT OF SELECTIVE ß1-BLOCKADE ON EMG SIGNAL CHARACTERISTICS DURING PROGRESSIVE ENDURANCE EXERCISE

5.1 INTRODUCTION

Chapter four showed similar neural recruitment patterns for submaximal cycling exercise to fatigue during both hot and cold environments. This finding was attributed to the effective thermoregulatory mechanisms of the body in dealing with extreme temperatures. To elucidate more about neural involvement in submaximal exercise to fatigue we decided to investigate the effect of ß-blocker ingestion. ß-blocker ingestion will reduce exercise endurance performance (500), the exact cause of this reduction remains unclear, however, there does appear to be some involvement from the CNS (136) (see section 1.4.3). To our knowledge only two studies have investigated the effects of ß-blocker ingestion on the EMG signal during exercise (136;492). In the study by Derman (136) MPFS were not determined and in the study by Tesch et al. (492), although MPFS were calculated it was done so by using an initial cycling work epoch of 90 W and normalising all subsequent data against it. However, our conclusion from Chapter 3 suggests that MVC would be a more effective method for normalising MPFS data.

Accordingly, we examined the effects of acebutolol, a selective ß1-blocker with intrinsic sympathetic activity on muscle metabolism, IEMG and MPFS during a MVC and successive, incremental, submaximal cycling rides to fatigue.

5.1.1 Question

The question addressed by this study is:

What is the effect of ß-blocker ingestion on neuromuscular recruitment patterns during submaximal fatigue?

5.2 METHODS

5.2.1 Subject selection and sampling

Ten healthy males volunteered for the study, who were physically active on a regular basis. Three subjects were unable to complete due to adverse effects such as headaches, dizziness and nausea whilst ingesting beta blockade. The mean age of the remaining subjects was 26.1 ± 2.1 years, height 181 ± 9 cm, weight 78.6 ± 9.7 kg and percent body fat 14.8 ± 2.7%. The mean lean thigh volume (LTV) was 6492 ± 928cc. All subjects were well informed about possible risks associated with the experiment and gave their informed consent before participation.

5.2.2 Preliminary testing

5.2.2.1 *PPO testing*

PPO was determined as described in chapter 2.

5.2.2.2 Anthropometry

The LTV and percent body fat was determined as described in chapter 2.

5.2.3 Experimental trial

5.2.3.1 *Tablet ingestion*

Following the progressive exercise tests, the subjects' ingested acebutolol for one of the two phases of the trial in a random order. The trials took place over one week periods with a 10 day wash out in between trials. Subjects were instructed to consume two 200 mg capsules between 0700 and 0900 h before breakfast for a period of seven days.

5.2.3.2 *Blood sampling*

On the last day of each phase the subjects were instructed to report the laboratory. An 18-guage Teflon cannula (Jelco, Johnson and Johnson, Halfway house, South Africa) was positioned in an antecubital vein and connected to a three way stop cock (Uniflex, Mallinckrodt, Hennef-Seig, Germany). This cannula was flushed periodically with 2-3 ml of sterile saline containing heparin (5 IU ml^{-1}) and was used for the collection of venous blood samples (10 ml) at rest and during exercise. Venous blood samples (10 ml) were drawn at rest, at the end of each 15 min work rate and at exhaustion. The samples were then divided into aliquots, which were put into an ice-cold tube containing potassium oxalate and sodium fluoride for later determinations

of lactate concentrations. The tubes were centrifuged at 3000-x g for 10 minutes at 4 °C immediately after the completion of the trial and the supernatants were stored at –20 °C for later analyses of plasma lactate. Plasma lactate concentrations were measured with spectrophotomeric (Beckman Model 35, Beckman Instruments Inc., Fullerton, Ca, USA) enzymatic assays (Lactate PAP, BioM (rieux, Lyon, France; NEFA half-micro test; Boehringer Manheim, Germany).

5.2.3.3 *MVC testing*

MVC testing was determined as described in chapter 2.

5.2.3.4 *Progressive exercise test*

Following MVC testing, the subjects then performed cardiovascular fatigue testing as described in chapter 4. During the final 5 minutes of each 15 min work rate and at exhaustion, subjects wore and breathed through a mask connected to an Oxycon Alpha automated gas analyser (Mijnhardt, Netherlands). Before each test, the analyser was calibrated with a Hans Rudolp 3 l syringe (Vacuumed, Ventra, USA) room air and a 5% CO_2: 95% N_2 gas mixture. Analyser outputs were processed by a computer, which calculated one min ventilation (V_E), oxygen consumption (VO_2) and carbon dioxide production (VCO_2) values for each breath. VO_2 peak values were the average of the highest VO_2 values measured over 60 s in the final work rate. Heart rate was recorded at rest and then recorded along with rating of

perceived exertion (RPE) (67) at 10, 25, 40 minutes and at exhaustion (Figure 5.1).

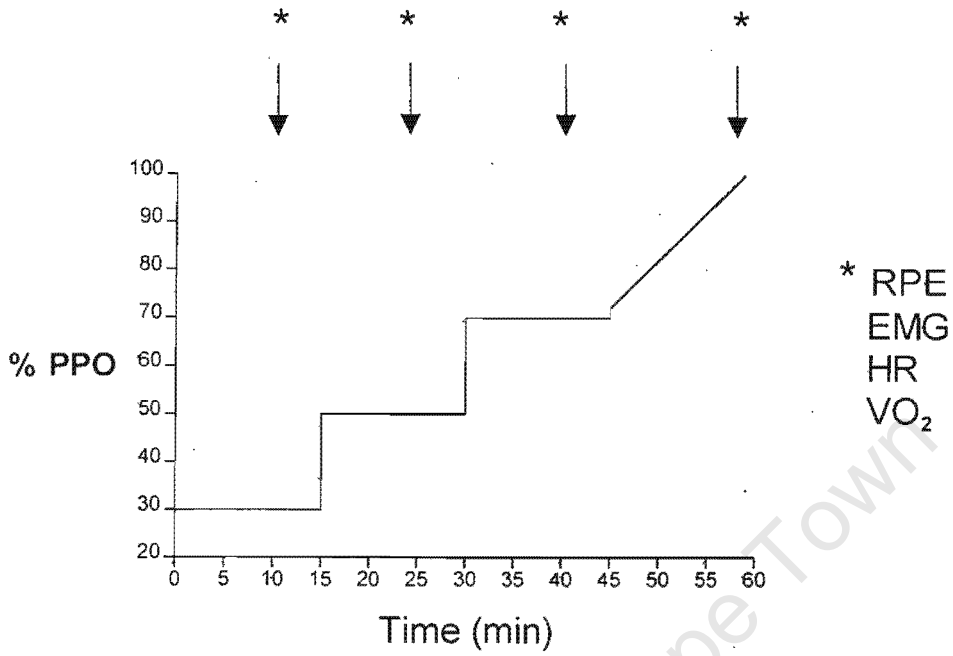


Figure 5.1. Progressive cycle exercise test protocol. Rate of perceived exertion (RPE), electromyography (EMG), heart rate (HR) and VO_2 were all recorded at 10, 25, 40 minutes and at exhaustion during the ride.

5.2.3.5 *EMG testing*

EMG methodology, sampling and signal processing was as described in chapter 4.

5.2.4 Statistical analyses

A two-way ANOVA for repeated measures was used to evaluate statistical significance of all the variables measured. A Scheffe's post hoc test was used to detect differences over time. Single comparisons between treatments were

analysed with a paired Students t-test using two-tailed values of P. Significance was accepted at $P \leq 0.05$. All data are expressed as means $\pm$ SD.

5.4 RESULTS

5.4.1 *Performance*

Significantly lower values ($P < 0.05$) during the cycle ride were found in BETA compared to CON for maximum power output (P_{WATT}) ($P < 0.01$) and time to exhaustion (TIME) in BETA (Table 5.1).

Table 5.1. Peak watts (PWATT) (W) reached at the point of cycling exhaustion and time taken to reach exhaustion (TIME) (min) for both control (CON) and subjects who have ingested ß-blocker (BETA).

	CON	BETA
PWATT	270.8 ± 111.6 **	197.45 ± 75.7
TIME	49.7 ± 23.2 *	40.33 ± 23.7

All values are mean ± SD

** - $P < 0.01$ PWATT CON vs BETA

* - $P < 0.05$ TIME CON vs BETA

5.4.2 *Heart rate*

Heart rate increased significantly less ($P < 0.05$) as a group (Table 5.2, Figure 5.2) for BETA in comparison to CON and both groups responded similarly over time. Mean heart rate for the total ride was 111.5 ± 30.0 $b.min^{-1}$ for BETA and 135.5 ± 38.3 $b.min^{-1}$ for CON.

Table 5.2 Heart rate ($b.min^{-1}$) captured at rest, 10, 25, 40 mins and exhaustion during the cycle ride for BETA and CON.

	CON	BETA
Rest	83.2 ± 15.8	69.8 ±13.7 *
10	114.0 ± 16.7	97.0 ± 6.2 *
25	138.4 ±16.2	112.4 ± 7.7 *
40	161.8 ±13.3	132.2 ± 4.6 *
EXH	180.0 ±9.6	146.3 ±30.0 *

All values are mean ± SD

- P < 0.05 CON vs BETA

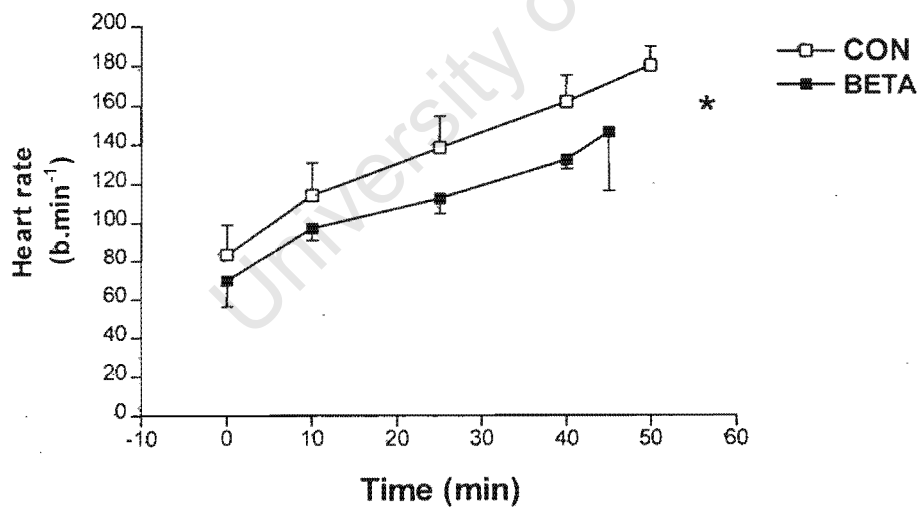


Figure 5.2. Heart rate values captured at rest, 10, 25, 40 mins and exhaustion during the cycle ride for the ß-blocker group (BETA) and the control group (CON) (* - P < 0.05 group effect).

5.4.3 *Submaximal VO_2*

In BETA the reduced heart rate was accompanied by a trend for lower submaximal VO_2 values with both groups increasing significantly over time ($P < 0.05$) (Table 5.3, Figure 5.3). Mean submaximal VO_2 values for the duration for the ride were 32.0 ± 8.3 $ml.kg^{-1}.min^{-1}$ for BETA and 38.1 ± 9.6 $ml.kg^{-1}.min^{-1}$ for CON.

Table 5.3 VO_2 ($ml.kg^{-1}.min^{-1}$) captured at 10, 25, 40 mins and exhaustion ride BETA and CON during the cycle ride.

	CON	BETA
10	25.4 ± 2.0	21.6 ± 3.0
25	36.3 ± 8.3	31.3 ± 5.7
40	43.6 ± 6.7	33.1 ± 5.9
EXH	47.1 ± 7.3	42.0 ± 5.1

All values are mean ± SD

$P < 0.05$ increase for both groups over time

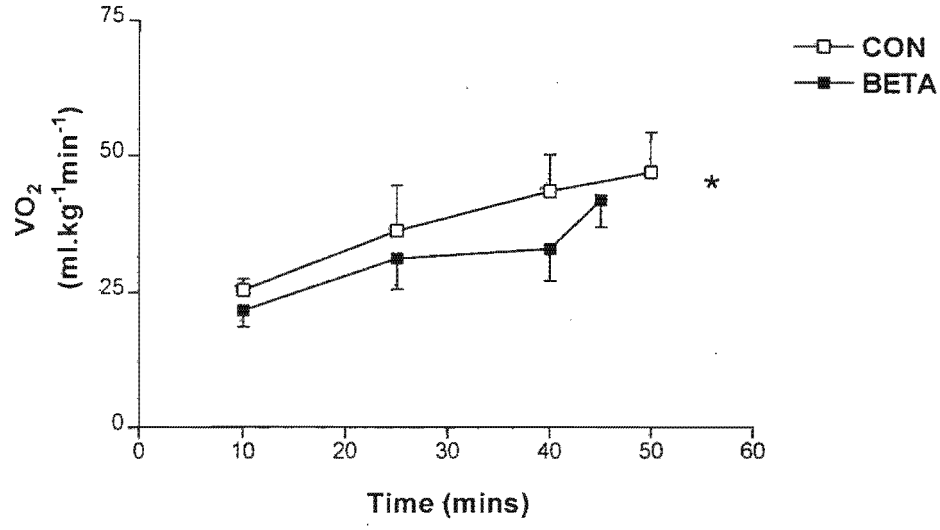


Figure 5.3. VO_2 values captured at 10, 25, 40 mins and exhaustion ride for the ß-blocker group (BETA) and the control group (CON) during the cycle ride (* - $P < 0.05$ increase for both groups over time).

5.4.4 *RPE*

RPE was significantly greater for BETA than CON ($P < 0.01$) (Table 5.4, Figure 5.4) and mean RPE values for the total ride were 6.1 ± 3.6 for BETA and 4.7 ± 3.6 CON.

Table 5.4 Rating of perceived exertion (units) captured at 10, 25, 40 mins and exhaustion ride for BETA and CON during the cycle ride.

	CON	BETA
10	9.0 ± 0.63	11.0 ± 0.84*
25	12.0 ± 0.70	14.0 ± 0.89*
40	15.0 ± 1.64	17.0 ± 1.30*
EXH	19.0 ± 0.58	19.0 ± 0.45*

All values are mean ± SD

*- $P < 0.01$ CON vs BETA

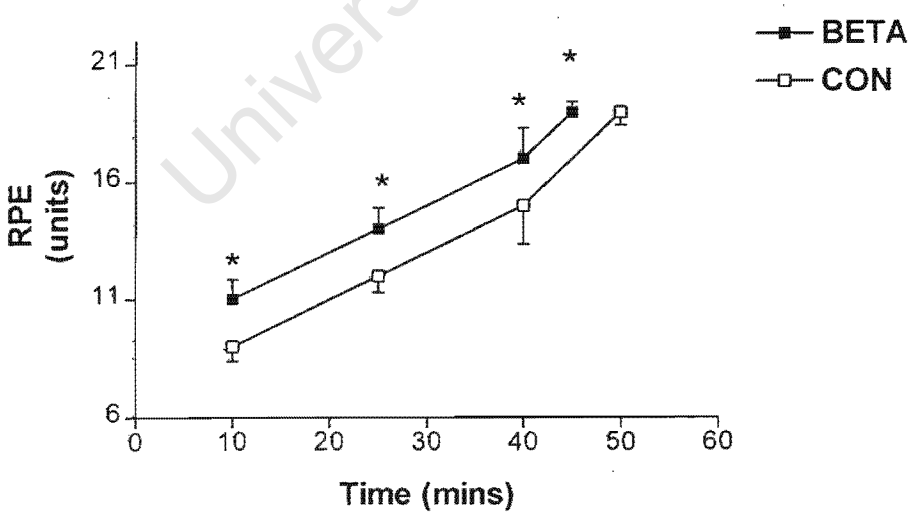


Figure 5.4. Rating of perceived exertion values captured at 10, 25, 40 mins and exhaustion ride for the ß-blocker group (BETA) and the control group (CON) during the cycle ride (* - $P < 0.01$ main effect).

5.4.5 IEMG

IEMG values showed a trend to increase in BETA in comparison to CON during the ride. The BETA group also showed a large standard deviation for IEMG data at exhaustion (Table 5.5, Figure 5.5). The mean IEMG for the total ride was 56.9 ± 25.4 % for BETA and 29.4 ± 4.0 % for CON.

Table 5.5. IEMG (%) captured at 10, 25, 40 mins and exhaustion during the cycle ride have been normalised as a percentage of Maximal voluntary contraction (MVC) for both CON and BETA.

	CON	BETA
10	5.2 ± 3.2	4.6 ± 1.4
25	10.3 ± 10.3	6.8 ± 1.3
40	8.7 ± 4.9	11.8 ± 5.6
EXH	10.5 ± 9.2	13.9 ± 1.9

All values are mean ± SD

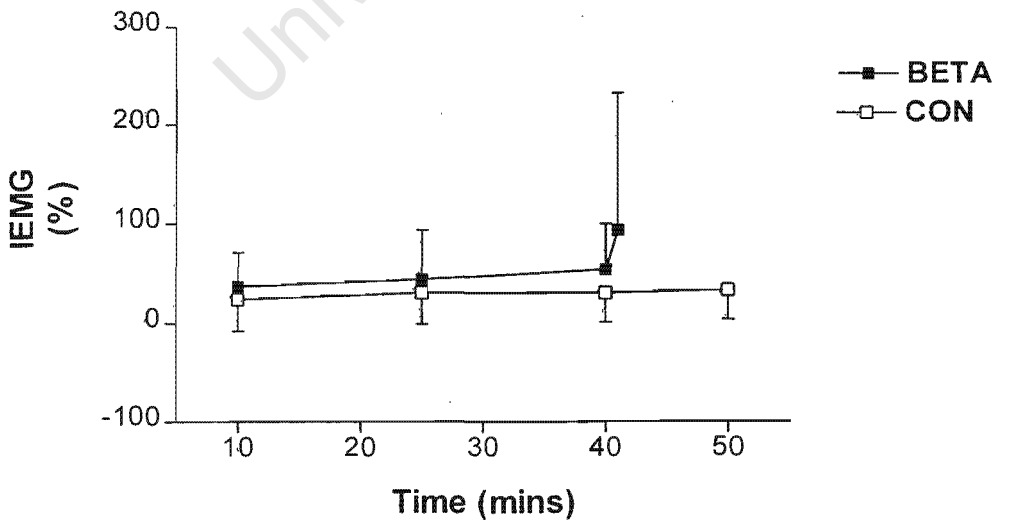


Figure 5.5. Mean IEMG values a normalised as a % of MVC for both BETA and CONTROL, taken at 10, 25, 40 min and exhaustion.

5.4.6 MPFS

There was a significant ($P < 0.05$) shift to the upper portion of MPFS in BETA as a group with a mean of 1.15 ± 0.03 in comparison to a shift to the lower portion of MPFS in CON, which showed an average of 0.78 ± 0.07 for the total ride (Table 5.6, Figure 5.6).

Table 5.6. MPFS (%) was calculated by taking frequency values at 10, 25, 40 mins and exhaustion throughout the cycle ride and dividing them by MVC frequency values for both CON and BETA.

	CON	BETA
10	0.705 ± 015	1.14 ± 0.3*
25	0.88 ± 0.15	1.13 ± 0.32*
40	0.78 ± 0.15	1.13 ± 0.32*
Exhaustion	0.77 ± 0.17*	1.19 ± 0.3*

All values are mean ± SD

* - $P < 0.05$ CON vs BETA

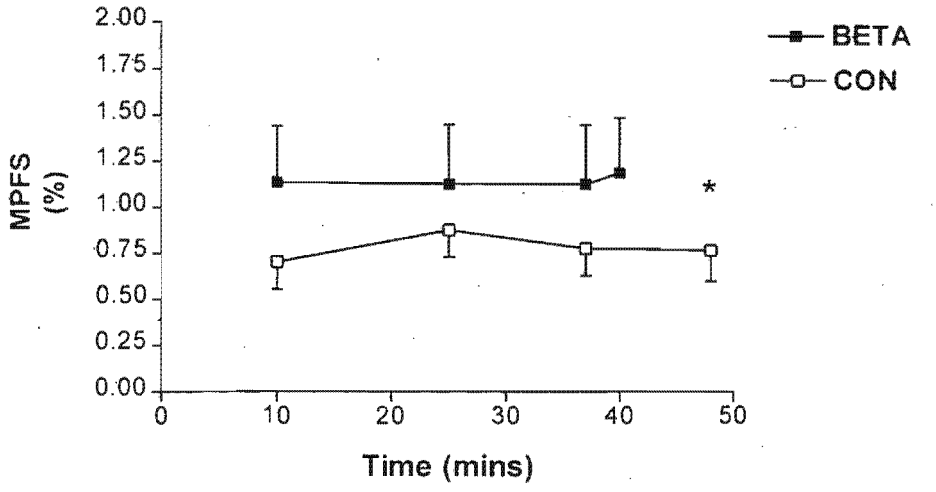


Figure 5.6. Mean power frequency spectrum (MPFS) values normalised against MVC for both BETA and CON, taken at 10, 25, 40 mins and exhaustion. (* - $P < 0.05$ group effect).

5.4.7 *Lactate*

Lactate concentrations were significantly less for BETA in comparison to CON (7.1 ± 4.1 mmol.l^{-1} BETA; 11.7 ± 3.5 mmol.l^{-1} CON) at exhaustion (Table 5.7, Figure 5.7) ($P < 0.01$) (F: group – 3.94, time – 69.6, interaction – 5.74).

Table 5.7. Lactate samples (mmol.l^{-1}) taken at rest (LACTATE REST), at 15 minutes during the ride (LACTATE 15) and at exhaustion (LACTATE EXH) in both CON and BETA groups.

	CON	BETA
LACTATE REST	2.1 ± 1.4	1.6 ± 0.4
LACTATE 15	2.2 ± 1.2	1.91 ± 0.8
LACTATE EXH	11.7 ± 3.5**	7.1 ± 4.1

All values are mean ± SD

** - $P < 0.01$ CON vs BETA over time and interaction

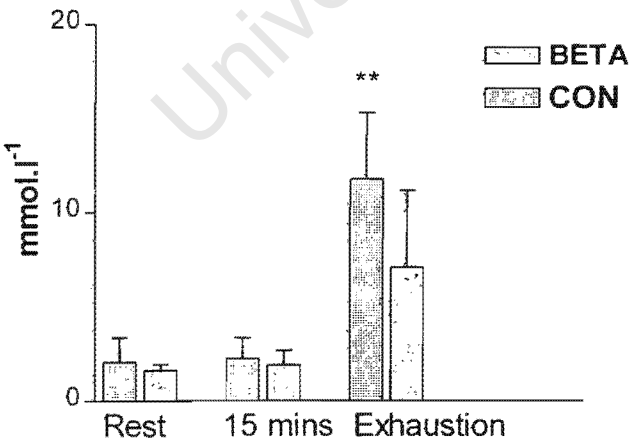


Figure 5.4. Lactate samples taken at rest, at 15 minutes during the ride and at exhaustion in both CON and BETA groups. (** - $P < 0.01$ CON vs BETA main effect)

5.5 DISCUSSION

The results of this study indicate that ß-blockade significantly affects neuromuscular recruitment activity during submaximal exercise. Maximal exercise capacity was reduced by ingestion of selective ß-1 blockade, as reported by most investigators (136;178;222-224;261;280;306;416;420;491;498;501;531). The impaired maximal exercise capacity may reduce heart rate and VO_2 as is shown in this study. It is also well documented that an increased perception of effort will reduce exercise performance in healthy individuals who have ingested ß-blocker (273;531), which is shown in this study by an increase in RPE for BETA. It is has been proposed that this increase in perception of effort results from an inability of healthy individuals to compensate for the decrease in heart rate by increasing stroke volume as it is has been maximised as an adaptation effect of exercise training (243). These changes in RPE may also be related to neuromuscular recruitment differences as shown in this study.

IEMG data firstly showed similar results to Derman et al. (136), where IEMG tended to increase in subjects who ingested ß-blockade. This could be due to a central mechanism projecting increased neural output due to the reduced maximal exercise capacity. Moritani and Yoshitake (382) showed a linear increase for IEMG/VO_2 in normal subjects. However the BETA group had a significantly lower VO_2, yet IEMG was higher. This would indicate that there is a higher neural input to compensate for the reduced VO_2. Another possibility is that there was a higher recruitment of non-fatigued muscle fibres (136), when subjects were on ß-blocker.

The large standard deviations for final force output and IEMG activity in the BETA group show that certain subjects are more vulnerable to reduced exercise tolerance when ingesting ß-blockade. This finding is supported by Derman (136) who showed that not all the subjects' performance capacities were equally affected following the ingestion of ß-blockade.

There was a significant shift to the upper portion of MPFS in BETA, which indicates an increase in firing rate to recruit more motor units (37) in response to the reduced exercise capacity (230;300;383;422). This shift towards the upper portion of MPFS could have been caused by an increase in MFCV (42) from an up regulation in central command (309;549). This change in central command may be an attempt to recruit more type II muscle fibers as ß-blockade will cause the impairment of type I fibers during cycling (279), resulting in a greater use of type II fibers which have a lower fatigue threshold. Furthermore, this finding is supported by Kupa et al. (307), who showed that the greater proportion of type II muscle fibers would result in a shift to the upper end of the spectrum. This is due to type II fibers having a greater maximum rate of repolarization and depolarisation compared to type I fibers and indeed produce an action potential that has a lesser duration (307). Action potentials that have a shorter duration, contribute high frequency components to the EMG spectrum that produce a greater value of MPFS (307). This increased use of type II fibers, which have a lower fatigue threshold may have also been a factor in the early termination of exercise in BETA.

However, it is also possible that the difference in MPFS could be from muscle changes. First, it has been suggested that the decline in MPFS is as a result of increased lactate concentrations causing a lowering of pH (275) and a consequent reduction in MFCV (74;132;363;383). In this study, lactate concentrations were significantly less for BETA, which does suggest a possible cause for the difference in MPFS. Conversely, it has also been shown in McArdle's disease patients that MPFS declined during MVC, in the absence of any lactate and MFCV changes, therefore suggesting that factors other than increasing lactate would influence MFCV (325). The change in MPFS could also be from an increase in muscle temperature in BETA, as the reduced cardiovascular capacity will be requiring a higher amount of effort to sustain the same given workload as CON. Bigland-Ritchie (42) showed that by heating a muscle it would increase conduction velocity and therefore produce a shift to the upper portion of MPFS.

Blood lactate concentrations were lower in the BETA group at fatigue, which is supported by some investigators (17;277). However, other studies show conflicting results (243). In our study, BETA subjects fatigued at lower power outputs, which will inevitably cause a decrease in absolute lactate values. Therefore, reduced lactate may be caused simply by reduced cycling intensity at fatigue. However, if as previously mentioned, type II fibers are preferentially recruited in the BETA group, higher lactate values would be expected in the BETA group.

In conclusion this study supports earlier studies that selective ß1-blockade will limit prolonged exercise endurance. Furthermore, this study has shown that

subjects who have ingested ß-blockers have altered EMG frequency spectrum, which could result from a change in the neuromuscular recruitment strategy in an attempt to generate more power to compensate for the reduction in exercise capacity.

CHAPTER SIX

CAFFEINE ALTERS NEITHER PACING STRATEGIES NOR PERFORMANCE DURING A 100 KM CYCLING TIME-TRIAL PERFORMANCE

6.1 INTRODUCTION

During chapters four and five we examined the mechanisms of fatigue during submaximal exercise using open loop cycle protocols. The merits and shortcomings of such a protocol were discussed in section 1.2.16. Therefore, to obtain a clearer picture of the mechanisms of submaximal fatigue, we decided to use a closed loop protocol (see section 1.2.15) to induce submaximal fatigue under a different condition to chapters four and five.

As we examined fatigue perturbations in the previous two chapters, the next question would be to determine the role of neural mechanisms during submaximal exercise induced fatigue after ingestion of a stimulant. The stimulant we decided to use was caffeine, which has been shown to have a substantial ergogenic effect during submaximal open loop cycle protocols (414) (see section 1.4.4, Table 1.2).

To our knowledge, very few studies have evaluated the effects of caffeine ingestion on performance during a laboratory trial in which the endpoint is predetermined. It is known that inter-test variability is substantially reduced in this form of closed loop trial compared to an open loop trial (458). However the large caffeine effect in the open-ended trials that have been reported (141) precluded the possibility that a false positive finding might have resulted from the relative insensitivity of that experimental method. However, an ergogenic effect of caffeine has also been found in runners (301;527) who completed closed performance time-trials in which a specified distance was completed in a faster time after caffeine ingestion. The durations of these

trials were relatively brief lasting from 4 minutes (527) (301). However there are good reasons that the effects of caffeine-ingestion should also be investigated during exercise in which the endpoint is predetermined and the duration of exercise is more prolonged, for example hours rather than minutes. First, the majority of recreational athletes for whom advice about caffeine-ingestion is provided by exercise scientists, probably compete in long distance, running and cycling events, including marathons and triathlons that last hours rather than minutes. Second, the effects of caffeine could conceivably differ depending not only on the type of exercise undertaken (closed vs open trials) but also on its duration. Third, the method of action of caffeine could conceivably be different under different circumstances. For example, it would be unlikely that a glycogen sparing effect would explain any ergogenic effect of caffeine during exercise of short durations whereas that effect could be important during more prolonged exercise, lasting some hours.

Accordingly in this study, we evaluated the effects of caffeine ingestion on performance during a 100 km cycling time trial, which included bouts of sprinting activity, a model previously used to study the effects of carbohydrate (83). In addition, performance in this model appears to be a measure of central neural drive, which falls during exercise (285;481). Thus a subconscious pacing strategy may determine performance during such exercise and the effects of stimulatory drugs like caffeine on this mental function.

6.1.1 Question

The question addressed by this study is:

What is the effect of caffeine ingestion on 100 km cycling performance, pacing strategy and the neuromuscular recruitment pattern?

6.2 METHODS

6.2.1 Subject selection and sampling

Fifteen competitive, endurance-trained male cyclists volunteered for this study, with eight completing the full trial. Seven of the original fifteen subjects were excluded; two because they were unable to achieve the required cycling speed and five experienced difficulty in cycling because of the dietry restrictions required of them. At the time of the study, the trialists were cycling between 200-500 $km.wk^{-1}$ and had completed at least one local, specified, annual 104 km Road Race under 3 hours during the previous 2 years. Subjects were well trained and accustomed to exercising for prolonged periods (3-4 h). The mean age of the 8 subjects who completed the trial were 23.5 $\pm$ 6.7 years, weight 66.8 $\pm$ 6.2 kg, body fat 10.6 $\pm$ 3.5 %, VO_{2MAX} 64.6 $\pm$ 7.9 $ml.kg^{-1}.min^{-1}$ and PPO 385 $\pm$ 61 W. All subjects were fully informed of the nature of the investigation, which was to evaluate the effects of caffeine or carbohydrate ingestion on cycling performance, after which they gave written informed consent. The study was approved by the Research and Ethics Committee of the Faculty of Health Sciences within the University of Cape Town.

6.2.2 Preliminary testing

To determine Peak Power Output (PPO), the modified protocol as described by Hawley and Noakes (240) was used. Subjects performed a 10-minute warm up on the Kingcycle. The starting power output was determined by multiplying the subject's body weight by 3.3 W. The load was subsequently increased every minute at a rate of 1 $W.kg^{-1}$ BW.150 s^{-1}. The subject was required to match a continuously increasing power output displayed in analogue form on the computer monitor. The test was terminated when the subject failed to match the target power. The highest mean power output achieved during any 60 s period was recorded as the subject's PPO. The PPO was subsequently converted into a predicted VO_{2MAX} value using the following equation as described by Keen et al. (288).

VO_{2MAX} ($l.min^{-1}$) = 0.011 PPO (W) + 0.08 1

(SEE = 0.15, r = 0.98)

6.2.2.1 *Kingcycle ergometry system*

All testing was conducted on a Kingcycle ergometry system (Kingcycle Ltd, High Wycombe, U.K.), which allows cyclists to ride on their own racing bicycles in the laboratory. After the front wheel was removed, the subject's bicycle was attached to the ergometry system by the front fork and supported by an adjustable pillar under the bottom bracket. The bottom bracket support was used to position the rolling resistance of the rear wheel correctly on an air-braked flywheel. A photo-optic sensor monitored the velocity of the flywheel in revolutions per second (RPS), from which an

IBM-compatible computer calculated the power output (W) that would be generated by a cyclist riding at that speed on a level terrain, using the following equation:

$$W = 0.000136\ RPS^3 + 1.09\ RPS$$

The Kingcycle was calibrated before both the incremental tests to exhaustion and time-trials. For the calibration, subjects were asked to accelerate to a workload of ~200 W and instructed to immediately stop pedalling as soon as they reached the desired workload, while remaining seated in their riding position. The bottom bracket support was then adjusted until the computer display indicated that the slowing of the flywheel matched a pre-determined reference power decay curve. The time taken for a laboratory simulated 20 km and 40 km time-trials on the Kingcycle ergometer system has previously been shown to be highly reproducible (CV 1.1 ± 0.9% and 1.0 ± 0.5% respectively) (252;412).

6.2.2.2 *Familiarisation trial*

After measurement of the PPO, each subject reported to the laboratory on four separate occasions. During the first visit, subjects familiarized themselves with the equipment and laboratory conditions, thereafter they completed a familiarization 100-km cycling time trial (TT) during which they ingested their own chosen fluid replacement solutions "ad libitum". All other conditions were identical except that subjects were also not given tablets, nor were they forced to drink at specific, regular intervals during the familiarization trial.

6.2.3 Experimental procedure

6.2.3.1 *Conditions*

For each subject, the 3 TT's were conducted at the same time of day in the environmental chamber (Scientific Technology Corporation, Cape Town, South Africa) at an ambient temperature of 27°C, a relative humidity (Rh) of 50 ± 0.9% and a wind velocity (v) of 15 ± 0.4 $km.h^{-1}$. The trials were randomised and subjects were blinded to the nature of each trial. The subjects were requested to perform the same type of training for the duration of the trial and to refrain from heavy physical exercise on the day before a TT. Subjects completed a nutritional information sheet on which they recorded their food and fluid intake for three days preceding the TT. Subjects were instructed to avoid any caffeine containing products for 48 hours before each time trial. They were specifically asked to abstain from all the obvious sources of caffeine including, coffee, tea, cola drinks, chocolate and over the counter caffeine containing pharmaceuticals. They were requested to report any deviations from these instructions. The day prior to the trial the subjects followed a prescribed diet, which consisted of 5 $g.kg^{-1}$ of carbohydrate and 1.3 $g.kg^{-1}$ protein (60% and 17% respectively). They were instructed to repeat the same dietary regimen before each subsequent trial.

In addition, before each experiment, subjects were issued with a standardised breakfast consisting of 30 g of cornflakes and 150 ml of 2% fat milk, which was consumed three hours prior to commencing the TT. Only subjects who

followed the standardised dietary and training protocol were allowed to continue with the study.

6.2.3.2 *MVC testing*

To normalise EMG recordings during cycling it was first necessary to perform MVC testing as described in chapter 2.

6.2.3.3 *EMG testing*

Prior to maximal isometric strength testing on the Kin-Com isokinetic dynamometer, the same EMG methodology and signal processing was used as described in chapter 4. EMG was captured midway into each sprint on the TT which was normalised against EMG data captured during MVC. EMG was captured at 5 second bouts during MVC, whereas during the TT 4 successive bursts of EMG activity representing the cycle contractions were selected to standardise measurement because subjects selected their own cadence. Four bursts of consecutive raw EMG data from the cycle contractions were collected midpoint of each 1 km sprint (10.5 km, 32.5 km, 52.5 km, 72.5 km and 99.5 km) and each 4 km sprint (22 km, 42 km, 62 km and 82 km) during each TT. All EMG data were normalised by dividing the value obtained midway during each sprint in the TT by the EMG value obtained during the MVC performed before the start of each TT. IEMG and MPFS data were therefore expressed as a percentage of this MVC data.

6.2.3.4 *Tablet and fluid ingestion*

Following isometric testing the subjects ingested gelatine capsules containing placebo (white flour) with 150 ml of either a sports electrolyte solution containing a sweetener (1.7% carbohydrate) (Pl) or the same solution with 7% carbohydrate without sweetener (Cho), or gelatine capsules containing caffeine (6 $mg.kg^{-1}$) with the 7% carbohydrate solution (Caf). This caffeine dose was selected because it is believed to produce an ergogenic effect without exceeding the International Olympic Committee's (IOC) legal limits (218). Moreover, caffeine doses of 5-6 $mg.kg^{-1}$ BW saturate the cytochrome P-450 system in the liver (214). Furthermore, because a considerable amount of caffeine is excreted through sweat during endurance exercise (301) and as exercise increases the expression of cytochrome P450 1A2 (513), decreasing peak plasma caffeine levels and half-life (108), it was considered necessary that subjects also ingest a maintenance dose of 0.33 $mg.kg^{-1}$ BW of caffeine every 15 minutes during the Caf trial. This maintenance dose was calculated to ensure that the overall caffeine dosage did not exceed 9 $mg.kg^{-1}$ BW. This dose maintains mean urinary caffeine levels below the IOC limit of 12 $\mu g^{-1}ml$ (147;213;414;503;506). Hence a bolus amount of 6 $mg.kg^{-1}$ BW caffeine allowed a maintenance dose of 3 $mg.kg^{-1}$ BW over the TT of approximately 2.5 hours. This allowed 9 x 15 min dosages, each containing 0.33 $mg.kg^{-1}$ BW. During the two other trials (Pl and Cho), flour containing gelatine tablets were ingested every 15 minutes. One hour after ingesting the initial solution and capsules, a resting blood sample was taken and body weight measured on a precision scale (Soehnle. Germany).

6.2.3.5 *100 km cycle time trial*

After a standardised 5 min warm up of easy cycling, subjects commenced the 100 km time-trial. Every 15 minutes the subjects were given either the caffeine or placebo capsules together with 150 ml of either the carbohydrate or placebo drinks. To mimic the stochastic nature of cycle road races, the time-trial included a series of sprints during which subjects were requested to ride "as fast as possible" according to the methods previously described (83). There were five 1 km sprints after 10, 32, 52, 72 and 99 km, as well as four 4 km sprints after 20, 40, 60 and 80 km (Figure 6.2). Subjects were instructed to complete the total distance in "the fastest time possible", taking into consideration the sprints that were included. Just before commencement of a sprint, the investigator gave a distance count down and instructed the cyclist to complete the sprint in the fastest possible time as soon as he reached the specific distance at which the sprint started. Subjects viewed a diagram of the "course profile" which graphically illustrated where the 1 km and 4 km sprints occurred, before and during each ride. Otherwise subjects received no external clues to influence their performance other than their elapsed distance and heart rate. Subjects were not informed of the elapsed time or the times for the sprints until completion of all three trials.

Throughout each trial, power output and elapsed time were monitored continuously and stored on computer. Heart rate was recorded using a Sport Tester monitor (Polar Electro, Kempele, Finland) and was displayed to the subject throughout the ride. Instantaneous power output and heart rate was recorded at each 500 m split of both the 1 km and 4 km sprints to provide an estimate of the average power output

for the sprint. Rating of perceived exertion was recorded in the middle of each sprint (Figure 6.2).

Upon completion of the TT, subjects had their body weight recorded and a final blood sample taken. Finally, subjects were asked whether they could determine which solution, caffeine or placebo, they had received and to describe the basis on which they made that choice.

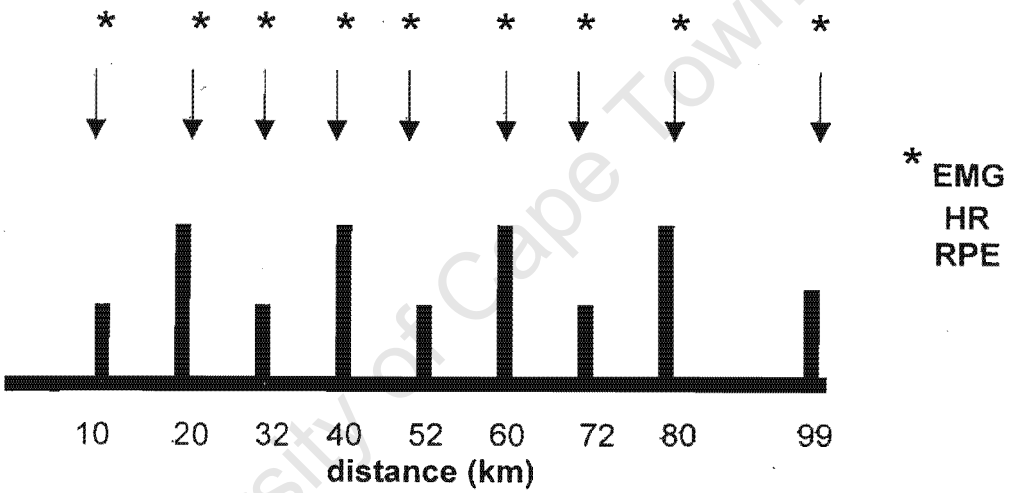


Figure 6.1. 100 km course profile consisting of 5-1km sprints (short bars) and 4 -4km sprints (long bars), which commenced at the distances stated below each bar. * = data captured midway during each sprint for EMG, HR and RPE.

6.2.3.6 *Blood samples.*

Fifteen ml venous blood samples were drawn by venipuncture into EDTA, sodium fluoride and potassium oxalate and SST® gel clot activator vactutainer tubes for determinations of caffeine, lactate and free fatty acid

(FFA) concentrations respectively. The tubes were centrifuged at 3000 x g for 10 minutes at 4°C immediately after the completion of the trial and the supernatants were stored at -20°C for later analyses. Plasma lactate concentrations were measured with spectrophotometric (Beckman Model 35, Beckman Instruments Inc., Fullerton, Ca, USA) enzymatic assays (Lactate PAP, BioM (rieux, Lyon, France; NEFA half-micro test; Boehringer Manheim, Germany). Serum FFA concentrations (FFA one and a half –micro test, Boehringer Manheim, Germany) were also determined by spectrophotometric enzymatic assay using commercial kits. Plasma caffeine, theophylline, paraxanthine and theobromine concentrations were measured using high-performance liquid chromatography (HPLC) (Gilson Inc., Middleton, WI). Plasma samples were processed as described by Tang-Liu et al. (1982) (487) with modifications. Briefly, plasma proteins were precipitated by the gradual addition of 2 ml of acetonitrile to 0.5 ml of plasma containing 20 ml of 1 $mg.ml^{-1}$ paracetomol as an internal standard. The samples were mechanically agitated and centrifuged at 3000 X g for 5 minutes, the supernatants were transferred to a fresh tube, frozen in liquid nitrogen and freeze dried for at least 24 hours. The dried samples were resuspended in 200 ml of the mobile phase (90 mM phosphate, pH 3.6; 2.3 mM tetrabutyl ammonium hydrogen sulphate; 6% acetonitrile) and stored at 4 ^{O}C until HPLC analysis. Twenty ml of each sample was injected and resolved on a 150 X 4.6 mm Ultracarb 5 ODS (20) analytical column protected by 30 X 4.6 mm ultracarb 5 ODS (20) guard column (Phenomenex, St. Torrance, CA). The methylxanthines were measured at 280 nm.

6.2.4 Statistical analyses

A two-way ANOVA for repeated measures was used to evaluate statistical significance of all the variables measured. A Scheffe's post-hoc test was used to reduce the possibility of incurring a type I error and automated checks for spericity were computed by the Statistica programme. The data was analysed by a 3 (condition) x 5 (time) and 3 (condition) x 4 (time) ANOVA for the 1 km and the 4 km sprints respectively. Significance was accepted at $P \leq 0.05$. Due to a large intersubject variation EMG data are expressed as means $\pm$ SE, while all other data as means $\pm$ SD.

6.3 RESULTS

6.3.1 *Plasma caffeine concentrations*

Subjects had low plasma caffeine concentrations in both non-caffeine trials, confirming their abstinence from caffeine-containing products before the trial (Table 6.1). Plasma caffeine and its metabolite paraxanthine concentrations were significantly higher during, and over time in Caf compared to both Pl or Cho trials ($P < 0.01$) (Table 6.1). Plasma theophylline and theobromine concentrations were also higher ($P < 0.05$) in Caf vs Cho and over time between Caf vs Cho (Table 6.1).

Table 6.1. Plasma concentrations of caffeine and its metabolites before and after the 100 km cycle time trials for both carbohydrate and caffeine, when subjects ingested either placebos (Pl), carbohydrate and placebo (Cho) or carbohydrate and caffeine (Caf) during exercise.

	Pl		Cho		Caf	
	Pre	Post	Pre	Post	Pre	Post
Caffeine (μM)	4.6 ± 7.2	2.5 ± 4.9	0.4 ± 0.6	1.1 ± 1.8	47.3 ± 12**	73 ± 27.8**
Theophylline (μM)	0.7 ± 0.6	0.5 ± 0.5	0.3 ± 0.4	0.5 ± 0.6	1.9 ± 2.1†	4.2 ± 4.7†
Paraxanthine (μM)	1.4 ± 1.2	1.3 ± 1.4	0.7 ± 0.4	0.6 ± 0.8	3.9 ± 2.8**	9.3 ± 7.2**
Theobromine (μM)	1.6 ± 2.3	1.4 ± 2	0.6 ± 0.7	0.5 ± 0.5	1.6 ± 1.1*	2.7 ± 1.3*

Values are means ± SD.

** - $P < 0.01$ Caf vs Cho and Pl

* - $P < 0.05$ the interaction of Caf vs Cho and changes over time.

† - $P < 0.05$ the interaction of Caf vs Pl and differences between groups.

Table 6.1.2. F values for group, time and interaction for Caffeine, Theophylline, Paraxanthine and Theobromine.

	Group	Time	Interaction
Caffeine	80.68	5.91	7.16
Theophylline	4.24	3.42	3.73
Paraxanthine	9.77	7.12	7.71
Theobromine	2.51	11.25	24.72

6.3.2 *Performance*

TT performance measured as average power during the TT or as time to complete the TT was not different in the 3 trials (Table 6.3). Although performance was faster in Caf, the quickest, average finish time occurred during the familiarization trial when subjects ingested their own fluid solutions "ad libitum". There was, however, a tendency for the first 1 km sprint to be faster in Caf than in Pl or Cho, with the reverse pattern in the last sprint (Fig 6.2 a). Similarly the second and third 4 km sprints were insignificantly faster in Caf than in Pl or Cho (Fig 6.2 b).

Table 6.2. Total time (mins) and average power (W) during 4 performance rides of 100 km

	Familiarization	Pl	Cho	Caf
Average power (W)		205.8 ± 56.4	217 ± 50.1	227.3 ± 63.1
Total time (mins)	155.6 ± 14.2	158.6 ± 16.4	157.8 ± 14.0	155.8 ± 15.3

Values are means ± SD.

Table 6.3. Time taken (mins) to complete 1 km sprints within the 100 km cycle time trials in subjects ingesting placebo (Pl), carbohydrate (Cho) or caffeine (Caf).

Km	Pl	Cho	Caf
10	1.12 ± 1.2	1.15 ± 1.2	1.1 ± 1.2
32	1.24 ± 1.9	1.19 ± 2	1.23 ± 2
52	1.22 ± 1.2	1.17 ± 1.2	1.2 ± 1.2
72	1.24 ± 1.4	1.21 ± 1.2	1.21 ± 1.3
99	1.15 ± 1.2	1.18 ± 1.2	1.36 ± 1.3

Values are means ± SD.

Table 6.4. Time taken (mins) to complete 4 km sprints within the 100 km cycle time trials in subjects ingesting placebo (Pl), carbohydrate (Cho) or caffeine (Caf).

km	Pl	Cho	Caf
20	5.22 ± 5.4	5.31 ± 5.4	6.04 ± 5.5
40	5.54 ± 5.7	5.34 ± 5.6	5.49 ± 5.5
60	5.41 ± 5.8	5.5 ± 5.7	5.46 ± 5.4
80	6 ± 5.7	5.37 ± 5.8	6.05 ± 5.8

Values are means ± SD.

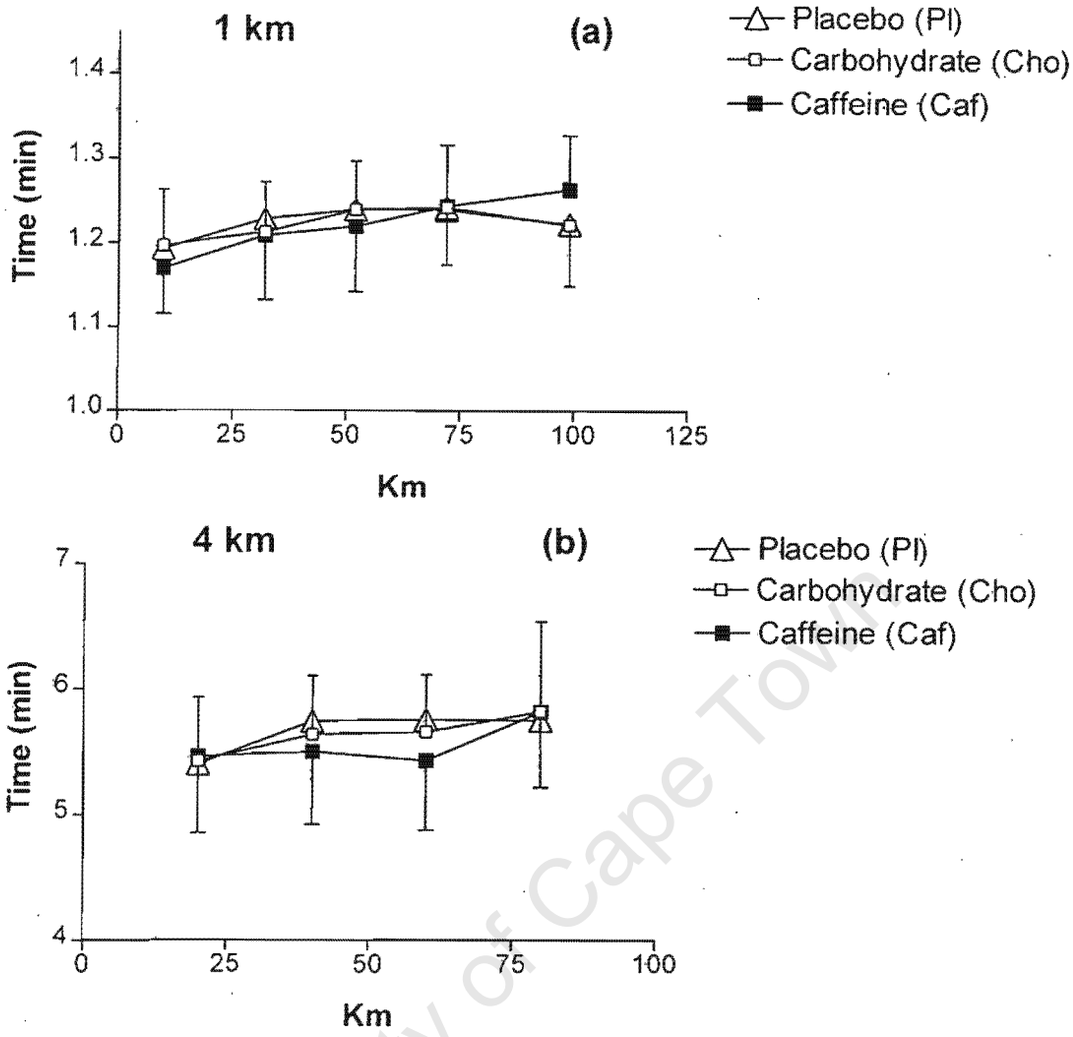


Figure 6.2. Time taken to complete 1 km (a) and 4 km sprints (b) within the100 km cycle time trials in subjects ingesting placebo (Pl), carbohydrate (Cho) or caffeine (Caf).

6.3.3 *Heart rate*

There was a trend for average heart rate to be higher during the 1 km sprints in Caf compared to Pl or Cho (Figure 6.3 a); this trend became significant during the 4 km sprints ($P < 0.05$) (Figure 6.3 b). Heart rate in all groups responded similarly over time. Peak heart rates during both the 1 km (184.0 $\pm$ 9.8 Caf; 177.0 $\pm$ 5.8 Pl; 177.4 $\pm$ 8.9 Cho) and 4 km (181.7 $\pm$ 5.7 Caf; 174.3 $\pm$

7.2 Pl; 175.6 ± 7.6 Cho) sprints were similar under the different trial conditions and tended to fall with successive sprints (Figure 6.3). Mean heart rate for the 100 km was significantly higher ($P < 0.01$) in the Caf group compared to the Cho group (181.6 ± 1.9 Caf; 175.5 ± 2.7 Cho).

Table 6.5. Heart rates (b.min-1) during 1 km sprints during the 100 km time trial for Caf, Pl and Cho.

km	Pl	Cho	Caf
10	177.4 ± 5.8	177.4 ± 9	184 ± 9.9
32	176.1 ± 6	176.5 ± 8.3	181.9 ± 6.7
52	173.4 ± 5.4	176.2 ± 7.7	182.7 ± 6.9
72	171.7 ± 4.9	174.6 ± 6.5	181.6 ± 6.8
99	172.5 ± 7.2	177.8 ± 5.3	181.5 ± 10.4

Values are means ± SD.

Table 6.6. Heart rates ($b.min^{-1}$) during 4 km sprints during the 100 km time trial for Caf, Pl and Cho

km	Pl	Cho	Caf
20	174.6 ±6.9	175.6 ± 7.6	181.7 ± 5.7*
40	171.3 ± 7.2	174.1 ± 8.3	181.8 ± 6.7*
60	172.3 ± 5.8	172.4 ± 6.7	181.5 ± 6.7*
80	170.6 ± 5.5	171.8 ± 6.4	177.3 ± 10*

Values are means ± SD.

* - $P < 0.05$ between groups and over time for Caf, and Pl and Cho.

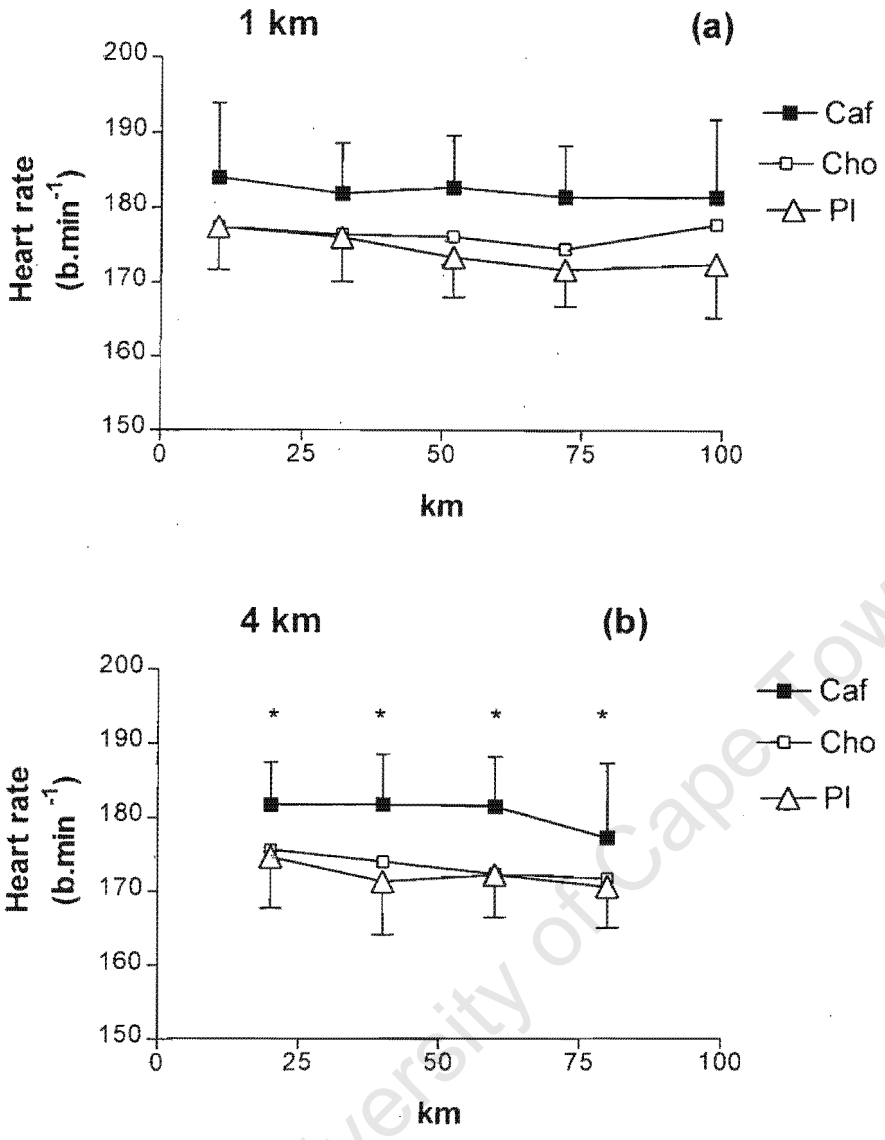


Figure 6.3. Average heart rates during 1 km (a) and 4 km (b) sprints for Caf, Pl and Cho (* - $P < 0.05$ between groups and over time for Caf, and Pl and Cho).

6.3.4 *IEMG*

Due to technical difficulties with the EMG instrumentation, complete EMG data could only be collected for Cho and Caf. IEMG expressed as a % of the IEMG recorded during the MVC are shown in Table 6.7 and 6.8 and Figure 6.4. There were no significant differences in IEMG values in any of the sprints for Caf or PI (Table 6.7, 6.8 and Figure 6.4). The values ranged between 15.2 ± 1.0 % and 41.3 ± 17.3 % in the 1 km sprints and between 19.9 ± 4.4 % and 26.3 ± 7.7 % in the 4 km sprints. There were no differences between groups or over time. The average IEMG was 25.4 ± 8.6 % for 4 km and 22.0 ± 2.4 for 4 km TT.

Table 6.7. IEMG (%) normalised against MVC during 1 km sprints during the 100 km time trial for Caf, Pl and Cho.

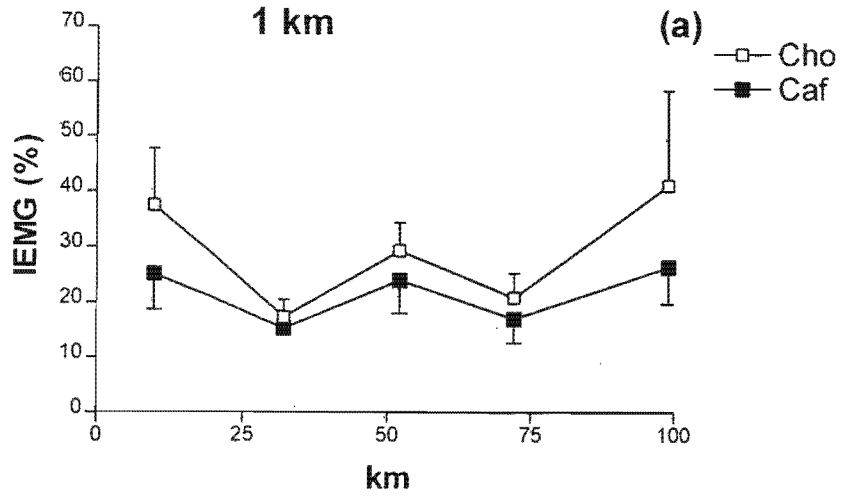
Km	Cho	Caf
10	37.5 ± 10.2	25.1 ± 6.4
32	17.3 ± 3.1	15.3 ± 1
52	29.3 ± 5.1	24 ± 6
72	20.9 ± 4.4	17 ± 4.4
99	41.2 ± 17.3	26.5 ± 6.6

Values are means ± SD.

Table 6.8. IEMG (%) normalised against MVC during 4 km sprints during the 100 km time trial for Caf, Pl and Cho.

Km	Cho	Caf
20	20.4 ± 4.6	23.6 ± 5.1
40	22 ± 4.4	19.1 ± 4.4
60	19.3 ± 2	22.2 ± 5
80	23.1 ± 4.5	26.3 ± 7.7

Values are means ± SD



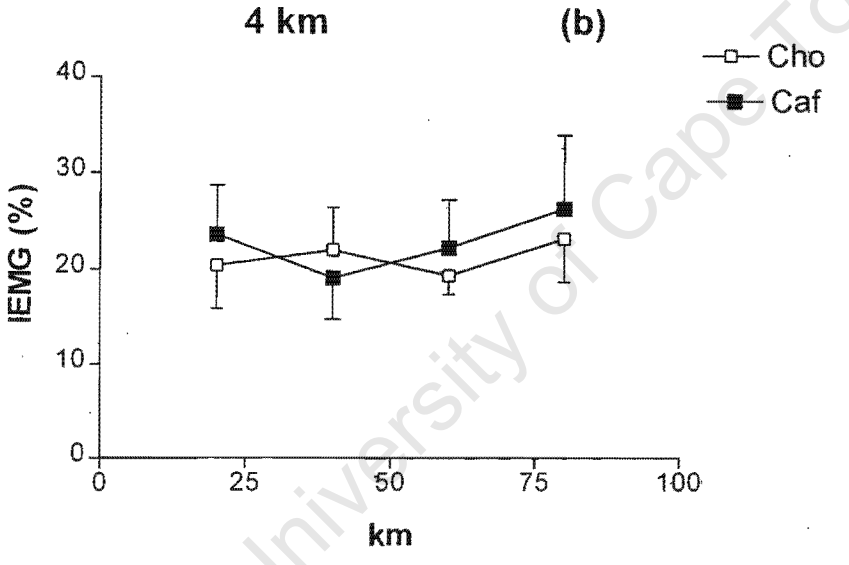


Figure 6.4. Integrated EMG (IEMG) values expressed as a % of values recorded during an isometric MVC, during 1 km (a) and 4 km (b) in Caf and Cho trials.

6.3.5 *MPFS*

The mean power frequency spectrum, expressed as a % of the MPFS measured during the MVC is shown in Table 6.9 and 6.10 and Figure 6.5.

There were no differences for MPFS between groups, over time or between groups the 1 km and 4 km time trials (Table 6.9, 6.10 and Figure 6.5). However, during MPFS in the first and last 4 km sprint there was a ~12% difference with the Cho group shifting to upper part of the spectrum and the Caf group shifting to lower part of the spectrum.

Table 6.9. MPFS (%) normalised against MVC during 1 km sprints during the 100 km time trial for Caf and Cho.

km	Cho	Caf
10	1.10 ± 0.06	1.02 ± 0.04
32	1.08 ± 0.09	1.06 ± 0.04
52	1.11 ± 0.09	1.05 ± 0.04
72	1.08 ± 0.07	1.16 ± 0.07
99	1.21 ± 0.06	1.13 ± 0.06

Values are means ± SD

Table 6.10. MPFS (%) normalised against MVC during 4 km sprints during the 100 km time trial for Caf and Cho.

km	Cho	Caf
20	1.13 ± 0.08	1.01 ± 0.04
40	1.12 ± 0.08	1.05 ± 0.06
60	1.13 ± 0.07	1.13 ± 0.10
80	1.20 ± 0.06	1.06 ± 0.04

Values are means ± SD

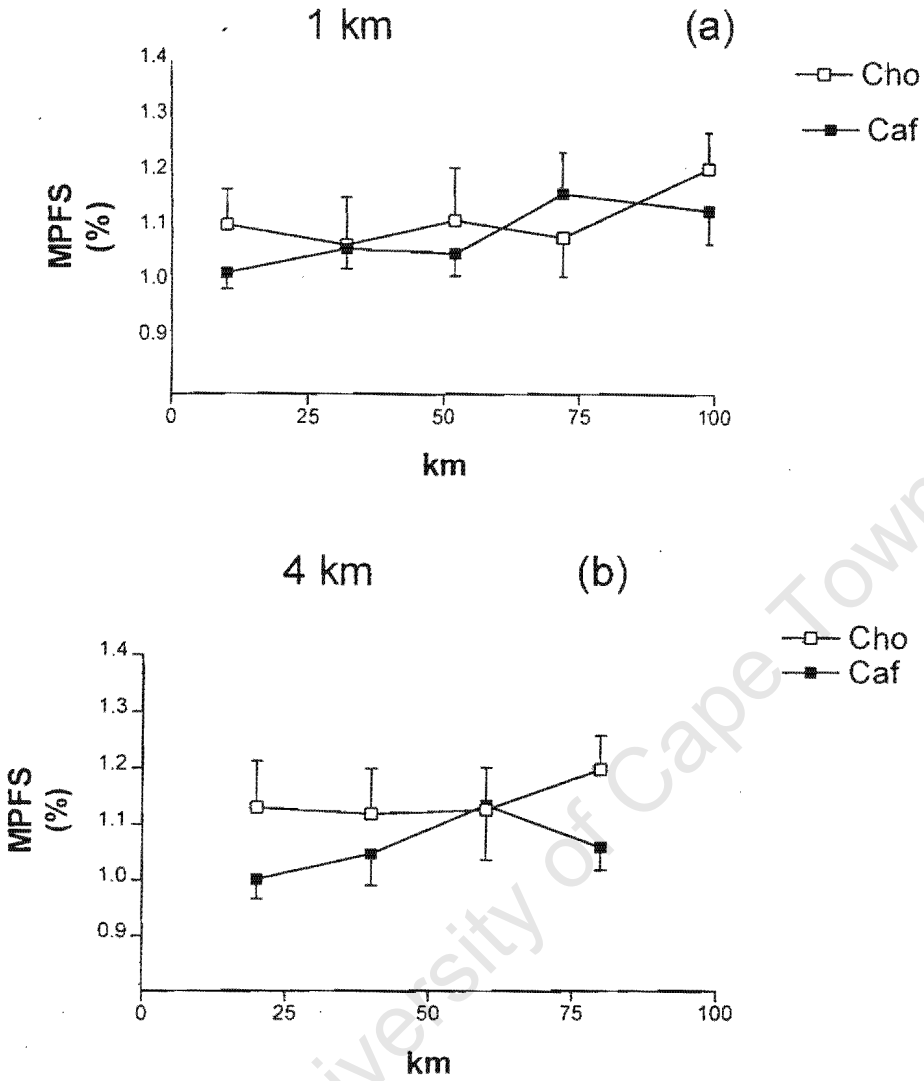


Figure 6.5. Mean power frequency spectrum (MPFS) values normalised against an isometric MVC during 1 km (a) and 4 km (b) sprints in Caf and Cho trials.

6.3.6 *Serum FFA and lactate concentrations*

Serum FFA and lactate concentrations increased significantly during exercise in all TT ($P < 0.01$). There were no differences in serum FFA and lactate concentrations between groups (Table 6.11, 6.13, Figure 6.6).

Table 6.11. Serum free fatty acid concentrations ($mmol.l^{-1}$) before (pre) and after (post) the TT for Pl, Cho and Caf

	Pl	Cho	Caf
Pre	0.2 ± 0.2	0.1 ± 0.1	0.3 ± 0.2
Post	1.6 ± 0.9*	1.6 ± 0.5*	1.9 ± 0.4*

Values are means ± SD

* - P < 0.01 Pre vs post

Table 6.12. Serum lactate concentrations ($mmol.l^{-1}$) before (pre) and after (post) the TT for Pl, Cho and Caf

	Pl	Cho	Caf
Pre	1.1 ± 0.5	1.3 ± 0.3	1.6 ± 0.5
Post	4.5 ± 1.7	4.1 ± 1.6	4.9 ± 1.4

Values are means ± SD

*- P < 0.01 Pre vs post

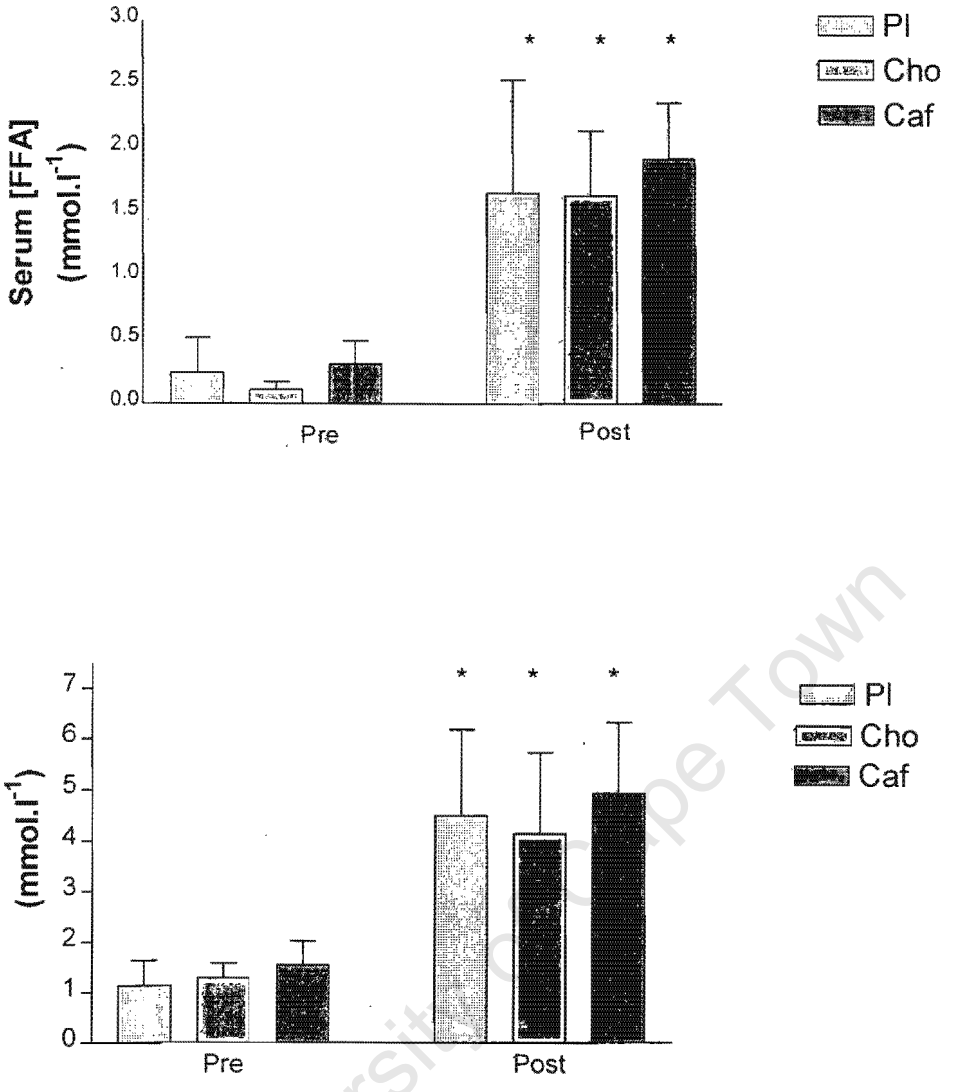


Figure 6.6. Serum free fatty acid (top) and lactate (bottom) concentrations before and after the TT in placebo (Pl), carbohydrate (Cho) and caffeine (Caf) trials. Serum FFA and lactate concentrations increased significantly during exercise but were not different between groups (* - $P < 0.01$ vs pre-exercise values).

6.3.7 *RPE*

RPE values rose progressively ($P < 0.01$) in successive 1 km (Table 6.13, Figure 6.7 top) and 4 km (Table 6.14, Figure 6.7 bottom) sprints. Peak RPE's

were also similar in the consecutive 1 km (19.8 ± 0.4 Caf; 19.6 ± 0.5 Pl; 18.7 ± 1.5 Cho) and 4 km (18.4 ± 1.9 Caf; 18.2 ± 1.8 Pl; 18.5 ± 1.5 Cho) sprints but there was no difference between Pl, Cho and Caf.

Table 6.13. RPE (units) during 1 km sprints during the 100 km time trial for Caf, Pl and Cho.

Km	Pl	Cho	Caf
10	15.4 ± 2.9	15.3 ± 2.5	15.0 ± 4.1
32	16.6 ± 2.1	15.7 ± 2	16.3 ± 3.1
52	17.2 ± 1.8	16.6 ± 2.1	16.6 ± 1.6
72	17.4 ± 1.6	17.4 ± 2	18.0 ± 1.1
99	19.6 ± 0.6	18.7 ±1.5	19.8 ± 0.4

Values are means ± SD

Table 6.14. RPE (units) during 4 km sprints during the 100 km time trial for Caf, Pl and Cho.

km	Pl	Cho	Caf
20	16.0 ± 2.5	15.7 ± 2.5	15.8 ± 3
40	16.2 ± 1.6	16.3 ± 2	16 ± 1.5
60	16.8 ± 2	17.2 ± 1.6	17 ± 1.1
80	18.2 ± 1.8	18.4 ± 1.5	18.4 ± 1.2

Values are means ± SD

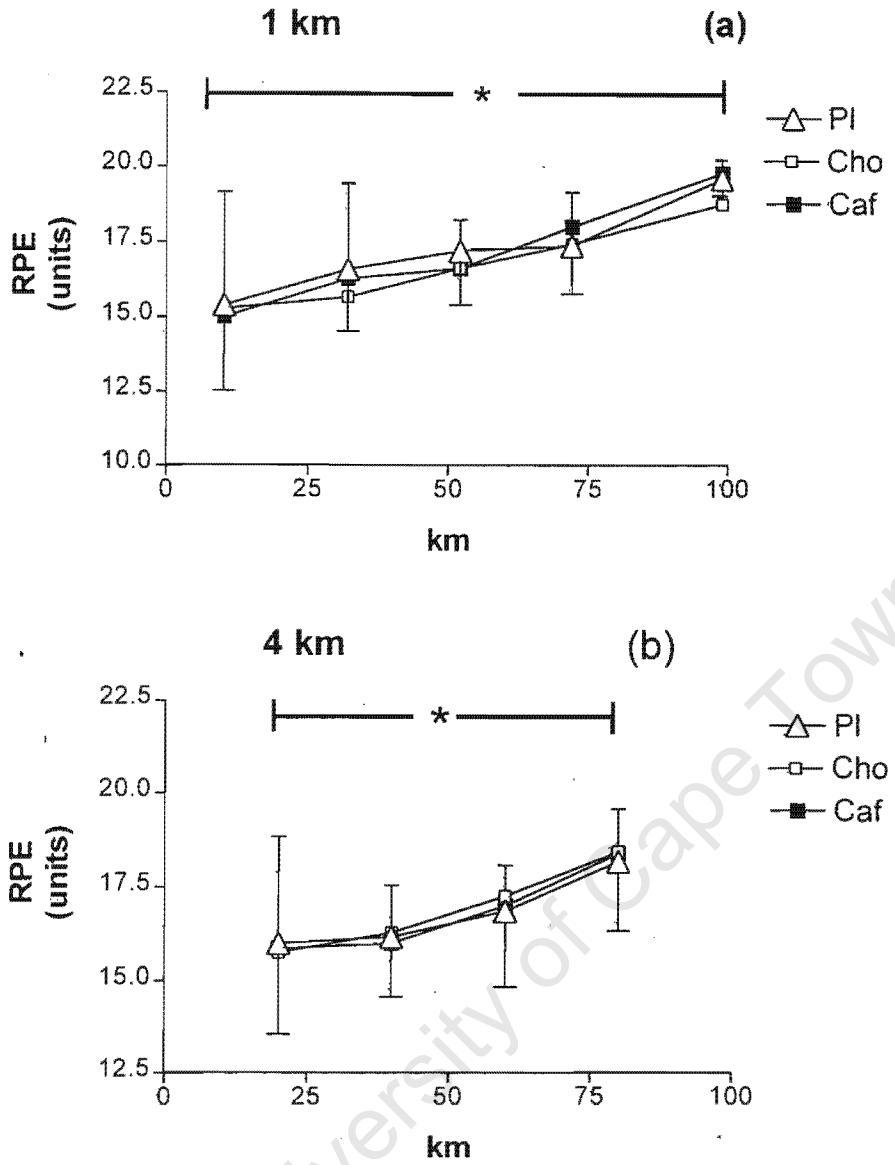


Figure 6.7. RPE values during the 1 km (a) and 4 km (b) sprints for the placebo (Pl), carbohydrate (Cho) and caffeine (Caf) trials. RPE values rose significantly (* - $P < 0.01$ for all groups in both 1 km and 4 km, but were not different between groups).

6.3.8 *Weight loss*

Weight loss during the three trials was similar and averaged ~1.-kg. (Table 6.15, figure 6.8).

Table 6.15. Percentage of weight (%) lost over the TT for Pl, Cho and Caf.

	Pl	Cho	Caf
	3 ±1.6	2.7 ±1.5	3.5 ±1.8

Values are means ± SD

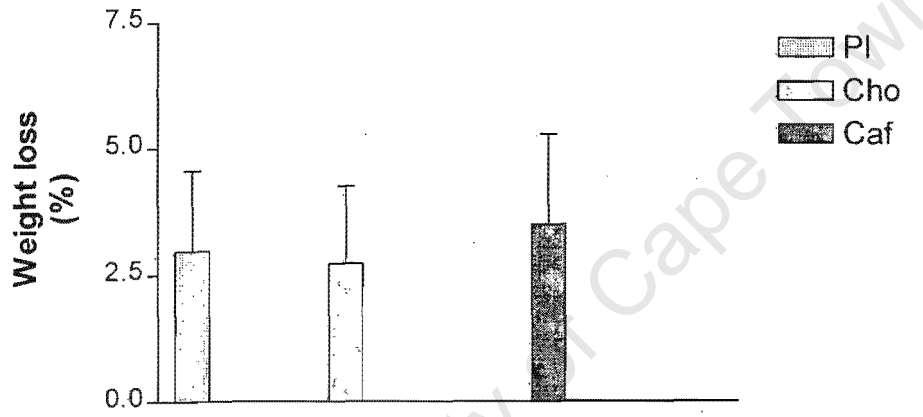


Figure 6.8. Percentage weight loss during the TT in placebo (Pl), carbohydrate (Cho) and caffeine (Caf) trials.

6.3.9 *Drug identification*

7 out of the 8 subjects correctly identified when they ingested caffeine, citing symptoms of reduced concentration, elevated heart rate, before during and after the trial and difficulty in sleeping the following night.

6.4 DISCUSSION

The bulk of current evidence suggests that caffeine ingestion enhances performance to quite a marked extent during more prolonged endurance exercise (465), although there appears to be little if any effect during high intensity exercise of short duration (141;212;218;415).

However, most of the evidence for this large effect comes from studies in which subjects begin exercise unaware of any fixed end point other than to continue exercising for "as long as possible". One result is that subjects do not have a fixed goal around which to plan their pacing strategy. We (459) and others (357) have shown that the coefficient of variance for such open-end testing is large. In contrast, performance during closed trials in which subjects begin exercise knowing exactly what is the endpoint in terms of distance to be covered or total exercise time, is far more reproducible (458). In addition, relatively few studies have evaluated the effects of caffeine on the performance of better performing athletes of the quality studied in this trial.

Accordingly, the most important finding of this study was that, in contrast to the large ergogenic effect measured in open-ended trials, caffeine did not significantly enhance overall performance during the 100 km cycling time trial that included bouts of high intensity exercise. This is consistent with other trials which show that any effect of caffeine is much greater in open-ended than in closed trials. For example, the magnitude of the ergogenic effect of caffeine in open-ended trials is of the order of 20-50% (214), whereas the average performance enhancement in closed trials was 1.8 % (range 1.7 –

2.5%) (34;79;301;338;527). In this study, subjects were 1.3% faster when using caffeine compared to placebo but 0.1% slower when ingesting caffeine than in the familiarization trial when they ingested their chosen drinks "ad libitum".

Plasma caffeine concentrations were low in the Pl and Cho trials but were markedly elevated in the Caf trial. Hence illicit use of caffeine before the Pl or Cho trials could not explain our failure to detect an ergogenic effect of caffeine. The significantly higher heart rates with caffeine ingestion, especially during the 4 km sprints, confirm that the ingested caffeine was physiologically active and was not due to an increase in cycling intensity.

Our second important finding was that caffeine ingestion did not significantly alter the pacing strategy. Thus, although subjects performed the first 1 km sprint slightly faster; and the last 1 km sprint slightly slower during the Caf trial than when they ingested either carbohydrate or placebo, this effect was not significant. Similarly, the second and third 4 km sprints were slightly but not significantly faster with caffeine. In contrast sprinting performances when ingesting placebo or carbohydrate were similarly reproduced (Figure 6.2). However, subjects had sight of their heart rate, which could have been used to control their pace to avoid fatigue.

Thus, despite a complete absence of all external, particularly temporal clues, subjects employed essentially the same pacing strategies when ingesting either carbohydrate or placebo, whereas caffeine ingestion may have modified imperceptibly the pacing strategy. Interestingly, in the studies of Bruce et al.

(79), caffeine enhanced the performance of rowers during a simulated 2000 meter trial specifically by increasing their speed only over the first 500m of the race. This suggests that, in that trial, caffeine may have acted directly on the subconscious brain centres that direct the early pacing strategies during exercise.

Indeed, the noteworthy feature of this and other studies (457;457;458;458;460;460;481) is the reproducibility of the pacing strategies used by subjects during closed trials of the type used in this study. This invites the question of the nature of the internal physiological cues determining that response since the subjects in all our trials receive no external cues other than the distance covered.

In this study we found that, whereas average performance time remained relatively unchanged, RPE rose progressively with consecutive 1 km and 4 km sprints, peak heart rate was essentially the same, in both 1 km and 4 km sprints and, if anything, decreased during the course of the exercise. Peak EMG activity was more variable, in contrast to our previous study in which EMG activity was significantly lower in the final 1 km and the last two 4 km sprints in subjects who modified their diets in the 3 days before exercise (481). However, sprinting performance in that trial also fell significantly in the final 1 and 4 km sprints, whereas in this study, performance was unchanged in the successive 1 and 4 km sprints.

Significantly, IEMG activity was about 12% higher during the 1 km than during the 4 km sprints, which is to be expected since subjects cycled faster during

the 1 km than the 4 km sprints. However, as also reported in our previous study (481), subjects recruited only ~25% of their maximal neuromuscular activation during the 1 km sprints and only ~22% during the 4 km sprints.

Skeletal muscle activation (Figure 6.4) and performance (Figure 6.2) was greater in the 1 km than the 4 km sprints. However, if peripheral metabolite accumulation or muscle glycogen depletion determines performance during the 4 km sprints or indeed the pacing strategy during the sustained exercise between the sprints, then even higher performance should not have been possible during the 1 km sprints. Rather, the higher metabolic rate during the 1 km sprints should have increased the concentration of fatigue-inducing metabolites, thereby progressively impairing performance. But this was not the case (Figure 6.2).

Indeed in a previous study employing shorter 40 km time trials, which also included repeated sprints, we (460) found that sprinting performance fell progressively as did blood lactate concentrations whereas pH increased, the opposite of the predicted effect if fatigue is regulated by peripheral metabolites.

Furthermore, only 22 – 25% of the total neuromuscular activation was recruited, even from the outset of the 1 and 4 km sprints (Figure 6.4). Hence something "constrains" neuromuscular activation so that less than 25% of the available muscle mass is ever recruited even from the start of exercise when muscle metabolite concentrations are likely to be the least perturbed. We have proposed that this constraint exists centrally in the brain (285;481).

According to this theory, the consistency of performance in all these trials results from a centrally determined pacing strategy that is uninfluenced by caffeine ingestion. Furthermore, the faster speeds during the 1 km than the 4 km sprints result simply from a greater neuromuscular activation. Since the neuromuscular activation does not rise during the repetitive sprints (Figure 6.4), the absence of a "peripheral" fatigue is confirmed, since the definition of peripheral fatigue requires a greater neuromuscular activation to maintain the same, or a lesser power output (379).

In summary, this study establishes that a substance considered ergogenic because of its stimulatory effects on the central nervous system and shown to have this effect during prolonged open-ended exercise (117;164;213;214;264;414) in which a subconsciously chosen pacing strategy is unnecessary, fails to improve performance when the trial has a defined endpoint so that a pre-determined pacing strategy is required. Furthermore, this and other studies (107) suggest that the pacing strategy is a reproducible, individual characteristic that may be resistant to the effects of stimulatory drugs like caffeine. If any drugs influence a centrally regulated pacing strategy, they could conceivably be as likely to produce a negative as a positive effect.

CHAPTER SEVEN

THE EFFECTS OF SUPRAMAXIMAL EXERCISE ON THE EMG SIGNAL

7.1 INTRODUCTION

The various manipulations used in previous chapters to investigate the involvement of neural mechanisms during exercise fatigue have been during first, hot environments and ß-blocker ingestion in an attempt to pertubate the normal exercising body and therefore reduce the time to onset of fatigue. Second, caffeine ingestion as an ergogenic aid to prolong the time to fatigue and improve performance. Chapters four to six have examined different environments on submaximal fatigue, four and five used open loop protocols and seven used a closed loop protocol. All of these studies been performed with the use of submaximal protocols, therefore the final question required to fully manipulate all aspects of fatigue is to determine the role of neuromuscular recruitment during a supramaximal protocol.

The WAT is reportedly the most effective method for measuring supramaximal performance capacity (25) and achieving high intensity fatigue, which is reviewed in detail in section 1.4.5. To the author's knowledge, no previous research has been performed in which skeletal muscle recruitment patterns have been investigated during a supramaximal ride to exhaustion using the WAT protocol. Accordingly, we set out to investigate the effect of the WAT on the EMG signal.

7.1.1 Question

The question addressed by this study is:

What is the effect of performing the Wingate supramaximal cycling protocol on the neuromuscular pattern?

7.2 METHODS

7.2.1 Subject selection and sampling

Ten healthy males volunteered for this study. The mean age and body mass of the subjects were 21.4 ± 2.6 years and 72.3 ± 8 kg respectively. All subjects were physically active and each signed an informed consent before the study. The Research and Ethics Committee of the University of Cape Town Faculty of Health Sciences approved the study.

7.2.2 Experimental trial

7.2.2.1 *MVC testing*

To normalise EMG data, MVC was determined as described in chapter 2.

7.2.2.2 *Progressive exercise test*

After performing the MVC, the subjects warmed up with light cycling and stretching. The WAT consisted of one 30 second sprint, performed on an

electrically braked cycle ergometer (Watsystem, ITC Corp, Bloomington, USA) with a preset load of 0.09 $kg.kg^{-1}$ BW (144). The subjects were given 5 seconds of loadless pedaling to reach maximum pedal speed and subsequently were instructed to sustain maximal cadence during the 30 seconds once the correct load was applied. All subjects received verbal encouragement throughout the 30 seconds.

The rate of fatigue during the WAT was calculated by using the following equation as described previously by McCartney et al. (352):

Fatigue index % = peak power (W) – lowest power (W) / peak power (W) x 100

7.2.2.3 *EMG testing*

EMG methodology and processing of the signal was as described in chapter 4. EMG was captured continuously throughout the 30 second cycle and divided into 6 five second epochs. The raw data were divided into 7 five second epochs, the first included all data collected during the second maximal isometric trial and during WAT at 5, 10, 15, 20, 25 and 30 seconds. The mean EMG activity at each epoch was calculated. The data for the first epoch was subsequently described as 100% EMG activity, and all subsequent data was normalised by using the first epoch as the denominator for subsequent epoch values.

7.2.3 Statistical analyses

Data are presented as means and standard deviations. Significant differences over time were assessed using a one way ANOVA with repeated measures. Where significant changes occurred an Scheffe's post hoc test was applied to identify individual differences over time and to avoid incurring a type I error. Relationships among variables were assessed using the Pearson correlation coefficient. Significance was accepted at $P > 0.05$.

7.3 RESULTS

7.3.1 *Fatigue index*

The mean rate of fatigue calculated from the decrease in power during WAT was 44.5 ± 8.6 % (Table 7.1). Data from subjects 9 and 10 could not be recorded due to technical difficulties with the cycle ergometer.

Table 7.1. Fatigue index for each subject throughout the 30 second Wingate Anaerobic Test (WAT)

Subject	Fatigue index %
1	53.8
2	34.0
3	38.0
4	49.8
5	45.8
6	50.7
7	51.7
8	32.1
Mean	44.5 ± 8.6

Values are means ± SD

7.3.2 *IEMG*

IEMG normalised to MVC showed no significant change over WAT (Figure 7.1b). However, there was a large individual variation with one subject showing a drop off in IEMG in the final 5 seconds (Figure 7.1a).

Table 7.2 IEMG (%), captured at 5 second time points during the 30 second Wingate, normalised to MVC.

Seconds	IEMG
5	89.7 ± 46.3
10	94.8 ± 56
15	111.7 ± 61.2
20	110.4 ± 69.3
25	113.2 ± 78.7
30	89 ± 40.8

Values are means ± SD

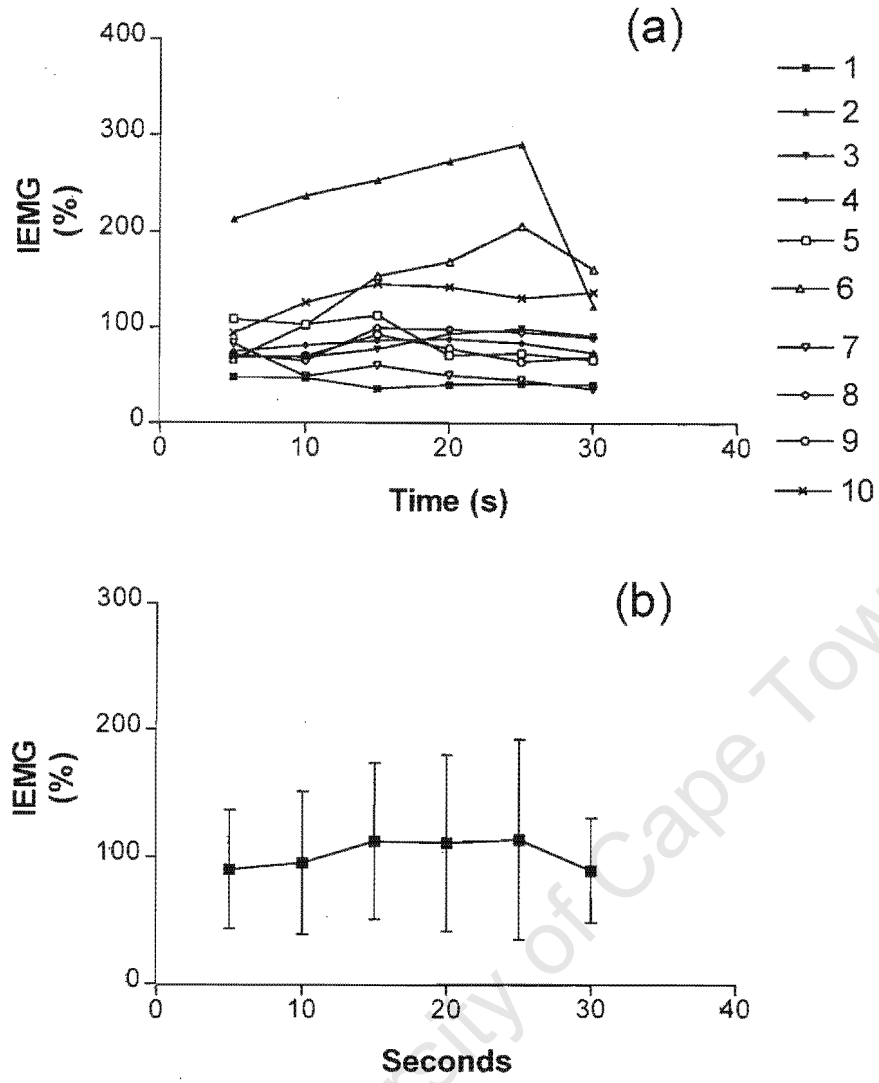


Figure 7.1. Total amplitude (IEMG) as a % of MVC at 5 second intervals for individuals (a) and as a group (b) over a 30 second Wingate Anaerobic Test (WAT).

7.3.3 *MPFS*

MPFS normalised to MVC was significantly reduced by 14.7% over the 30 second WAT ($P < 0.01$) (Table 7.3) (Figure 7.2b) and the individual variation was far less then for IEMG (Figure 7.1a).

Table 7.3. MPFS values, captured at 5 second time points during the 30 second Wingate, normalised to MVC.

Seconds	MPFS
5	0.99 ± 0.07** *
10	0.97 ± 0.06**
15	0.95 ± 0.07**
20	0.9 ± 0.08
25	0.88 ± 0.11
30	0.84 ± 0.11 *

Values are means ± SD

** - $P < 0.01$ 5s vs 25s

5s vs 30s

10s vs 30s

15s vs 30s

* - $P < 0.05$ 5s vs 20s

30s vs 10s

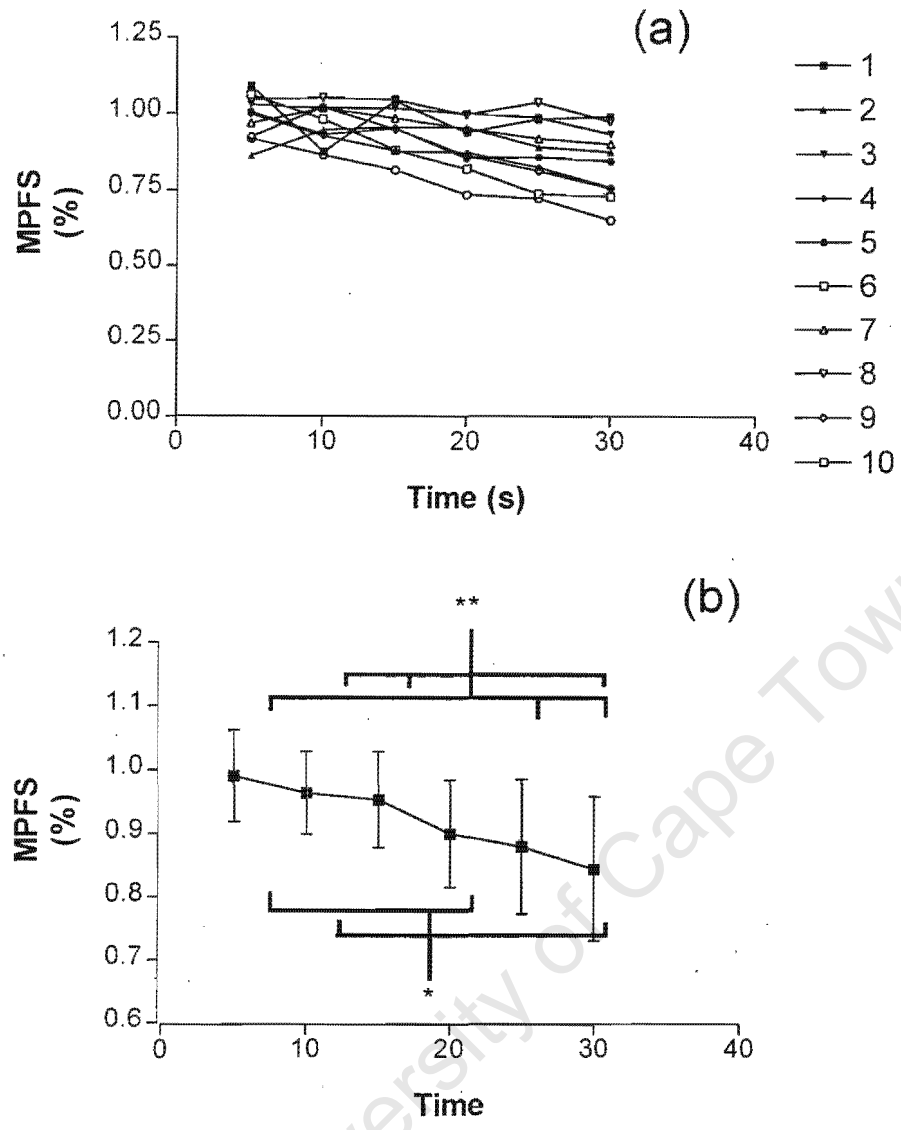


Figure 7.2. Mean power frequency (MPFS) at 5 second intervals during WAT for individuals (a) and as a group (b). Significant (* - $P < 0.05$ and ** - $P < 0.01$) reduction over 30 seconds.

7.3.4 *Correlation*

A correlation of r = 0.56 (NS) was found between fatigue index % and MPFS % decline during WAT (Figure 7.3).

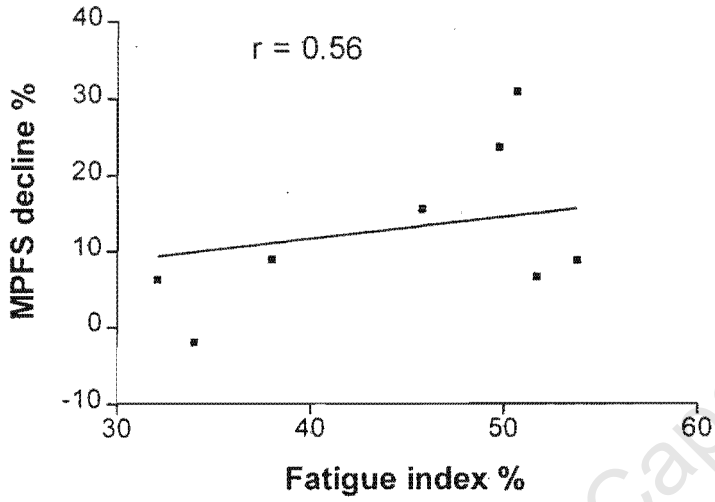


Figure 7.3. Correlation between MPFS % decline and fatigue index during WAT.

7.4 DISCUSSION

The main finding in this study was the significant drop off in MPFS coupled with a relatively unchanging IEMG despite a mean fatigue index (FI) of 45%. The decline in force during the WAT was similar to previous investigators (25). The MPFS decline could be caused by a decrease in pH levels and consequently slowing down of the muscle fiber conduction velocity (MFCV) (549). This decline in MPFS is similar to what happens in both isometric and isokinetic fatiguing protocols (230). This occurrence could be as a result of a reduced central drive (309;549), changing synchronization of motor units (418), motor unit firing time statistics and recruitment of different motor units (309;502). The precise reason for the drop in MPFS in this study remains unclear, however it could be that the 30 second time frame for the feedback loop between central motor drive and intramuscular metabolism at this high level of fatigue and angular velocity is too short but would have come into effect given more time.

IEMG showed no significant change throughout the WAT, our suggestion is that this could be caused by minimal afferent signal from the active muscles to influence a reduction in central drive, thus offering no protective mechanism to initiate changes to adapt to the high level of fatigue that is occurring. It has been suggested that additional motor units (MU) are progressively recruited to compensate for the reduction of contractility due to the impairment of fatigued MU's, resulting in an increasing IEMG (379). This will also cause a decline in the propagation velocity of the action potentials along the muscle fibres (324;511) and the recruitment of new, non fatigued, motor units to replace the

fatigued ones (50). These mechanisms will cause an increase in the duration of the recorded motor-unit action potentials resulting in a reduction in MPFS. However, because the larger motor units become recruited, a larger IEMG is usually recorded. In our study the IEMG showed no significant changes throughout the WAT, thus agreeing with our suggestion.

Intramuscular metabolism during exercise fatigue involves a lowering of pH, which leads to a decline of neural drive, resulting in a loss of force (58;275). However, in the present data, there is no change in IEMG, which does not indicate that there was a decline in neural drive. Indeed, Vandewalle et al. (507) using a 45 second WAT protocol found a decrease in IEMG amplitude. This is in agreement with the findings of Bigland Ritchie et al. (44), Bigland Ritchie et al. (41), Moritani et al. (378) and Petrofsky and Lind (423) who found a decline in IEMG and MPFS throughout MVC. It was concluded that these observations were the result of a decline in central drive to ensure the optimal force output by avoiding peripheral transmission failure (44;47;380). It has been argued that this response may be a safety mechanism protecting the muscle from going into rigor through depletion of ATP (394;477). However, Hussain et al. (263) discovered prolonged post WAT diastolic hypotension. Such prolonged recovery kinetics of femoral arterial flow indicates the continued effect of vasodilator metabolites, which suggests that the mechanism of fatigue during a WAT is predominantly humoral as opposed to neural (263). Because of the ischaemia that occurs in the WAT, the importance of tissue oxygenation differences becomes relevant. When skeletal muscle becomes ischaemic no known protective mechanism is used to prevent cellular damage from occurring, unlike other tissues in the body, for

example the brain (404). Therefore, during a 30 second WAT when skeletal muscles become ischaemic a protective mechanism may not be employed.

Kent-Braun (290) suggested that the relationship between pH and both the decline in force and neural drive are likely to be within a feedback loop between central motor drive and intramuscular metabolism throughout fatigue. Hussain et al. (263) showed a fall in pH from a baseline value of 7.35 $\pm$ 0.01 to 7.15 $\pm$ 0.02 immediately after the WAT. A rise in H^+, has been shown to interfere with Ca^{2+} binding to troponin by reducing the evident binding constant (188;514), this could impede muscular contraction (175). Moreover, Nakamaru and Schwartz (391) discovered the pH dependent association between the sarcoplasmic reticulum and Ca^{2+}, and proposed the possible interference of excess H^+ in excitation-contraction coupling resulting in a decline in the developed force. Juel (275) identified a reduction in membrane excitability and attributed it to the pH dependency of the sodium-potassium pump (275), which might cause a decrease in (74;132;363) resulting in a drop in the spectral frequency (324;485). However, Brody (73) electrically stimulated the hamster diaphragm, whilst decreasing the bath pH resulting in a significant decrease in both spectral power and MFCV. They concluded that substrates other than pH were responsible for conduction velocity changes.

Hussain et al. (263) also showed that mean venous lactate rose from a baseline value of 1.7 $\pm$ 0.15 to 12 $\pm$ 0.67 mmol immediately after the WAT and continued to rise, peaking at 6 mins with a value 13.6 $\pm$ 0.72 mmol. However, venous lactate may not necessarily be indicative of intramuscular lactate levels as WAT causes almost >80% ischaemia (403). Therefore lactate

infusion into the blood during exercise may be minimal resulting in alacticacidaemia (138) despite high lactate concentrations occurring in the active skeletal musculature. Furthermore, Linssen et al. (325) showed that MFCV did not decline throughout MVC in patients with McArdle's disease, when the spectral frequency did drop. This would suggest that factors other than increasing lactate will influence MFCV. Finally, Naeijie and Zorn (388), Lindstrøm et al. (324), Stulen and DeLuca (485); Hägg (232) showed that an MPFS change may occur without a concomitant change in MFCV which also suggests other mechanisms may be responsible.

It has been postulated that changes in muscle temperature may be an important factor in fatigue and MFCV changes (42). However, an increase in muscle temperature during the WAT is an unlikely cause of fatigue as 30 seconds is too short to see a significant rise in muscle temperature. Petrofsky and Lind (423) showed that the curve form of the association between time and IEMG during fatiguing contractions was dependent on the temperature of the muscle. Furthermore, the relationship between IEMG and MPFS signal is influenced by variations in the muscle temperature (423) as a result of possible changes in the sarcolemma resistance and skin temperature (547). Therefore, the influence of temperature on IEMG and MPFS may be important for longer events, but not for this short duration.

Interestingly, the individual variation for IEMG was far greater than for MPFS. Subject 2 displayed a far higher overall IEMG (over 200% of MVC) than other subjects and then rapidly declined in the final 5 seconds, however also showed one of the lowest fatigue indexes. This subject may have

subconsciously attempted the WAT submaximally as opposed to supramaximally, which could be evidence of a protective mechanism as described by St Clair Gibson et al. (481).

In conclusion, during WAT, MPFS was attenuated with no decline in IEMG, The significant reduction in MPFS may have been caused by a drop off in central command or from an accumulation of metabolites in the periphery. However, it is possible that there is a reduction in central drive and a 30 second period is too short for the feedback loop between central drive and intramuscular metabolism to affect motor unit recruitment strategies. Subsequently, further investigation is required to measure metabolite accumulation during the WAT and neural firing pattern changes.

CHAPTER EIGHT

SUMMARY AND CONCLUSIONS

The hotly debated issue within the scientific community of whether the cause of exercise fatigue is centrally or peripherally mediated has risen exponentially over the years with the steady advancement of scientific technology. This debate is often objective and intriguing, however, it is frequently between scientists who are either cemented in their paradigms and too proud to accept constructive criticism of their work and ideas or those who are willing to take risks, step away from the beliefs of the herd and venture into the unknown: "The most beautiful thing we can experience is the mysterious, it is the source of all true art and science" (Enstein (57)).

The novel data and conclusions generated from this thesis may hopefully help clarify the neural mechanisms involved in the exercise fatigue under different circumstances.

In the first part of this thesis, we examined the most effective EMG methodology to use when investigating neuromuscular recruitment patterns during both submaximal cycle fatigue and MVC. We began by testing 7 different capture rates (32, 64, 128, 256, 512, 1024 and 1984 Hz) during MVC, and submaximal cycling at 50% intensity and at exhaustion. We found that data is consistent therefore reliable if captured over 326 Hz for 50% intensity cycling, 503 Hz for cycling at exhaustion and 1604 Hz for MVC. However, during scientific testing it is impractical to use different capture rates for different activities and intensities, therefore we chose the highest capture rate available on our machinery of 1984 Hz to ensure the collection of reliable data for both MVC and cycling. Consequently, to compare data from all the activities and intensities we decided to use the highest capture rate available on our EMG machinery, which is 1984 Hz.

The second study examined the most effective EMG normalisation method for intersubject comparison. We found that MVC will record a higher IEMG than fixed maximal cycle pedal contractions and one maximal cycle revolution and it appears that MVC has fewer variables to affect both IEMG and force output, therefore will be the most effective in describing maximal muscle recruitment activity.

The third study examined was the effect of hot (35 °C) and cold (15 °C) environments on submaximal exercise to fatigue using 10 healthy subjects. The cycle protocol was open loop, which involved three successive 15 min rides at 30%, 50% and 70% of their peak power output and then at increasing [15 $W.min^{-1}$] work rates to exhaustion. No differences in performance were seen because of possible effective thermoregulatory mechanisms. This was shown by a significant increase in skin temperature, a higher heart rate and similar rectal temperature when cycling in the hot environment, which suggests that heat is effectively being conducted out of the body, resulting in controlled core temperature without compromising performance from an elevation in heart rate. There were significant differences in thermal comfort values, however it is likely that effective thermoregulation resulted in similar perceived exertion for both groups. Finally, because core temperature remained unchanged, the afferent command to the CNS from the periphery resulted in similar EMG data, which is the efferent command from CNS to the working muscles in both groups. Therefore, this study indicates a number of factors that will effect exercising under hot and cold conditions. The hot environment caused changes in skin temperature and heart rate, but not in

rectal temperature. EMG was not altered in the presence of elevated skin temperature and thermal comfort. RPE was the same for both the hot and cold groups, suggesting that peripheral mechanisms resulted in unchanged central drive. Therefore, it appears that during the hot environment used in this study, effective peripheral thermoregulation mechanisms control core temperature, resulting in an unchanged neuromuscular recruitment strategy.

In the fourth study the next condition used to shorten the time to exercise fatigue was the effect of ß-blocker ingestion on 7 healthy subjects. These subjects performed the same open loop cycle protocol as the previous study. We found that ingestion of selective ß1-blockade will impair maximal exercise capacity. This study showed first, an increased perception of effort and a decrease in heart rate in subjects who ingested ß-blocker. It has been proposed that this is due to the inability of healthy subjects to compensate for the decrease in heart rate by increasing stroke volume as it has been maximised as an adaptation effect from exercise training. Second, there was shift in EMG frequency to the upper end of the EMG spectrum, which could have been caused by an increase in conduction velocity from an upregulation in central command to recruit more type II fibers in response to impaired type I fiber function. Finally, blood lactate concentrations were lower at exhaustion in the group who ingested ß-blocker, which could have been as a result of lower power outputs, which will inevitably cause a decrease in lactate concentrations. Therefore this study has shown that ß-blocker ingestion will limit prolonged exercise performance and will have an altered neuromuscular recruitment strategy which could be in an attempt to generate more power to compensate for the reduction in peripheral exercise capacity.

The third and fourth studies discussed above examined fatigue perturbations during open loop protocols. Therefore for the fifth study we analysed the effect of a stimulant on a closed loop protocol to observe the effect on pacing strategy as well as neuromuscular recruitment patterns. Accordingly, we examined the effects of caffeine ingestion on performance during a 100 km cycling time trial that included bouts of 1 and 4 km sprints on eight highly - trained cyclists who participated in 3 separate trials: i) Placebo tablet with 1.7% carbohydrate drink (1 tablet and 150 mml of drink 1 hour before cycling and every 15 minutes during cycling); ii) Placebo tablet with 7% carbohydrate drink (same quantity and dose interval as i; iii) Caffeine tablet with 7% carbohydrate drink (a 6 mg/kg tablet of caffeine) 1 hour before cycling and a (0.33 mg/kg tablet of caffeine) every 15 minutes during cycling. Drink dosage and quantity the same as i and ii.

This study showed that first, in contrast to the large ergogenic effect measured in open-ended trials, caffeine did not significantly enhance overall performance during the 100 km cycling time trial that included bouts of high intensity exercise. Second, plasma caffeine concentrations were low in the placebo trials but were markedly elevated in the trial where caffeine was ingested. Hence non-compliance by subjects ingesting small quantities of caffeine before the placebo trials could not explain a failure of any performance difference between trials. Third, caffeine ingestion modified the pacing strategy to one in which more work was done earlier in the trial. Since subjects were not informed, the effect must have occurred at those subconscious centres that control pacing strategies during exercise. Fourth,

Heart rate values were also significantly higher with caffeine ingestion, especially during the 4 km trials, confirming a stimulatory effect. Finally, no significant differences were found between groups for any of the EMG data. Therefore, despite markedly elevated serum caffeine concentrations, subjects ingesting caffeine did not perform better during a 100 km cycling time trial that included repeated sprinting. Caffeine increased exercising heart rate significantly and altered the pacing strategy so that more work was performed early in the trial. Hence caffeine may be without ergogenic benefit during endurance exercise in which the athlete begins exercise with a defined, predetermined goal measured as speed or distance.

All the previous studies have manipulated neural fatigue by using submaximal protocols. Therefore to manipulate as many aspects of neural fatigue as possible, we decided to examine neuromuscular recruitment strategies during supramaximal exercise. Accordingly, for the final study we used the Wingate test to induce high intensity fatigue on ten healthy subjects. The Wingate involves pedalling as fast a possible against a preset load of 0.09 $kg.kg^{-1}$ body weight for 30 seconds, whereupon most subjects reach their peak power output at 5 seconds and steadily decline until completion. EMG data and rate of fatigue was recorded throughout the cycling. This study showed that throughout the 30 second Wingate, the EMG spectrum significantly reduced whilst EMG amplitude showed no significant change, but showed a trend towards dropping off in the final 5 seconds. The following possible causes are proposed:

Therefore, during the Wingate test, the EMG frequency spectrum was compressed with no decline in EMG amplitude, however EMG amplitude displayed a decreasing trend over the final 5 seconds in some individuals. The significant reduction in EMG frequency spectrum may have caused by a drop off in central command or from an accumulation of metabolites in the peripheral skeletal muscle. It is however possible that there is a reduction in central drive and a 30 second period is too short for the feedback loop between central drive and intramuscular metabolism to affect motor unit recruitment strategies.

Specifically the conclusions resulting from the data generated by this thesis are:

What is a reliable EMG capture rate to be used for both MVC and submaximal to fatigue cycling?

1. Reliable data is captured over 326 Hz for cycling at 50% of peak power output, 503 Hz for cycling at exhaustion and 1604 Hz for MVC.

What is the most effective method for normalising the EMG signal for submaximal cycling to enable intersubject comparison?

2. MVC will record a higher EMG amplitude than fixed maximal cycle pedal contractions and one maximal cycle revolution and it appears that MVC has fewer variables to affect both IEMG and force output, therefore it will be the most effective in describing maximal muscle recruitment activity.

To gain further understanding of the neural involvement in exercise fatigue, what are the effects of differing exercising fatiguing conditions on the neuromuscular recruitment pattern?

3. The type of fatigue occurring will affect the neuromuscular recruitment pattern. It is proposed that the fatigue may be central or peripheral, which can develop separately or combined, depending on the specific task and the conditions that constitute it has been given. This thesis has shown that in some conditions there is a distinct change in central neural recruitment strategy, possibly to serve as a protective mechanism. In others however there appears to be no change either because of an effective peripheral haemodynamic response that regulates the system or because of an ineffective neural response mechanism that fails to protect the body. The specific effects of the different conditions were answered by the following studies:

What is the effect of both hot and cold conditions on neuromuscular recruitment patterns during submaximal fatigue?

4. The heat protocol caused changes in skin temperature and heart rate, but not in rectal temperature. EMG was not altered in the presence of elevated skin temperature and thermal comfort. Perceived exertion was the same for both hot and cold protocols, suggesting that peripheral mechanisms resulted in unchanged central drive. Therefore, it appears that during the heat protocol, effective peripheral thermoregulation mechanisms control core temperature,

resulting in an unchanged neuromuscular recruitment strategy in the environmental conditions used in our study.

What is the effect of ß-blocker ingestion on neuromuscular recruitment patterns during both submaximal fatigue and MVC?

5. This study and other studies show that selective ß1-blockade will limit prolonged exercise endurance. This study has shown through significant shifts to the upper end of the EMG spectrum that there is an altered neuromuscular recruitment strategy, which could be in an attempt to generate more power to compensate for the reduction in exercise capacity

What is the effect of caffeine ingestion on 100 km cycling performance, pacing strategy and neuromuscular recruitment pattern?

6. Despite markedly elevated serum caffeine concentrations, subjects ingesting caffeine did not perform better during a 100 km cycling time trial that included repeated sprinting. Caffeine increased exercising heart rate significantly and altered the pacing strategy so that more work was performed early in the trial. Hence caffeine affects both peripheral and central systems, but may be without ergogenic benefit during endurance exercise in which the athlete begins exercise with a defined, predetermined goal measured as speed or distance.

What is the effect of performing the supramaximal cycling protocol on the neuromuscular recruitment pattern?

7. During the Wingate test, the EMG frequency spectrum was attenuated with no decline in EMG amplitude, however this amplitude displayed a trend to decrease over the final 5 seconds in some individuals. The significant reduction in frequency spectrum may have caused by a drop off in central command or from an accumulation of metabolites in the peripheral skeletal muscle. It is however possible that there is a reduction in central drive and a 30 second period is too short for the feedback loop between central drive and intramuscular metabolism to affect motor unit recruitment strategies.

CHAPTER NINE

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CHAPTER TEN

APPENDIX

ANTHROPOMETRICAL DATA SHEET

NAME : _____ AGE : _____ DATE : _____
WEIGHT : _____

SKINFOLDS (mm)

	1	2	av		1	2	av
Triceps	___	___	___	Calf	___	___	___
Biceps	___	___	___	Thigh	___	___	___
Subscap	___	___	___	Abdomen	___	___	___
Suprailliac	___	___	___				

DIAMETER (cm)

Humerus	___ ___	Transverse chest	_____
Femur	___ ___	A-P chest	_____
Biacromial	___ ___	Bi-iliac	_____

GIRTH (cm)

Relaxed Arm	___ ___	Above knee	___ ___
Con. arm	___ ___	Abdominal	___ ___
Calf	___ ___	Forearm	___ ___
Chest	___ ___	Wrist	___ ___
Sub-gluteal	___ ___	Ankle	___ ___
Mid-thigh	___ ___	Bitroch	___ ___

HEIGHT (cm)

Stature : _____ Sub-gluteal - knee _____

BIOELECTRICAL IMPEDANCE

RESISTANCE : _____ HEIGHT : _____
HUMERUS DIAMETER : _____(cm) WEIGHT : _____
DATE : _____

Peak Power Output TEST

DATE: .....

TIME OF TEST: .....

WEIGHT:Pre.....Post.....

WORKLOAD: Start @ 3.3W/kg

RAMPRATE: 1W/kg /150 sec

3.3W/kg	Watts	2:30 min:s
4.3		5:00 min:s
5.3		7:30
6.3		10:00
7.3		12:30
Stop		

PPO		(W)
Power:Weight		(W/kg)
Maximum Heart Rate		(bpm)

Appendix 3 – Maximal voluntary contraction

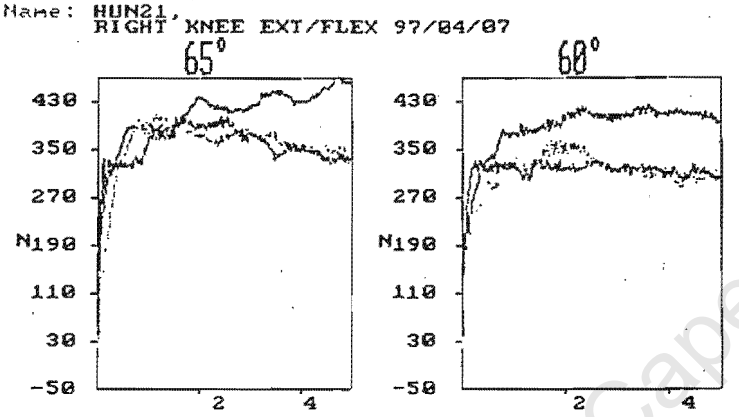
KIN-COM TEST RESULT
Version: 5.13

SPORTS SCIENCE INSTITUTE OF S.A.
BOUNDARY ROAD
NEWLANDS
(021)6866968

Test Date : 97/04/07
Name : HUN21,
Diagnosis : TRIAL
Physician : GIBSON
Clinician :
Involved Side : Body Weight: 78 Kg
Tested Joint : KNEE Side: RIGHT File: A4.CHT
Muscle Group : EXT/FLEX
Lever Arm Length: 31 cm Start Angle: 65 deg Stop Angle: 60 deg
Sets Specified : 3

ISOMETRIC CURVES

Name: HUN21,
RIGHT KNEE EXT/FLEX 97/04/07



Angle	Min Contr Force (N)	Peak Force (N)	Time to Peak (sec)	Mean Force (N)
65	16	468	4.7	407
65	16	406	1.1	353
65	16	403	1.6	353
60	16	423	3.6	381
60	16	364	1.8	306
60	16	331	0.4	312

Assessment: hun21

Appendix 4 – Normalisation study subject information and informed consent

SUBJECT INFORMATION

This study, conducted at the Sport Science Institute of South Africa, is investigating electromyographic normalisation methods. The following measurements and tests will be performed:

Anthropometry: weight, height and body fat %.

Maximum contraction of the quadriceps muscles on the Kin Com dynamometer.

Maximum contraction on a fixed pedal, whilst seated on a cycle ergometer at knee angles of 60° and 108°.

Maximum one revolution of both pedals whilst seated on the cycle ergometer.

Measurements to be taken whilst on the Kin Com and cycle ergometer: electromyography of the Rectus Femoris and power output.

I understand that I will be free to withdraw from the study at any time and that I will not be subjected to any pressure whatsoever not to withdraw. All the information that is collected during the course of the investigation will be treated with the strictest confidentiality and will be used for scientific research purposes. Names and personal particulars will not be released under any circumstances. I will be free to ask any questions about the procedures and results of the study.

I, the undersigned, have read and understood the purposes and procedures involved in the scientific study. I also understand that my participation is on a voluntary basis.

Date: _____

Subject: _____

Signature: _____

Investigator: _____

Signature: _____

Witness: _____

Signature: _____

Appendix 5 – Normalisation study data

Date_____ Code_____

Data Capture

EMG

Isometric Kin Com

Isometric pedal 60° _____

Isometric pedal 108° _____

One revolution max _____

_____

Work

Isometric Kin Com

One revolution max _____

_____

Appendix 6 – Heat study subject information and informed consent

SUBJECT INFORMATION

This study, conducted at the Sports Science Institute of South Africa, is investigating muscle activity during exercise in the heat. The following measurements/tests will be performed:

visit 1:
Anthropometry: weight, height, bodyfat

Peak Power Output (PPO) and maximal aerobic capacity (VO2max) to be performed on a cycle ergometer in thermo-neutral conditions

visit 2:
Isometric max. test of the quadriceps muscles

Cycling test in 15°C in the environmental chamber, using the following protocol:
15 minute ride at 30% of PPO
15 minute ride at 50% of PPO
15 minute ride at 70% of PPO
15 W increase every minute to exhaustion

Measurements taken whilst on the cycle ergometer in the environmental chamber:
-oxygen uptake
-heartrate
-electromyography of the quadriceps muscles
-skin temperature
-rectal temperature
-before and after the test bodyweight will be measured to calculate sweatrate

visit 3:
Cycling test in 35°C, conducted as above.

All the information collected during the course of the trial will be treated with the strictest confidentiality and will only be used for the purpose of scientific research.

INFORMED CONSENT

I, the undersigned, voluntarily agree to participate in this study. I will be free to withdraw from the study at any moment, without having to justify my decision. I will be free to ask any questions about the procedures and results of the study. I understand that I will receive R50 on completion of the trial as remuneration.

The Sports Science Institute of South Africa or any of the investigators is in no way liable for any harm or damage suffered by me as a result of the trial.

I, the undersigned, have read and understood the purposes and procedures involved in the scientific study.

Subject: _____
Signature: _____

Date: _____

Investigator: _____
Signature: _____

Date: _____

Appendix 7 – Heat study data

Date_____ Code_____

Test 1

Completion time_____

Time	RPE	Thermal comfort	Rectal	Bicep	Sternum	Thigh	Calf	EMG
Rest								
10								
25								
40								
Exh								

Test 2

Completion time_____

Time	RPE	Thermal comfort	Rectal	Bicep	Sternum	Thigh	Calf	EMG
Rest								
10								
25								
40								
Exh								

Appendix 8 – β Blocker study subject information and informed consent

INFORMED CONSENT

I, the undersigned, have been fully informed about the dangers inherent in participation in this trial. I also understand that the following measurements/tests may be conducted on myself before, during and after participation in the trial or any of its components:

Oral daily intake of 400 mg Acebutolol at breakfast for a period of seven days.
SIDE-EFFECTS AND SPECIAL PRECAUTIONS:
Those common to β-blockade include: hypotension, bradycardia, gastro-intestinal effects and depression- have been met with infrequently.

Anthropometry: Weight, height, girths, diameters, segmental lengths and volumes, body fat.

VO_{2MAX} and PPO to be performed on the Lode cycle ergometer using laboratory oxycon.

Isometric muscle function of Rectus Femoris on Kin-Com whilst recording electromyographic (EMG) data.

Protocol for Lode cycle:
15 minute ride at 30% of PPO
15 minute ride at 50% of PPO
15 minute ride at 70% of PPO
15W increase every minute to exhaustion

Tests to be performed whilst on the cycle ergometer:

1. VO_{2MAX} to be recorded from the lab oxycon
2. EMG to be attached to Rectus Femoris
3. Catherization for blood samples – lactate

I understand that some of the above tests are invasive and have certain risks. I also understand that some of the investigators undertake to inform me of any negative side effects or findings as soon as results of tests become available. The University of Cape Town or any of the investigators is in no way liable for any harm or damage suffered by me during the course of the trial or arising from the trial.

INFORMED CONSENT

I, the undersigned, voluntarily agree to participate in this study. I will be free to withdraw from the study at any moment, without having to justify my decision. I will be free to ask any questions about the procedures and results of the study. I understand that I will receive R50 on completion of the trial as remuneration.

The Sport Science Institute of South Africa or any of the investigators is in no way liable for any harm or damage suffered by me as a result of the trial.

I, the undersigned, have read and understood the purposes and procedures involved in the scientific study.

Subject: _____
Signature: _____

Date: _____

Investigator: _____
Signature: _____

Date: _____

Appendix 9 – β Blocker study data

Date_____ Code_____

Test 1

Completion time_____

Time	VO_2	RPE	Power	Time	EMG
15				10	
30				25	
45				40	
Exhaustion				Exhaustion	

Test 2

Completion time_____

Time	VO_2	RPE	Power	Time	EMG
15				10	
30				25	
45				40	
Exhaustion				Exhaustion	

UNIVERSITY OF CAPE TOWN

LIBERTY LIFE CHAIR OF EXERCISE AND SPORTS SCIENCE IN THE
BIOENERGETICS OF EXERCISE RESEARCH UNIT
OF THE MEDICAL RESEARCH COUNCIL (MRC)
AND THE UNIVERSITY OF CAPE TOWN
Sports Science Institute of South Africa Boundary Road Newlands 7700
PO Box 115 Newlands 7725
Telephone (021) 6867330 International 27-21 6867330 Fax (021) 6867530

Dear Subject

Thank you for taking part in the performance trial sponsored by Energade to determine the effects of varying quantities of caffeine, sodium and glucose on performance. From this study, we aim to determine your performance under varying conditions. The trial will be of high quality intensity, which will enhance your overall performance.

In participating in this study, you will receive the overall results of the study in addition to your individual results, which will include:

1) VO2 max (maximum oxygen consumption)
2) Peak Power Output (PPO)
3) % body fat and muscle mass
4) Maximal Isometric Force Output

When taking part in the study, it is important not to alter your training or dietary intake. We want to know what YOU are doing.

Thanks again and enjoy!!

Signature removed

Angus Hunter

MSc Exercise Science

UNIVERSITY OF CAPE TOWN

LIBERTY LIFE CHAIR OF EXERCISE AND SPORTS SCIENCE IN THE
BIOENERGETICS OF EXERCISE RESEARCH UNIT
OF THE MEDICAL RESEARCH COUNCIL (MRC)
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Sports Science Institute of South Africa Boundary Road Newlands 7700
PO Box 115 Newlands 7725
Telephone (021) 6867330 International 27-21 6867330 Fax (021) 6867530

SUBJECT INFORMATION SHEET FOR PERFORMANCE TRIAL

Requirements: You will be a well-trained male cyclist between the ages of 18 and 35, accustomed to riding long periods (3-4hrs)

THIS TRIAL WILL INCLUDE 5 VISITS TO THE SPORTS SCIENCE INSTITUTE:

1) Initial testing: total time 1hr

Peak oxygen consumption (VO2 peak) – from which peak power output (Wpeak) and maximum heart rate (Hrmax) will be determined.

On the same day as the VO2 peak test, body composition measurements will be taken. These will include: the sum of seven skinfolds (to determine % body fat) and leg measurements (to determine lean thigh volume).

At this session you will be given a booklet with the following questionnaires to complete:

- Informed consent - to acknowledge what's required of you as a subject and that you agree to take part in the trial.
- Training history questionnaire – to assess your training for the past 15 years.
- Training logbook – to accurately log your training for 8 weeks during the study.
- Three-day dietary record – to accurately record your dietary intake for one weekend day and for the three days prior to the time trial.
- Diet plan – to be followed the day before the time trial.

- Habitual caffeine users will be requested to abstain from caffeine use for 48 hrs before each time trial, which will include sources such as tea, coffee, cola drinks, chocolates or any caffeine containing pharmaceuticals

2) Experimental Trial: total time 4hrs

- **You must not exercise the day before this test!!**
- You cannot drink anything but water for 10 hours before the test.

- Breakfast will be given to you 3 hours prior to the trial

Bring your bicycle and upon arrival at the laboratory we will it calibrate on the Kingcycle.

MVC testing
You will perform 4 x 5 second maximal voluntary contractions on the Kin Kom isokinetic dynamometer testing the knee extensors.

Body mass
Body mass will be measured before and after the time trial to measure the average sweat loss.

EMG
EMG electrodes will be placed on your mid thigh for EMG recordings during the MVC's and throughout the time trial.

Heart rate monitor
You will be required to wear a heart rate monitor throughout the duration of the time trial.

Urine sample
Urine sample to be taken before and immediately after the time trial.

Blood sample
A blood sample will be taken from the subcutaneous vein before and immediately after the trial

Time trial
The time trial must be performed at the same time of day under the same conditions in the environmental chamber.

Hydration
Before commencing the time trial you will required to either ingest 400ml of a Energade drink and then receive the same solution at a rate of 15ml/kg/body mass per hour throughout the ride or receive sublingual ice treatment.

Tablets
Before commencing the trial you will be required to ingest a concoction of tablets, which will consist of either sodium and/or caffeine both before and during the trial.

Cycle protocol
After performing 5 minutes self paced warm up you will begin the 100km trial. The time trial will include a series of sprints;
5 x 1Km sprints, after 10, 32, 52, 72, and 99 Km
4 x 4Km sprints after 20, 40, 60 and 80 Km
You will be instructed to complete the total distance, as well as the sprints in the fastest time possible. The only feedback given to you during the time trial will be your elapsed distance and heart rate.

If you have any questions, please contact me immediately.
Thanks for taking part!!!

Angus Hunter

Tel: 686 7330 ext 264 (w) 083 362 5936 (emergancy)
Email: ahunter@sports.uct.ac.za

I, the undersigned, voluntarily agree to participate in the research trial investigating Energade Sports Drink with varying. quantities of caffeine and sodium. I have been given a full explanation of this trial and have been given the opportunity to ask questions on all aspects pertinent to this study. I understand that I am free to withdraw from this trial at any time without having to justify my decision. I understand that I will receive R500 on the successful completion of this trial as remuneration.

All the information collected during the course of the trial will be treated with the strictest confidentiality and will only be used for the purpose of scientific research.

I the undersigned have read and understand the purposes and procedures involved in the scientific study.

Signed by the volunteer:.....

Date:.....

I confirm that I have witnessed the above signature:

Signed:.....

Date:.....

Appendix 11 – Caffeine study diet information

THREE DAY DIETARY RECORD FORM:

It is essential that you eat as you normally do, don't change your eating patterns or choices just because you are keeping a record.
Record your dietary intake on **TWO weekdays** and **ONE weekend day**.

When filling in the form:

- Record the approximate time that you ate/drank the meal/beverage.

- Record the food/beverage consumed and give a detailed description of the food:
  - use brand names if possible (e.g. Tussers cheese, Trim mayonnaise)
  - state how the food was cooked (steamed, boiled, roasted, fried etc.)
  - record if the meat was fatty or lean, was it crumbed?
  - was the chicken skin eaten?
  - record the type of margarine used and approximately how much (e.g. 1 tsp.)
  - state what type of milk was used (2%, full cream or skim/fat-free milk)
  - record the type of cheese used (cheddar, feta, low fat cottage cheese, fat-free cottage cheese, brie etc.)
  - when eating canned food, state whether it is canned in oil or water or tomato?
  - record any additions to food such as cream, sugar etc.
  - when eating mixed foods such as stew or stir-fry, describe in as much detail the contents of the dish. If you prepared the food, record the quantities that you put in and divide by the proportion eaten.

- Record the AMOUNT of food consumed. Either record
  - the weight (in grams) or
  - the volume (e.g.. 250 ml) or
  - use a household measure (1/2 cup, 1 tsp. etc.) or
  - the dimension (e.g.. 10 cm of boerewors, 20 cm diameter pizza) or
  - draw the food on the back of the page (e.g.. the size of a chop)
- Always report the COOKED weight
- When using household measures, record if it is a heaped or level spoon, what type of spoon (dessert, table or teaspoon)

Try and be as specific as possible, rather over-describe than under-describe as this will improve the reliability of the analysis. Use the back of the page if necessary.
If you are not certain about anything, please contact me immediately.

Sacha West BSc Med (Hons) (SA)
Tel: 686 - 7330 x 297 (w) by Email: sachaw@sports.uct.ac.za

DIETARY RECORD FORM:

NAME: _____ DAY: _____ DATE: _____

Time:	Food/beverage type:	Description/brand name or method of preparation:	Amount:

Is this representative of your USUAL daily intake?

Yes No Explain: _____

_____

Type of physical activity/training:	Time (minutes)

Did you take any supplements ? (vitamins or minerals or other supplements?)

No Yes ,

if yes, what (brand name, dosage etc.)_____

_____

Dietary intake 24 hours before trial: 75 kg body weight

(360 g carbohydrate; 90 g protein)

The following items can be spread throughout the day as you like:

- 2 cups (250 ml) of milk/yoghurt
- 8 teaspoons of sugar/jam/syrup/honey
- 500 ml carbohydrate drink (caffeine free!) e.g. Fanta/Refuel or 750 ml Energade or 600 ml Powerade

* Items like marmite/bovril/fish paste, salad dressing, condiments, sauces & gravy can be included as you like.
* Diet drinks (sugar and caffeine free) e.g. Tab etc. can be included as you like. NO alcohol.

Breakfast	4 slices of bread **OR** 3 slices of bread + 1 med. banana **OR** 1½ cups cooked porridge + 1 slice bread **OR** 2 cups cereal + 1 slice bread (sugar/jam/milk from allowance) **OR** 3 slices bread and another 1 slice as a mid-morning snack
Snack (mid-morning or -afternoon)	1 fruit (size of tennis ball or 2 golf balls) Milk/yoghurt/drink from allowance
Lunch	4 slices of bread **OR** 2 rolls **OR** 2 slices bread + 1 big muffin (margarine/butter/mayonnaise/avocadopear can be used as a spread) + 60 g cheese (2 x thin slices, size of a slice of bread each) OR 60 g lean ham/chicken/beef/turkey roll (± 3-4 thin slices) OR 2 chicken drumsticks or 1 thigh (60 g, boneless) + salad veggies (e.g. lettuce/tomato/cucumber/mushroom)
Snack (mid-afternoon)	1 big banana OR 2 Slices of bread (+jam/honey/syrup)
Supper	2½ cups pasta + ½ c veggies **OR** 2 c rice/pasta + 1 c veggies **OR** 2 c mashed potato + 1 c veggies **OR** 2 med. Potatoes + 1 cup sweetcorn + 150 g meat (size of 1½ x packs of cards) **OR** 150 g chicken (1 breast + 1 drumstick) **OR** 200 g fish + salad veggies (e.g. lettuce/tomato/cucumber/mushroom) (optional)

** All weights are *cooked* weights

MRC/UCT BIOENERGETICS OF EXERCISE RESEARCH UNIT

Training History

Name: _____ **Subject Code:** _____

Date: _____ **Study Code:** _____

Instructions: *We are interested in finding out more about your history of sports participation and physical activity. Please complete the following questionnaire as accurately as possible. List any activities in which you have participated regularly in the past, and estimate for how long and how often you participated.*

LEISURE ACTIVITY HISTORY

	Time/Age Period											
	15-20yrs			*20-25 yrs*			*25-30 yrs*			*35-40 yrs*		
Activity/ Sport	yrs	mos/ yr	hrs/ wk	yrs	mos/ yr	hrs/ wk	yrs	mos/yr	hrs/ wk	yrs	mos/ yr	hrs/ wk
For office use												

	Time/Age Period											
	40-45yrs			*45-50 yrs*			*50-55 yrs*			*>55 yrs*		
Activity/ Sport	yrs	mos/ yr	hrs/ wk	yrs	mos/ yr	hrs/ wk	yrs	mos/yr	hrs/ wk	yrs	mos/ yr	hrs/ wk
For office use												

Examples of sporting activities include:

1. jogging
2. swimming
3. cycling
4. walking
5. squash
6. badminton
7. netball
8. football/soccer
9. rugby
10. aerobic dance/step
11. martial arts
12. volleyball
13. strength& resistance training
14. hiking
15. rock climbing
16. tennis
17. golf
18. canoeing
19. dancing
20. skating

RETROSPECTIVE TRAINING HISTORY

Sporting Activity: _____ Age sporting activity began: _____

*Complete the following table for each sporting activity that you have participated in regularly over the past 10 years. Begin with the most **recent** year.*

	Months/Year	Hours/ wk	Distance/ wk*	Races/ yr**	Level ***	Interval#	Injuries > 3 mos##	Comment
Year			(km)	(no)	Comp/ social	Yes or no	Yes or no	

where:

* Distance where applicable;
**Races or matches;
***Competitive or social;
Interval or high-intensity training included, yes or no;
Injuries lasting more than 3 months

Sporting Activity: _____ Age sporting activity began: _____

*Complete the following table for each sporting activity that you have participated in regularly over the past 10 years. Begin with the most **recent** year.*

	Months/Year	Hours/ wk	Distance/ wk*	Races/ yr**	Level ***	Interval#	Injuries > 3 mos##	Comment
Year			(km)	(no)	Comp/ social	Yes or no	Yes or no	

where:

* Distance where applicable;
**Races or matches;
***Competitive or social;
Interval or high-intensity training included, yes or no;
Injuries lasting more than 3 months

TRAINING DIARY

Name:

Subject code:

Use one row of boxes for each type of workout (you can have a number of workouts on one day). Put any comments in the box at the bottom of the page. Link the comments to the sessions.

Show the date and time of the session.

A = AM
P = PM

1 = cycling 2 = running 3 = swimming 4 = weight training 5 = other (specify in Comments)

1 = legs 2 = arms 3 = abdominals
4 = back 5 = most muscles

I = interval training
C = continuous training

V = Very hard
H = Hard
M = Moderate
E = Easy

If possible, show heart rate range during the training session. State whether measured with a heart-rate monitor or manually.

day month year | time A or P | type of session | muscles | duration of workout (min) | Approx. distance covered (km) | I or C | effort VHME | heart-rate range

1 2 3 4 5 6 | 7 | 8 | 9 | 10 11 12 | | | 13 | 14 15 16 - 17 18 19

1 2 3 4 5 6 | 7 | 8 | 9 | 10 11 12 | | | 13 | 14 15 16 - 17 18 19

day month year | time A or P | type of session | muscles | duration of workout (min) | | | effort VHME | heart-rate range

1 2 3 4 5 6 | 7 | 8 | 9 | 10 11 12 | | | 13 | 14 15 16 - 17 18 19

1 2 3 4 5 6 | 7 | 8 | 9 | 10 11 12 | | | 13 | 14 15 16 - 17 18 19

day month year | time A or P | type of session | muscles | duration of workout (min) | | | effort VHME | heart-rate range

1 2 3 4 5 6 | 7 | 8 | 9 | 10 11 12 | | | 13 | 14 15 16 - 17 18 19

1 2 3 4 5 6 | 7 | 8 | 9 | 10 11 12 | | | 13 | 14 15 16 - 17 18 19

day month year | time A or P | type of session | muscles | duration of workout (min) | | | effort VHME | heart-rate range

1 2 3 4 5 6 | 7 | 8 | 9 | 10 11 12 | | | 13 | 14 15 16 - 17 18 19

1 2 3 4 5 6 | 7 | 8 | 9 | 10 11 12 | | | 13 | 14 15 16 - 17 18 19

Comments

100 km TIME-TRIALS

TIME OF DAY: .....

LABORATORY: .....

WEIGHT: Pre - TT1.....kg TT2.....TT3.....TT4.....

Post –TT1.....kg TT2.....TT3.....TT4.....

	10-11	20 24	32-33	40 - 44	52-53	60 - 64	72-73	80 - 84	final km	overall time	average watts
TT1 time											
TT2											
TT3											
TT4											

Comments:.....

.....

TIME	DISTANCE	HEART RATE	POWER OUTPUT
	5		
	10	10.5	10.5
	11		
	15		
	20	20.5	20.5
	21	21.5	21.5
	22	22.5	22.5
	23	23.5	23.5
	24		24
	25		
	30		
	32	32.5	32.5
	33		
	35		
	40	40.5	40.5
	41	41.5	41.5
	42	42.5	42.5
	43	43.5	43.5
	44		44
	45		
	50		
	52	52.5	52.5
	53		
	55		
	60	60.5	60.5
	61	61.5	61.5
	62	62.5	62.5
	63	63.5	63.5
	64		64
	65		
	70		
	72	72.5	72.5
	73		
	75		
	80	80.5	80.5
	81	81.5	81.5
	82	82.5	82.5
	83	83.5	83.5
	84		84
	85		
	90		
	95		
	99	99.5	99 99.5
	100		100

Appendix 14 – Caffeine study EMG data

Date_____ Code_____

EMG Data Capture

Time	TT1	TT2	TT3
File			
5			
10.5			
22.5			
32.5			
42			
52.5			
55			
62			
72.5			
82			
95			
99.5			

Appendix 15 – Wingate study subject information and informed consent

SUBJECT INFORMATION

This study, conducted at the Sport Science Institute of South Africa, is investigating neuromuscular activity during high intensity exercise. The following measurements and tests will be performed:

Anthropometry: weight, height and body fat %.

Isometric max: Maximum contraction of the quadriceps muscles on the Kin Com dynamometer.

Wingate test: 30 seconds of high intensity cycling.

Measurements to be taken whilst on the Kin Com and cycle ergometer: electromyography of the Rectus Femoris and power output.

INFORMED CONSENT

I, the undersigned, voluntarily agree to participate in this study. I will be free to withdraw from the study at any moment, without having to justify my decision. I will be free to ask any questions about the procedures and results of the study. I understand that I will receive R50 on completion of the trial as remuneration.

The Sport Science Institute of South Africa or any of the investigators is in no way liable for any harm or damage suffered by me as a result of the trial.

I, the undersigned, have read and understood the purposes and procedures involved in the scientific study.

Subject: _____
Signature:

_____

Date: _____

Investigator: _____
Signature:

_____

Date: _____

Appendix 16 – Wingate study data

Date_____ Code_____

Wingate

Peak power W	
Average power W	
Total work J	
Fatigue index %	

Appendix 17 – Filtering of electromyographical data

```
function [yout]=zegait(dummy)

This file reads in thedata and gets the linear envelope
contact mark.omalley@ucd.ie
This is for a gait cycle

load b:\s5t3n12.txt;
dat=s5t3n12;


starttime=0;
endtime=5.00;
endpoint=1984*(endtime-starttime);

offset=1;

Low passfilter characteristics

nfiltl=2;
wcl=5/(1984/2);

High pass filter characteristics

nfilth=2;
wch=15/(1984/2);

Beginning the algorithm

x=dat(offset:endpoint);

y=x-mean(x); % removing the mean

Here it can be high pass filtered

[b,a]=butter(nfilth,wch,'high');

y = filtfilt(b,a,y);

y=abs(y); % rectifying the signal


Low pass filter

[b,a]=butter(nfiltl,wcl);



be sure to filter in both directions to make sure the filtered data has zero phase
make a data vector properly pre- and ap- pended to filter forwards and back
so end effects can be obliterated.
```

```
for i=1:(size(y,1)/10);

yout(i)=y(1+(i-1)*10);

end


t=1:(size(y,1)/10);
t=t/(size(y,1)/10);
t=t*(10)*(1);

yout=yout';
yout=yout/max(yout);
t=t';
```

Appendix 18 – Electromyographic fast fourier transformation algorithim

```
samp=1984; %sampling rate in Hz
durn=1.0; %duration of sample in secs
subj=1; %number of subjects

The following are the files which contain the
EMG data to be analysed. The first file in the
list is the initial section of data, the second
is the latter portion which is to be compared with it

fid =fopen('f:\angus2\hun51b.txt');
fid =fopen('f:\angus2\hun51d7.txt');

k=zeros(subj,500*durn);
EMG=zeros(2*subj,samp*durn);
q=zeros(2*subj,500*durn);
ratio=[];

for i=1:2*subj;
  z=fscanf(i+2,'%f',inf);
  EMG(i,:)=(z(1:samp*durn))';
end;

fouriera=(fft(EMG'))';
fourier=abs(fouriera(:,1:500*durn));

for count=1:2*subj;
 for i=1:500*durn;
   q(count,i)=sum(fourier(count,1:i))/sum(fourier(count,:));
 end;
end;

for count=1:subj
 x=abs(q(count*2-1,:)-.05);
 pta(count)=find(x==min(x));
 x=abs(q(count,:)-.95);
 ptb(count)=find(x==min(x));

 for i=100*durn:350*durn %pta(count):ptb(count);
  temp=abs(q(2*count-1,i)-q(2*count,:));
  k(count,i)=find(temp==min(temp));
 end;
 f=100*durn:350*durn;%pta(count):ptb(count);
 ratio(count)=mean(k(count,100*durn:350*durn)./f);
end;

fclose('all');
```

Appendix 19 – Filtering, calculation of amplitude (RMS) and fast fourier transformation algorithm for electrical data in the caffeine study.

POO Flexcomp .pdf File EMG Processing Tool Ver 2.1 for MATLAB 4.0

The language used by the programmers in writing the program is quite simply and easy to follow. The writing in bold are commands that the program could recognise. There are three parts that have to be followed in order to get the required answers and analyses.

Part 1

function [] = poo(file01, file02, file03, file04, file05, file06, file07, file08, file09, file10, file11, file12, file13, file14, file15, file16, file17, file18, file19, file20, file21)

usage: poo file.pdf <file2.pdf> <file3.pdf> <file4.pdf>...<file21.pdf>

POO is great. It has, however, been written without any definite header information. Therefore it is only recommended to run this utility on data that you know to have been sampled at 1984Hz. (The program should nonetheless tell you if the EMG has not been sampled at this rate. First you will be prompted for the length of data in seconds that you wish to process.

POO then plots the EMG data contained in file.pdf. Next, you will be asked to select an area of interest on the graph for processing. The left hand side of the graph will be the start of the time period that you specified for processing. Keep an eye on the graph's title - it will give timing information. The button on the bottom left can be used to see the data not on screen that will be selected when 'return' is pressed. Alternatively one can just enter the start time in seconds at the prompt.
POO will then plot the selected raw EMG, the filtered EMG and the EMG spectrum. If a second filename is specified, the process will repeat for that file, and the frequency shift of the second EMG selection will be expressed as a percentage of the first. Note that the same sample length of data will be taken from the second file as from the first. If you want to compare data from different parts of the same file, enter the filename twice on the command line argument.

If more files are specified, the spectral shift of these files will be expressed in terms of the frequency spectra of the first file.
In other words,
THE FIRST EMG FILE SHOULD CONTAIN THE NORMALISATION EMG DATA

A table of ratios of pertinent data is then outputed to the screen and to an ascii file. The default file name is results.txt and is in the root directory. i.e. c:\results.txt

Filtering Information (as by zegait.m):

The signal's mean is removed.

A 2nd 3dB @ 15Hz Butterworth high pass filter is used to remove the low frequency motion artifact. Note that this filter was used for gait cycle EMG. For isometric work a lower cutoff frequency is advisable but not necessary.

POO Flexcomp .pdf File EMG Processing Tool Ver 2.1 for MATLAB 4.0

The language used by the programmers in writing the program is quite simply and easy to follow. The writing in bold are commands that the program could recognise. There are three parts that have to be followed in order to get the required answers and analyses.

Part 1

function [] = poo(file01, file02, file03, file04, file05, file06, file07, file08, file09, file10, file11, file12, file13, file14, file15, file16, file17, file18, file19, file20, file21)

usage: poo file.pdf <file2.pdf> <file3.pdf> <file4.pdf>...<file21.pdf>

POO is great. It has, however, been written without any definite header information. Therefore it is only recommended to run this utility on data that you know to have been sampled at 1984Hz. (The program should nonetheless tell you if the EMG has not been sampled at this rate. First you will be prompted for the length of data in seconds that you wish to process.

POO then plots the EMG data contained in file.pdf. Next, you will be asked to select an area of interest on the graph for processing. The left hand side of the graph will be the start of the time period that you specified for processing. Keep an eye on the graph's title - it will give timing information. The button on the bottom left can be used to see the data not on screen that will be selected when 'return' is pressed. Alternatively one can just enter the start time in seconds at the prompt.
POO will then plot the selected raw EMG, the filtered EMG and the EMG spectrum. If a second filename is specified, the process will repeat for that file, and the frequency shift of the second EMG selection will be expressed as a percentage of the first. Note that the same sample length of data will be taken from the second file as from the first. If you want to compare data from different parts of the same file, enter the filename twice on the command line argument.

If more files are specified, the spectral shift of these files will be expressed in terms of the frequency spectra of the first file.
In other words,
THE FIRST EMG FILE SHOULD CONTAIN THE NORMALISATION EMG DATA

A table of ratios of pertinent data is then outputed to the screen and to an ascii file. The default file name is results.txt and is in the root directory. i.e. c:\results.txt

Filtering Information (as by zegait.m):

The signal's mean is removed.

A 2nd 3dB @ 15Hz Butterworth high pass filter is used to remove the low frequency motion artifact. Note that this filter was used for gait cycle EMG. For isometric work a lower cutoff frequency is advisable but not necessary.

The signal is then rectified.

The signal's amplitude envelope is calculated using a 2nd 3dB @ 5Hz Butterworth low pass filter.

Finally the data is subsampled
names(1,:) = 'file01';
names(2,:) = 'file02';
names(3,:) = 'file03';
names(4,:) = 'file04';
names(5,:) = 'file05';
names(6,:) = 'file06';
names(7,:) = 'file07';
and so on...

SUBTLETY: The signals are filtered both ways through the digital filters. This means that all the signal's frequencies will travel through the filter at the same speed, leading to no distortion. This also doubles the order of the filters, which means that the 3dB cut-off points of the filtering have been moved to about 18 and 4 Hz for the high and low pass filters respectively.

Open the files - check that they are all real - and don't reopen any files
for i = 1:nargin
filename = deblank(filez(i,:));
if(i==1)
fid(i) = fopen(filename,'r');

elseif(strcmp(filez(i,:),filez(i-1,:)));
fid(i) = fid(i-1);
else
fid(i) = fopen(filename,'r');
end
end

There are a few commands which will prompt the user when errors or incorrect commands are entered. The program then points out the error by displaying messages.

disp(' ') means display to the user;
[ ] is where the user will type in their commands

disp('Remember: Flexcomp pdf files are stored in directories named the same as their reference number.')
disp('The first number is the recording session, the second number is the channel number.');
disp('Example: c:\flexpat\fred01\0003_02.pdf -> Patient fred01, session 3, channel 2 data.');

Jump in 29 bits into the file and read what I believe to be the sampling rate marker

```
fseek(fid(i),28,'bof');
temp = fread(fid(i),6,'int8') - [0 8 33 4 58 0]';
if(sum(abs(temp)))
disp error('This file does not appear to have been sampled at 1984Hz.  Exiting...')
end
```

Jump in 150 bits into the file from its start

```
fseek(fid(i),150,'bof');
```

Read in the data. That big long number converts from ADC values to volts. It was also determined empirically by comparing max/min values with that provided by the flexcomp program.
e.g. EMGdata = 0.20757511668611e-6 * fread(fid(i),'short');

Find out the total number of samples, along with other info and output to screen.

```
datalen(i) = length(EMGdata);
datamax(i) = max(EMGdata);
datamin(i) = min(EMGdata);

disp(['File no. ', num2str(i),' (', deblank(filez(i,:)),'), samples:
',num2str(datalen(i)),', EMG Max: ',num2str(datamax(i)),'V, EMG Min:
',num2str(datamin(i)),'V'])
```

Create a time vector for plotting

```
time = (0:1/1984:(datalen(i)-1)/1984)';

timemax(i) = max(time);
```

Plot out the data, each one on a separate figure. The figures are plotted through the MATLAB 4 program.

```
figure
plot(time,EMGdata)
xlabel('time (secs)')
ylabel('amplitude (volts)')
title(['File no. ',num2str(i),': ',filez(i,:)])
set(gca,'XLim',[0 timemax(i)])
end
```

Here we clear all variables except the things we need. We do this out of memory
Important : clear EMGdata time temp ans global tselection i time EMGdata timemax

Loop through all the figures

```
for i = 1:nargin
```

```
Load back in the EMG from the current file
fseek(fid(i),150,'bof');
EMGdata = 0.20757511668611e-6 * fread(fid(i),'short');
time = (0:1/1984:(datalen(i)-1)/1984)';

The setting-up run, where the amount of data (in seconds) to be processed is decided.


Prompt for the user and all comments marking statements executed when i==1 only
are marked N.
if(i==1)

N  Blurb for user

disp('The file(s) have been displayed on separate figures.  Specify the amount of data
in seconds from the left hand side of the screen that you wish to process.  Maximum
time allowed is the shortest EMG data set.')

disp('Hitting 'return' will select just the displayed EMG for processing.')

tselection = input(['ENTER TIME IN SECONDS (max ',num2str(min(timemax)),']:


N  Check the selection time is not too large here
while (tselection > min(timemax))
disp(' ')
tselection = input(['Time specified too large.  Enter number (max
',num2str(min(timemax)),')  (ctrl-c to quit): ']);
end

N  Decide which mode we are going into...fixed time or screen selection
if(isempty(tselection))
mode = 0;

disp('Only plotted data will be processed')
else
mode = 1;
disp(['Now processing file number ',num2str(i),' called ',filez(i,:)])
disp([num2str(tselection), ' second(s) of data will be processed starting from left hand
side of plot.'])
end

N Close down all the figures and start selecting the start (and if appropriate) the
data end-points
close all

N Plot the EMG channel
figure;
plot(time,EMGdata)
```

```
ylabel('amplitude (V)');
xlabel('time (s)');
set(gca, 'XLim', [0, timemax(i)]);


N  Now a routine for data selection - the tricky stuff is just to give data on cursor
position in the title of plot and the small buttons on the bottom
we_are_not_happy = 1;
while(we_are_not_happy)
zoom xon
zoom(1)
set(gcf,'units','pixels');
grid

disp('Click and drag so that the left hand side of the displayed data corresponds to the
desired start of the EMG selection.  Right mouse button zooms out.')

set(gcf,'WindowButtonMotionFcn', 'axislimits=get(gca,''XLim'');mainscreen =
get(0,''PointerLocation'');thefigure = get(gcf,''Position'');jiggerypokery1 =
[mainscreen(1) - thefigure(1),mainscreen(2) -
thefigure(2)];jiggerypokery2=get(gca,''CurrentPoint'');title([num2str(axislimits(
1)),'' to '',num2str(axislimits(2)),'' secs displayed, now at
'',num2str(jiggerypokery2(1)),'' secs.'']);');
if(mode)
uicontrol('String','View Proposed Data','Position',[20, 5, 140,
20],'Callback','v=get(gca,''XLim'');global tselection;set(gca,''XLim'',[v(1) (v(1) +
tselection)]);');
uicontrol('String','Reset Plot','Position',[160, 5, 100, 20],'Callback','global time
EMGdata timemax i;plot(time,EMGdata);set(gca,''XLim'',[0
timemax(abs(i))]);zoom xon;grid on;ylabel(''amplitude (V)'');xlabel(''time
(s)'');set(gcf,''WindowButtonMotionFcn'',
''axislimits=get(gca,''''XLim'''');mainscreen =
get(0,''''PointerLocation'''');thefigure = get(gcf,''''Position'''');jiggerypokery1 =
[mainscreen(1) - thefigure(1),mainscreen(2) -
thefigure(2)];jiggerypokery2=get(gca,''''CurrentPoint'''');title([num2str(axislimi
ts(1)),'''' to '''',num2str(axislimits(2)),'''' secs displayed, now at
'''',num2str(jiggerypokery2(1)),'''' secs.'''']);');
end


if(mode)
choosetime = input('Press return when satisfied, or enter a time:  ');
else
disp(['Press 'return' when satisfied'])
pause
end
```

```
N  Now find out what data was selected
N  This just gets the start and stop of the x-axis on the plot
vv(:,i) = get(gca, 'XLim')';
if(~isempty(choosetime))
vv(1,i) = choosetime;
end

N  Change the limits if a specific amount of time was selected
if(mode)
vv(2,i) = vv(1,i) + tselection;
end

if (vv(1,i)>timemax(i))

disp('The specified start time is after the end of the data sequence')

N  Check that in the case of screen selection mode that the time selected is less
than the smallest data sequence
elseif(vv(2,i)-vv(1,i)>min(timemax))

disp(['The screen selected data is longer than an entire other EMG file of
',num2str(min(timemax)),' secs.'])
disp('Please select a shorter data sequence.')

N  Check that the calculated end point is within the data sequence
elseif (vv(2,i) > max(timemax(i)))

disp('The ending time of the selected data sequence is outside the range of the data.')
if(mode)
choosetime = [];
disp('Please select an earlier start to the data sequence.');
else
disp('Please select an earlier start to the data sequence.')
end
else
we_are_not_happy = 0;
end

end  end of while loop

If we are in plotted data only mode, determine the tselection time for further files
if((~mode)&(i==1))
tselection = vv(2,i)-vv(1,i);
end

Close the figure
close
```

That's the end of the set-up where the time of processed data is decided and the normalising data is obtained.

Part 2

We have a general setup now to process the files that contain the data to be normalised.

All comments in this section are marked by D. It is essentially the same process with different error checking and no time selection.

else
D Blurb for user

disp(['Now processing file number ',num2str(i),' called ',filez(i,:)])
disp([num2str(vv(2,1)-vv(1,1)), ' second(s) of data will be processed.'])

D Plot the EMG channel
figure;
plot(time,EMGdata)
title(['Recorded EMG data in file ', num2str(i),': ',filez(i,:),'.'])
ylabel('amplitude (V)');
xlabel('time (s)');
set(gca, 'XLim', [0, timemax(i)]);

D Now a routine for data selection - the tricky stuff is just to give data on cursor position in the title of plot and again, that small button
we_are_not_happy = 1;
while(we_are_not_happy)
zoom xon
zoom(1)
set(gcf,'units','pixels');
grid
disp(' ')
disp('Click and drag so that the left hand side of the displayed data corresponds to the desired start of the EMG selection. Right mouse button zooms out.')
disp('Press return when satisfied.')
set(gcf,'WindowButtonMotionFcn', 'axislimits=get(gca,''XLim'');mainscreen = get(0,''PointerLocation'');thefigure = get(gcf,''Position'');jiggerypokery1 = [mainscreen(1) - thefigure(1),mainscreen(2) - thefigure(2)];jiggerypokery2=get(gca,''CurrentPoint'');title([num2str(axislimits(1)),'' to '',num2str(axislimits(2)),'' secs displayed, now at '',num2str(jiggerypokery2(1)),'' secs.'']);');
uicontrol('String','View Proposed Data','Position',[20, 5, 140, 20],'Callback','v=get(gca,''XLim'');global tselection;set(gca,''XLim'',[v(1) (v(1) + tselection)]);');
uicontrol('String','Reset Plot','Position',[160, 5, 100, 20],'Callback','global time EMGdata timemax i;plot(time,EMGdata);set(gca,''XLim'',[0 timemax(abs(i))]);zoom xon;grid on;ylabel(''amplitude (V)'');xlabel(''time (s)'');set(gcf,''WindowButtonMotionFcn'',

```
''axislimits=get(gca,''''XLim'''');mainscreen =
get(0,''''PointerLocation'''');thefigure = get(gcf,''''Position'''');jiggerypokery1 =
[mainscreen(1) - thefigure(1),mainscreen(2) -
thefigure(2)];jiggerypokery2=get(gca,''''CurrentPoint'''');title([num2str(axislimi
ts(1)),'''' to '''',num2str(axislimits(2)),'''' secs displayed, now at
'''',num2str(jiggerypokery2(1)),'''' secs.''''])'');');
                pause
```

Now find out what data was selected

This just gets the start and stop of the x-axis on the plot
vv(:,i) = get(gca, 'XLim')';

A time selection is at this stage always specified
vv(2,i) = vv(1,i) + tselection;

Check that the calculated end point is within the data sequence
if (vv(2,i) > max(timemax(i)))
disp(' ')
disp('The ending time of the selected data sequence is outside the range of the data. Please select an earlier start to the data sequence.')
else
we_are_not_happy = 0;
end

end of while loop

Close the figure
close

Part 3

END OF FIRST FILE/OTHER FILE DIVISIONS

We then take the raw data - this business of adding two very small irrational numbers to both limits is to make the likelihood of selecting exactly a sampling time as the start time of the data selection, which will result in that time having one more sample and messing up the subsequent analysis. This can happen if one chooses zero time as the start of the EMG data selection.

index = find((time<=vv(2,i)+pi/3000000)&(time>=vv(1,i)+pi/3000000));

EMG = EMGdata(min(index):max(index));

So far so good, we have selected our data - let's process it, one at a time (we are still in the original filename loop at the very top.

Plot the raw EMG in the top of the figure
figure
subplot(3,1,1); plot(time(min(index):max(index)),EMG)
title(['Raw Selected EMG']);
ylabel('amplitude (V)');
set(gca,'XLim',vv(:,i));

Then get the linear envelope and find the area under the curve and RMS
We process using the zegait.m file algorithms. The following is a slight modification of that file

Start of zegait.m

Low pass filter characteristics
nfiltl=2;
wcl=5/(1984/2);

High pass filter characteristics
nfilth=2;
wch=15/(1984/2);

Beginning the algorithm
y=EMG-mean(EMG); removing the mean

Here it can be high pass filtered
[b,a]=butter(nfilth,wch,'high');
y = filtfilt(b,a,y);

RMS(i) = sqrt(mean(y.^2));

y=abs(y); rectifying the signal

Low pass filter
[b,a]=butter(nfiltl,wcl);

Be sure to filter in both directions to make sure the filtered data has zero phase.
Make a data vector properly pre- and ap- pended to filter forwards and back
so end effects can be obliterated.
y = filtfilt(b,a,y);

yout = y(1:10:length(y));
tout = time(min(index):10:max(index));

End of zegait.m

Plot the envelope
subplot(3,1,2); plot(tout,yout);

```
title('Filtered selected EMG');
ylabel('amplitude');
set(gca,'XLim', vv(:,i));

Calculate the area under the sampled curve
area = 0;
for nn = 1:size(tout)-1;
arrea = arrea + (tout(nn+1)-tout(nn)) * ( yout(nn) + (yout(nn+1)-yout(nn))/2);
end
area(i) = arrea;

Output this info to the screen
disp(['RMS is ', num2str(RMS(i)), '.'])

Finally plot the frequency spectrum and calculate the cumulative power spectrum

Now we calculate the frequency spectrum of our selected data
fEMG=fft(EMG);


We plot the fft data.  All this code just makes sure that the correct frequency axis
is displayed.  See the Matlab demo to find out more.
fEMG(1) = 0;
n = length(fEMG);
if(rem(n,2)~=0)
n = n - 1;
end

Note that the amplitude variable stores the fft amplitude information for each file
in the command line argument.
amplitude(:,i) = abs(fEMG(1:n/2));
nyquist  = 1984/2;
freq = (1:n/2)'/(n/2)*nyquist;
subplot(3,1,3); plot(freq, amplitude(:,i))
title('Selected EMG frequencies');
ylabel('amplitude');
set(gca,'XLim', [freq(1) freq(length(freq))]);
xlabel('frequency (Hz)');


Here we reclear all the data which may affect the next run through.  We hold on to the
frequency data if there are files to be compared later
clear global EMGdata
clear global time
global time EMGdata
end
```