Effects of Antecedent Exposures and Physiological Perturbations on Perceived Exertion, Muscle Activation and Performance During Open and Closed Loop Exercise

BY

SACHA JANE WEST

EFFECTS OF ANTECEDENT EXPOSURES AND PHYSIOLOGICAL PERTURBATIONS ON PERCEIVED EXERTION, MUSCLE ACTIVATION AND PERFORMANCE DURING OPEN AND CLOSED LOOP EXERCISE

BY

SACHA JANE WEST

Thesis Presented for the Degree of
DOCTOR OF PHILOSOPHY
In the Department of Human Biology,
Faculty of Health Sciences
UNIVERSITY OF CAPE TOWN
October 2006

MRC/UCT Research Unit for Exercise Science and Sports Medicine
Sports Science Institute of South Africa
Boundary Road, Newlands, 7700
South Africa

DECLARATION

PhD THESIS TITLE

EFFECTS OF ANTECEDENT EXPOSURES AND PHYSIOLOGICAL PERTURBATIONS ON PERCEIVED EXERTION, MUSCLE ACTIVATION AND PERFORMANCE DURING OPEN AND CLOSED LOOP EXERCISE

I, **Sacha West**, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgments indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the University of Cape Town to reproduce for the purpose of research either the whole or any portion of the contents in any matter whatsoever.

SIGNATURE: Signed by candidate

DATE: 01/10/06

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I wish to acknowledge and express my sincere thanks to the following people:

Associate Professor Alan St Clair Gibson, for his mentorship over the past 8 years, and his guidance that has helped shape this thesis. His infectious enthusiasm for research has both motivated and inspired me throughout my PhD.

Professor Vicki Lambert, for her continuous support, motivation and expert advice throughout the last 6 years. She has stimulated my interest in other spheres of exercise science and instilled me with a sense of confidence in my work.

Professor Tim Noakes, for his inspiration, teaching me to challenge set paradigms and ideas, and for creating a unique and great work place.

My parents, for their financial assistance and always encouraging me to follow my heart – their love, support, guidance and understanding is endless, thank you!

My sisters, **Josie-Kate and Gabrielle**, for their love, support and friendship, and for always being there through the difficult times.

My best friend, **Debbie Bryson**, for her invaluable friendship – she has always reminded me not to take life so seriously. Thank you for your help, support and encouragement throughout my thesis.

Jeanine Cameron, for her "extreme" patience and understanding, support and love.

Candy Bubb, for her support, encouragement and loyal friendship.

The **Broads** and all **my friends**, for their support, understanding and ongoing patience.

The **research subjects** who participated in these trials for their willingness to give of their time and bodies. It was a pleasure working with you all, making my research that much more fun, rewarding and interesting.

Julia Goedecke, for her invaluable contribution to Chapters 3 and 4, and for her assistance and motivation throughout my thesis. Her friendship, advice and willingness to always listen have helped me through some difficult times.

Ross Tucker, for his friendship, challenging discussions, editing my work and for always being available to answer any questions.

Amanda Claassen, for being such a helpful and efficient 'testing partner', but more importantly for her support and friendship over the past three years.

Dr Hugh Mullany, formerly of University of Dublin, for his assistance and expertise with the electromyography, and his generous provision of EMG analysis software.

Judy Belonje and Hendriena Victor, for assisting me in the laboratory and for performing the analysis of blood samples from the studies included in this thesis.

Paul Rossouw and the **administration staff**, for their assistance with printing and the research administration.

Lynne Smith, Liesl van Niekerk and Lize Havemann, for their subject recruitment, assistance during testing, endless hours in the laboratory, and data collection, as part of their Honours degree.

Yolande Harley, Karen Heath, Angus Hunter, Liesl Grobler, Mark Kirkman, Sharhied Taliep, for their friendship and helpful discussions.

My **fellow students** at the UCT/MRC Research Unit for Exercise Science and Sports Medicine, for their friendship and assistance.

The **UCT/MRC Research Unit for Exercise Science and Sports Medicine** and the **University of Cape Town**, for their very generous financial assistance for the duration of my thesis.

Finally, the **examiners**, for their expertise and time spent reviewing this thesis.

PUBLICATIONS ASSOCIATED WITH THE THESIS

Full Papers

West SJ, Goedecke JH, van Niekerk L, Collins M, St Clair Gibson A, MacDonald IA, Noakes TD, Lambert EV (2006). Effects of elevated plasma adrenaline levels on substrate metabolism, effort perception and muscle activation during low-to-moderate intensity exercise. *Pflugers Arch – Eur J Physiol 451: 727-737.*

Havemann L, **West SJ**, Goedecke JH, MacDonald IA, St Clair Gibson A, Noakes TD, Lambert EV (2006). Fat adaptation followed by carbohydrate loading compromises high-intensity sprint performance. *J Appl Physiol 100(1): 194-202.*

West SJ, Smith L, Lambert EV, Noakes TD, St Clair Gibson (2005). Submaximal force production during perceptually guided isometric exercise. *Eur J Appl Physiol 95(5-6): 537-542.*

Abstracts

Goedecke JH, van Niekerk L, **West S**, Collins M, St Clair Gibson A, Noakes TD, Lambert EV (2003). The effects of epinephrine on substrate metabolism and ratings of perceived exertion during moderate-intensity exercise. *South African Sport Med J 15(1): 31.*

Goedecke JH, van Niekerk L, **West S**, Collins M, St Clair Gibson A, Rauch L, Noakes TD, Lambert EV (2002). Plasma epinephrine – Effects on substrate metabolism and effort perception during exercise. *J Endocrinol Metab Diab South Africa 7(1): 20.*

Professional Presentations

8th Annual Congress of the European College of Sport Science, Salzburg, Austria, July 2003 – Poster presentation: "Muscle recruitment patterns during exercise:

Response to epinephrine infusion, short term high-fat feeding, and exhaustive single-limb exercise".

THESIS ABSTRACT

The concept of fatigue has been widely studied. However, the models of exercise fatigue that have emerged are often limited and inconsistent, which emphasises the difficulty involved in establishing a unified theory of fatigue. Furthermore, much of the research has attempted to identify a single physiological factor or system as the cause of fatigue development, but this may be ambiguous when observing the exercising body as a whole. As a result of this, various definitions have been used to describe fatigue, namely: *"any exercise-induced reduction in the ability of a muscle to generate force"*, to being classified as *"central or peripheral in origin"*, as well as being viewed as *"a conscious sensation rather than the consequence of physiological responses"*, and more recently it has been defined as *"the result of complex interactions of multiple physiological systems and the brain"*. This highlights that fatigue may involve a number of different physiological factors and processes that are integrative as part of a complex dynamic system, and therefore, it may seem more plausible to examine the development of fatigue using an integrative approach.

An integrative model, such as Borg's effort continua, includes the perceptual, the performance, and the physiological continuum, respectively, and centres on the relative importance of each continuum's contribution to fatigue associated with a specific task or event. The model suggests that the variables in all three continua are not linearly related, and the contribution of each continuum to fatigue may be 'weighted' differently in each individual for a given exercise task. Another integrative model, proposed by Ulmer, focuses on a feedforward/feedback system within a central regulatory system that integrates both afferent and efferent information to perform an exercise task, as well as antecedent experiences, training, metabolic reserves and actual metabolic rates, and the time necessary to complete the exercise task (teleoanticipation). Integrative models such as these, suggest that numerous factors from multiple physiological systems in the body should be examined, which provides an ideal means/platform in which to explore the exercising body as a whole.

Therefore, this thesis used this integrative approach to address the complex and multifaceted nature of the exercise regulatory system, which creates the experience of fatigue during exercise, by investigating the perceptual, the physiological, and the performance continua, and their response to various antecedent exposures and physiological perturbations. Accordingly, the aim of this thesis was to examine the effect of various antecedent exposures and physiological perturbations on the relationship between the perceptual (perceived exertion), the physiological (metabolic milieu), and the performance (overall exercise performance) during both open and closed loop exercise at either a self-paced or constant workload. We hypothesized that the antecedent exposures and physiological perturbations would alter the perceptual and physiological milieu and, as a result, impact on perceived exertion, muscle activation levels and overall exercise performance. A further hypothesis tested was that the variables in the perceptual, the physiological and the performance continuum of Borg's effort continua would not be linearly related but rather "weighted" differently during each specific exercise protocol.

In an attempt to examine the effect of antecedent fatiguing exercise on the integrative response to subsequent exercise and the experience of fatigue, a sequential single-limb exercise model was used. In the first study, we aimed to determine whether the humoral effects and physiological responses elicited during exercise to exhaustion of the first limb (Leg 1) influenced performance, perceived exertion and muscle activation levels in the previously rested limb (Leg 2). We hypothesized that the prior exercise of Leg 1 would alter the metabolic milieu, and therefore, increase effort perception and muscle activation levels resulting in reduced exercise capacity of the previously rested limb. Seven healthy males performed single-leg cycling to exhaustion on a recumbent bicycle at 30% of the two-legged peak power output (PPO). The rested leg remained completely inactive during the cycling bout of the exercised leg. Subjects ingested a 10% glucose polymer solution (500ml) at 30 min of exercise to maintain euglycaemia. The time to fatigue was not significantly different between Leg 1 and 2 (60 ± 6 vs 56 ± 8 min, Leg 1 vs Leg 2 respectively) (p=0.07). Epinephrine and norepinephrine concentrations were significantly higher at the start of exercise in

Leg 2. Epinephrine concentrations remained significantly higher throughout exercise ($p<0.01$) in Leg 2 compared to Leg 1. Submaximal VO_2 was comparable throughout both trials. Heart rate and general RPE were significantly lower at the start of exercise in Leg 2 ($p<0.05$), but increased similarly after 10% of the elapsed exercise time. Both general and leg RPE increased at the same linear rate in both legs when expressed relative to elapsed time. The EMG amplitude was significantly higher in Leg 2 than Leg 1 throughout the trial ($p<0.05$). In summary, antecedent exhaustive exercise did not influence performance during a subsequent bout of single-limb exercise with the previously rested leg, despite differences in catecholamine concentrations, heart rate, RPE and EMG activity at the onset of exercise in Leg 2. During exercise in Leg 2, these variables become similar to values measured in Leg 1, with the exception of epinephrine concentrations and EMG activity, which remained elevated until termination of exercise. The mechanism underlying this altered neuromuscular strategy remains unclear, but these data suggest that the increased muscle activation levels may be associated with increased circulating epinephrine concentrations. Perceived exertion was, however, unrelated to muscle activation and epinephrine concentrations but was related to the exercise duration. Therefore, the rate of change in perceived exertion associated with prior local muscle fatiguing exercise appeared to be dependent rather on the exercise duration, irrespective of either antecedent exercise, an altered physiological milieu or increased muscle activation levels.

In the second study of this thesis, we sought to further examine the interaction between the humoral effects of increased SNS activation (circulating epinephrine concentrations) and exercise intensity on perceived exertion and muscle activation levels during dynamic exercise. Specifically, we wished to differentiate the role of raised plasma epinephrine (Epi) concentrations from direct sympathoadrenal activation associated with moderate-intensity exercise, on muscle activation levels and perceived exertion, as well as cardiopulmonary responses and fuel metabolism during low-intensity exercise. Two groups of subjects (MOD, n=6; LOW, n=7) cycled on two occasions for 90 min. Subjects in the MOD group cycled at 68% VO_{2max} with saline infusion (MOD 68% Sal), and at 34% VO_{2max} with Epi infusion (MOD 34% Epi). Subjects in the LOW group cycled

twice at 34% VO_{2max}, with either Epi (LOW 34% Epi) or saline infusion (LOW 34% Sal). Epinephrine infusions (0.015 Epi μg/kg/min) started at 15 min. During exercise at 34% VO_{2max} with Epi infusion (MOD 34% Epi and LOW 34% Epi), plasma [Epi] increased to levels similar and even slightly higher than those reached during exercise at 68% VO_{2max} with saline infusion (MOD 68% Sal) (~1.9 vs. 1.4 nM, at 75 min). Mean plasma glucose and lactate concentrations were significantly higher during exercise at 34% VO_{2max} with Epi (LOW 34% Epi) than saline infusion (LOW 34% Sal) (5.1±0.6 vs. 4.4±0.3 mmol/l, $p<0.01$ and 2.1±0.8 vs. 1.3±0.5 mmol/l, $p<0.01$, respectively). However, elevated [Epi], without increased exercise intensity, did not alter glycogenolysis. Furthermore, there were no effects of Epi infusion during exercise at 34% VO_{2max} on muscle activation levels, RPE, heart rate, oxygen consumption, [FFA], respiratory exchange ratio and intramuscular triglyceride utilization. Therefore, it appears that elevated circulating levels of epinephrine as a result of Epi infusion, and the metabolic changes associated with it during low-intensity exercise, are not sufficient afferent feedback for cardiopulmonary and peripheral responses or changes in perceived exertion and muscle activation levels which we found at moderate- and higher-intensity exercise. Furthermore, these data suggest that at low exercise intensities, muscle activation levels and perceived exertion are not directly associated with the metabolic milieu.

Further to this study, we examined another potential model of increased sympathetic activation during exercise. Previous studies have shown that ingestion of a high-fat diet (HFD) alters glycogen stores and increases sympathetic activation and perceived exertion during exercise and training. We proposed to further study the relationship between metabolic activity and muscle activation levels and perceived exertion by studying the link between altered fuel and hormonal responses associated with ingestion of an antecedent HFD, and perceived exertion and muscle activation levels. The aim of the third study therefore, was to determine the effect of an antecedent HFD, followed by 1-d of carbohydrate (CHO) loading, on, heart rate variability as a proxy measure of sympathetic activation, perceived exertion, muscle activation levels and performance during steady-state exercise and a 100-km cycling time-trial (TT).

Eight well-trained cyclists completed two trials in a randomised, single-blind crossover design separated by a two-week washout period, ingesting either a high-CHO diet (HCD) (68% CHO energy) or an iso-energetic high-fat diet (HFD) (68% fat energy) for 6 days, followed by 1-d of CHO-loading (8-10g CHO/kg). On day 1, 3 and 5, subjects visited the laboratory for supervised training sessions. On day 7, subjects performed a 1-hr cycle at 70% of VO_{2peak} and on day 8, completed a 100-km performance TT interspersed with high intensity sprint bouts. Although not significant, there was a tendency for an increase in low frequency power spectrum for heart rate variability following the high-fat intake ($p=0.056$) during both training and exercise trials. Heart rate was significantly increased at the start and during the steady-state cycle after the ingestion of a HFD ($p<0.05$). Both perceived exertion and EMG amplitude were comparable during the steady-state trial. Overall 100-km TT performance was not different between diets, however 1-km sprint power output following HFD-CHO was lower ($p<0.05$) compared to HCD-CHO. Despite a reduced power output with HFD-CHO, heart rate, perceived exertion and EMG amplitude were not different between trials. Therefore, ingestion of an antecedent HFD for 6 days, followed by 1-d of CHO-loading, did not effect the overall 100-km TT performance but reduced high-intensity sprint power performance which occurred at similar levels of muscle activation, effort perception and heart rate. The relationship between self-paced exercise performance and these variables was thus altered during the 1-km sprints. The mechanism associated with the decrement in performance during the sprints is not clear, but could possibly be related to increased sympathetic activation, or altered contractile function. Furthermore, the reduced power output during the 1-km sprints did not affect overall exercise performance which suggests a compensatory increase in performance during the non-sprint cycling sections.

It is well known that carbohydrate availability, and therefore, the maintenance of euglycaemia appears essential in preserving exercise capacity during prolonged exercise. This is, in part, dependent on having intact and appropriate glucose counter-regulatory responses in the face of falling blood glucose. This has been shown to function in both a feedback manner, or feedforward, anticipatory manner. Glucose counter-regulation has previously been shown to be attenuated after exposure to hypoglycaemia. It is unclear how antecedent exposure to

hypoglycaemia may affect the subsequent integration of fatigue and associated central and peripheral control mechanisms. Thus, the fourth study investigated the effects of antecedent hypoglycaemic exposure on performance, perceived exertion and muscle activation levels during a subsequent (next day) bout of steady-state exercise, interspersed with high-intensity sprints, and self-paced exercise. Ten healthy, well-trained cyclists performed two, 2-day trials in a randomised, single blind crossover design separated by a one-month washout period. On day 1, subjects were exposed to either 2 consecutive 80-min bouts of hypoglycaemia (2.9±0.1 mmol/L) separated by 40 min of euglycaemia (HYP trial), or 200 min of euglycaemia (5.2±0.1 mmol/L, EUG trial), using the hyperinsulinemic glucose clamp procedure. On day 2, subjects performed 90 min of cycling exercise at 70% of $VO_{2\ max}$, with a 10 kJ (~20 sec) sprint performed every 15 min. At the end of 90 min, a self-paced 200 kJ cycling TT was performed. Water (only) was ingested ad libitum during the exercise trial. During the steady-state exercise, plasma glucose, epinephrine concentrations, heart rate, RPE and EMG amplitude were comparable between HYP and EUG conditions. However, oxygen consumption was significantly increased ($p<0.05$) after HYP compared to EUG exposure with a decrease in RER ($p=0.05$). Furthermore, norepinephrine concentrations were significantly lower after HYP exposure during subsequent steady-state exercise ($p<0.05$). There were no significant differences in plasma glucose, catecholamine concentrations, heart rate and effort perception after the 200 kJ TT between HYP and EUG trials. The EMG amplitude was increased after HYP exposure at the start of the TT, but was comparable at the end (trial x time interaction, $p<0.01$). Individual exercise times showed that 7 (out of 10) subjects average sprint times and 6 (out of 10) subjects TT performance were improved after antecedent HYP vs EUG exposure, however these differences were not statistically different. In conclusion, these results suggest that prior exposure to hypoglycaemia had no consistent effect on performance, perceived exertion, muscle activation levels and the metabolic counter-regulatory responses to subsequent prolonged exercise (70% $VO_{2\ max}$). However, oxygen consumption and RER were altered and the sympathetic neural response (norepinephrine concentrations) was blunted. Prior hypoglycaemia increased muscle activation levels at the onset of the self-paced TT despite no change in

performance, perceived exertion, plasma glucose, catecholamine concentrations and heart rate. The altered muscle activation at the onset of the TT may possibly be related to the blunted sympathetic neural response found during the preceding exercise bout although this cannot be conclusively determined from these data. The changes in norepinephrine concentrations, however, were not directly related to perceived exertion or performance.

The previous four studies employed either constant workload or self-paced dynamic exercise protocols (both open and closed loop) to examine the relationship between force production, muscle activation levels and perceived exertion. The fifth study, however, used an isometric exercise protocol and explored perceived exertion, specifically examining the effect of prior exposure to a perceptual anchor (maximal voluntary contraction) and antecedent fatiguing exercise on the perceptual response during subsequent isometric contractions. By using perceived exertion as a regulator of exercise intensity, we hypothesized that antecedent exposure to a maximum voluntary contraction would establish an improved perceptual response, and that antecedent fatiguing exercise would negatively impact on an individual's ability to judge feelings of exertion during subsequent isometric exercise.

Thirty young adults performed isometric knee extensions on an isokinetic dynamometer, which included five different trials: the first trial was standard for all subjects (standard naïve test) and the remaining four trials were randomly performed. During the standard naïve test, subjects were asked to produce force at perceived contraction intensities (25%, 50% and 75% of their maximum voluntary contraction (MVC)) in a random order. The 100% MVC was performed as the final intensity, and thus, the preceding submaximal intensities were produced in a naïve state, i.e. without antecedent maximal exposure. All intensities, including the 100% MVC, were randomly performed in the other four trials (control tests 1 and 2, post 20% MVC and post 100% MVC tests). Post 20% MVC and post 100% MVC tests included fatiguing isometric exercise at 20% and 100% MVC respectively, which were performed prior to the test protocol. Absolute force increased with increasing intensity ($p<0.001$) during all tests. During the standard naïve test, absolute force at 25% and 50% MVC was significantly lower

(p=0.009) compared to control test 2, post 20% MVC and 100% MVC tests, and relative force was lower in the standard naïve test at all intensities compared to all other tests (p<0.001). Absolute and relative forces were most accurate at 50% MVC during the five experimental trials (-12.06 N and –2.42%, respectively). Prior fatiguing isometric exercise did not affect the subsequent perceptual response range. Therefore, in this model, naïve isometric force was most accurate at 25% MVC but under-produced (perceptually over-estimated) at higher contraction intensities preceding a maximal voluntary contraction (100% MVC). Subsequent exposure to an MVC improved subjects' perceptual responses during the higher contraction intensities and resulted in a better judgement of force guided by perceptual feelings of exertion. Antecedent fatiguing isometric exercise did not alter the subjects' subsequent perceptual response range, irrespective of whether it was submaximal or maximal fatiguing exercise. It appears, however, that antecedent fatiguing exercise may have increased overestimation of force at the low perceptual intensity of 25% MVC. Furthermore, the ability to match absolute force with target contraction intensities was most accurate at 50% MVC during all five experimental conditions and poor at opposite ends of the force continuum. This suggests that during isometric exercise the brain mechanism functions well at medium-range exercise intensities but is inaccurate at high and low intensities. It is likely that this altered relationship between perceived exertion and exercise intensity at high and low intensities may exist during dynamic exercise.

In summary, antecedent exposures and physiological perturbations induced by the interventions did not alter perceived exertion or overall exercise performance during any study. These studies collectively demonstrate that perceived exertion appears to be dissociated from changes in metabolic activity, muscle activation levels and force output during dynamic steady-state or self-paced exercise, and the relationship between them may be regulated by an as yet undescribed brain mechanism. The RPE increased linearly in all exercise trials, irrespective of the mode of exercise, exercise intensity and duration. Therefore, the generation and regulation of RPE may largely be a function of increased exercise duration, with initial values determined, in part, by antecedent exposure or prior experience and knowledge of the end point of exercise. These findings support Borg's theory of an effort continua that the perceptual, physiological and performance variables

are not all linearly related to each other but rather their contribution complements each other during exercise and is "weighted" differently during each specific exercise task.

The interpretation may, however, be different during incremental exercise to volitional fatigue compared to self-paced exercise of a known duration. By using perceived exertion as a regulator of exercise intensity during isometric exercise, antecedent exposure to perceptual anchors may be necessary to establish improved judgement of force production. In addition, the finding of poor force production at opposite ends of the force continuum suggests that while the brain mechanism functions well at medium-range intensities, it is prone to error at high and low isometric exercise intensities It is possible that this altered relationship between perceived exertion and exercise intensity, particularly at high and low intensities, may occur during dynamic exercise.

Antecedent exposures and physiological perturbations used in the present thesis had no impact on overall exercise performance, although force production and muscle activation levels were altered during both open and closed loop exercise at a self-paced and constant workload. Antecedent fatiguing single-limb exercise altered muscle activation levels in the previously rested leg during a subsequent bout of exercise, which was associated with increased catecholamine concentrations. In addition, antecedent high-fat feeding compromised high-intensity sprint power performance during prolonged self-paced exercise, which may be related to increased sympathetic activation or altered contractile function. Antecedent hypoglycaemia altered muscle activation levels at the onset of a self-paced TT which may be associated with blunted sympathetic neural response measured during the preceding exercise bout. These findings suggest a possible association between altered sympathetic activation, force production and muscle activation levels during dynamic exercise, however, only with a concomitant increase in exercise intensity, as was demonstrated by the study involving the perturbation of epinephrine levels during low-intensity exercise.

Overall performance during the different dynamic exercise trials was, however, not affected by these alterations in force production, muscle activation levels and

sympathetic activation. Thus, in the context of an integrative regulatory system, the changes in the physiological systems elicited by the various stimuli (antecedent exposures and physiological perturbations), appear to have caused compensatory responses in the peripheral and central processes to ensure overall exercise performance was maintained.

LIST OF ABBREVIATIONS

ANOVA – analysis of variance
bpm – beats per minute
cal - calories
CHO – carbohydrate
d - day
EMG – electromyography
Epi – epinephrine
EUG – antecedent euglycaemic group
FFA – free fatty acid
g – grams
g/kg – grams per kilogram
HCD – high carbohydrate diet
HCD-CHO – 6 day carbohydrate diet + 1 day carbohydrate loading
HCl – hydrochloric acid
HF – high frequency
HFD – high fat diet
HFD-CHO – 6 day high-fat diet + 1 day carbohydrate loading
HRV – hear rate variability
HYP – antecedent hypoglycaemic group
hr - hour
IMTG – intramuscular triglyceride
kg – kilogram
kJ - kilojoule
km – kilometer
LF – low frequency
LFstand – low frequency standing
LFsup – low frequency supine
LOW – low group
min - minute
MOD – moderate group
MVC – maximum voluntary contraction
Norepi – norepinephrine
PPO – peak power output
PROT - protein
RER – respiratory exchange ratio
RPE – ratings of perceived exertion
rpm – revolutions per minute
Sal - saline
SD – standard deviation
SS – steady-state
TG – triglyceride
TT – time-trial
Ve – ventilation volume
VCO_2 – carbon dioxide production
VO_2 – oxygen consumption
VO_2max – maximum oxygen consumption
vs – versus
W - watts
wk - week

CHAPTER 1

REVIEW OF THE LITERATURE

1.1 INTRODUCTION

Physical activity is a universal phenomenon that is being encouraged worldwide to improve quality of life, as well as in the prevention and treatment of chronic diseases. While exercise is beneficial from a health perspective, athletes continue pushing the boundaries/confines of their bodies to achieve greater performances. Fatigue however, remains a limiting factor to exercise performance, and a controversial area of study that exercise scientists continue to extensively research.

A difficulty with reviewing fatigue in the literature is the variety of definitions, which depend, in part, on the methodological and theoretical approach that has been used to examine and understand it. Fatigue has long been classified as being either central or peripheral in origin with the peripheral model focusing on the metabolic changes in the exercising muscle (Taylor et al, 1997), while the central fatigue model highlights the role of neural drive or motor command (central nervous system) (Enoka, 1992). A more common definition used to describe fatigue is any exercise-induced reduction in the ability of a muscle to generate force or power (Gandevia, 2001) in the presence of an increased perception of effort (Enoka and Stuart, 1992). Fatigue can also be viewed as a conscious sensation, in some instances evoked in anticipation rather than as a consequence of physiological responses (St Clair Gibson et al, 2003). More recently however, fatigue has been described as the result of complex interactions of multiple physiological systems and the brain (Lambert et al, 2005). This would likely involve both feedforward anticipatory mechanism as well as a feedback response.

Previously, Borg established an effort continua which consisted of three main categories, namely the perceptual, the physiological and the performance (Borg, 1977) (Figure 1). The effort continua represent the relationship between the physiological demands of the exercise performance and the perception of effort associated with that performance. In Borg's model (Borg, 1977), the perceptual continuum refers to an individual's subjective experience or feeling in response to

a stimulus (situation or an exercise task) and is fundamental to our behaviour and how we adapt to a situation. The physiological continuum includes changes in variables such as heart rate, plasma glucose and lactate, muscle gycogen, catecholamine concentrations, VO_2 and ventilation etc and are measured using physical methods. The performance continuum includes maximal and submaximal exercise performances and are measured using power output changes and time taken to complete different exercise activities. According to Borg, the variables in all three continua are not linearly related to each other, however they all complement each other and contribute information providing a better understanding of fatigue and a complete picture of an individuals exercise performance (Borg, 1977). Therefore, for a given exercise task in a specific individual, the contribution of each continuum to fatigue may be "weighted" differently.

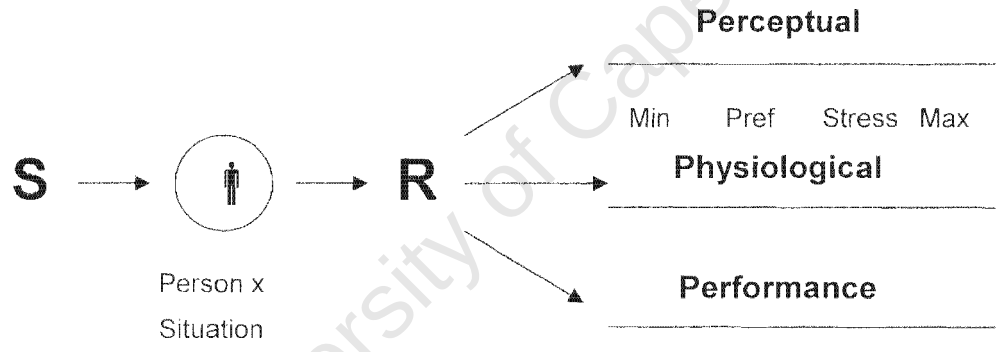


Figure 1: Schematic diagram of Borg's three effort continua: the perceptual, physiological and performance. S denotes the stimulus (exercise task) that interacts with the situation and the person (O is the observer). R is the response to each of these continua. Intensity in all continua varies from minimum (Min) over a preferred or adaptation level (Pref) and stress level (Stress) to a maximal (Max) intensity.

Although fatigue is regarded by some as a form of failure of physiological systems, it may also play a positive role in preventing the exercising body from exceeding its limits thus maintaining physiological homeostasis. Recently, Ulmer (1996) proposed a model of a central integrative system which regulates exercise performance by integrating both efferent and afferent information. St Clair Gibson

et al (2004) proposes that this integration may represent an attempt to prevent catastrophic failure of any particular system. The subsequent model is based on a feed forward and feedback control system which includes a "regulation centre" that controls complex algorithms taking into account both afferent and efferent signals, as well as antecedent experiences, training, metabolic reserves, actual metabolic rate and the time necessary to complete an exercise task, and as a result alters/modifies muscle output accordingly. This theory proposed by Ulmer is defined as teleoanticipation (Ulmer, 1996).

More recently, based on Ulmer's model, Hampson et al (2001) suggested that perception of exercise intensity is subconsciously set based on prior experience, which results in the programming of efferent signals. The central nervous system then interprets the afferent feedback against expected outcomes resulting in the conscious perception of effort. This regulatory system therefore, by means of an input/output "black box" (central programmer/brain), allows exercise intensity to be accurately controlled as not to exceed the biomechanical and metabolic limits of the body (Figure 2).

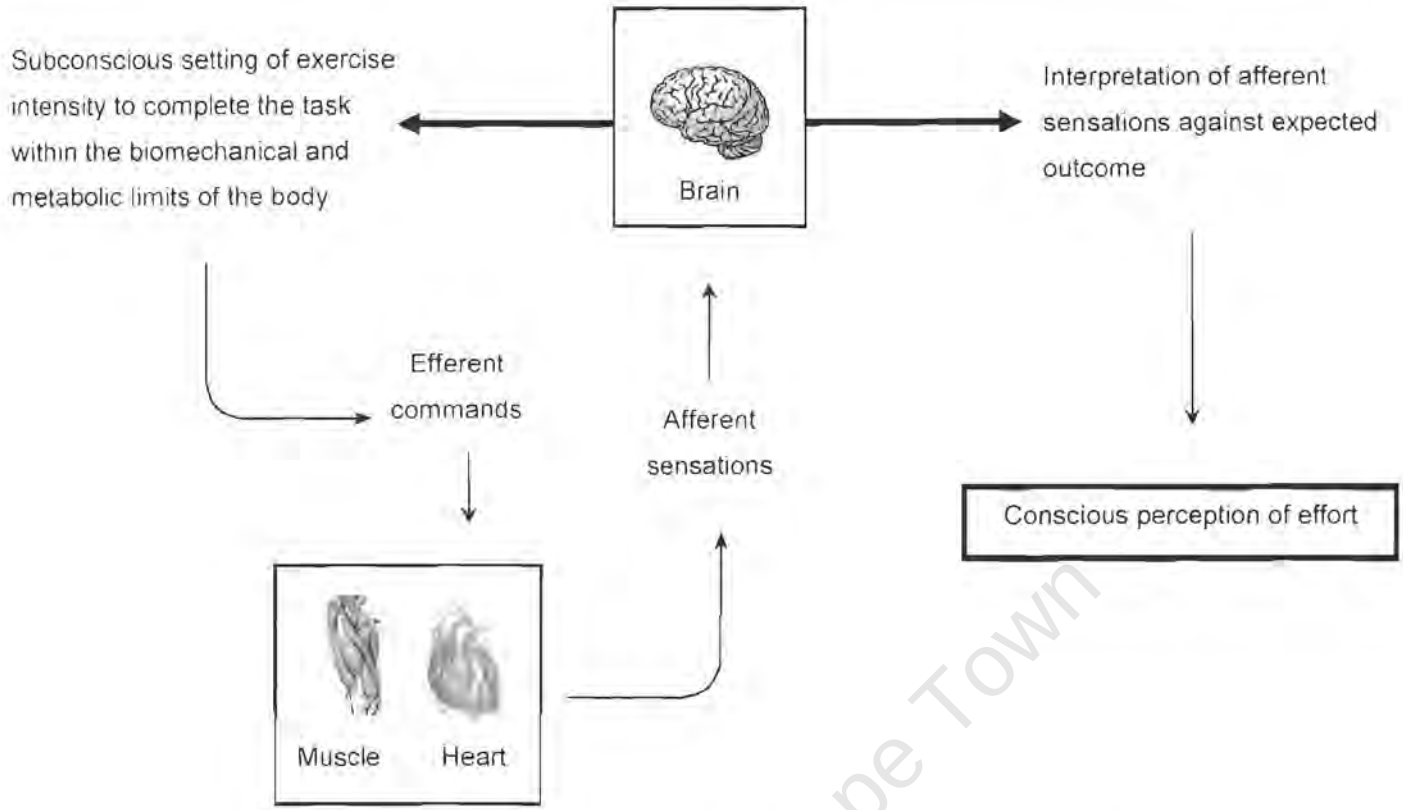


Figure 2: A modified schematic diagram of the teleoanticipation and perceived exertion model, first proposed by Hampson et al. (2001).

Therefore in the context of an integrative model of fatigue during exercise, there is no single regulation system, but rather a complex interplay between both feed forward and feedback regulation of exercise performance where all physiological systems interact continuously to maintain homeostasis. The "gain" and time constant of the gain of the system is the amount of change a variable deviates from baseline after reacting to a perturbation, and the speed at which a system returns to baseline respectively. The success and strength of a physiological system is therefore related to the gain and time constant of the gain of the different system variables and the efficiency of its physiological function and signalling processes.

Although there is integrative control of the different systems, factors such as training, muscle reserve, physiological perturbations and prior or antecedent activity initiate changes in the peripheral and/or central physiological systems

which ultimately alter the gain and time constant of the activity in the different systems.

Therefore, the primary objective of this literature review is to provide insight into the integrative regulatory system controlling exercise and the relationship between the 'sensation of fatigue' (perceived exertion), muscle activation levels (motor output/command) and performance (force production) during open and closed loop exercise at either a self-paced or constant workload, and to focus on various antecedent exposures and perturbations that may alter these three variables.

1.2 PERCEIVED EXERTION AND PERFORMANCE REGULATION

Perception of effort can be defined as the "subjective intensity of effort, strain, discomfort, and/or fatigue that is experienced during physical exercise" (Robertson and Noble, 1997). It is an area of research that was first studied in the 1800's, and has since developed into an established scientific field undertaken by exercise physiologists, clinicians, psychologists and physical educators. As the individual's subjective level of effort, the value of perceived exertion evaluation has proved to be a useful supplement to the measured physiological variables in the field of exercise physiology. The use of ratings of perceived exertion (RPE) as an investigative tool has yielded clinical applicability and has become a popular instrument in assessing effort perception.

1.2.1 Methods of Measurement

It is necessary in the assessment and interpretation of perceived exertion during exercise to use valid and appropriate scaling methodology. A variety of psycho-physiological methods have been developed over the years to investigate "effort perception".

1.2.1.1 Category Scales

The category scaling methods are the most popular and frequently used in clinical and experimental type settings. These scales are labelled with a fixed set of numbers, which are directly linked to verbal anchors e.g. 9 represents "very light" and 15 represents "hard" on the scale. One of the first of these scales to be developed was Borg's 21-point graded scale, which was highly correlated with heart rate at different exercise intensities (r = 0.80 to 0.90) (Borg, 1973). This 21-point scale was subsequently changed to a 15-point scale that ranged from 6 – 20, which had a stronger linear relationship between heart rate and exercise intensity. Borg's 15-point scale has been shown to be a reliable and valid tool for evaluating whole body exertion during exercise (Eston et al, 1987; Eston and Williams, 1988; Ceci and Hassmen, 1991; Dunbar et al, 1992). An advantage of the Borg 15-point RPE scale is that because its ratings increase linearly with exercise intensity, heart rate and oxygen consumption, it is easy to compare RPE with common measurements of exercise intensity. A disadvantage of the Borg 15-point scale however, is that it has an obvious upper limit which may cause the subject to follow a conservative perceptual strategy during experimental testing.

However, certain physiological variables do not increase linearly with exercise intensity, and therefore, Borg developed the 10-point category scale with ratio properties (Borg, 1982) that not only followed the growth of perceptual responses but also the exponential growth of nonlinear physiological responses, i.e. lactate metabolism and pulmonary ventilation (Noble, 1982; Noble et al, 1983). The category ratio scale is therefore suggested to be suitable for identifying local sensations of effort during exercise. An advantage of this scale is that is does not have a fixed endpoint so subjects have the option to choose something more than the maximum should they experience a perception stronger than experienced before. The category ratio scale is not recommended instead of the 15-point RPE scale, but the two should be used in conjunction with each other to determine the extent to which the cardiopulmonary and peripheral variables contribute to the overall sense of effort (Borg, 1982).

Another category scale developed and utilized to measure perceived exertion is the Pittsburgh perceived exertion scale, a 9-point category scale (Stamford and Noble, 1974; Robertson et al, 1979a; Robertson et al, 1979b). Although ratings on this scale correlate well with the 21-point Borg scale (r = 0.92), it is not known whether it correlates well with the more utilized 15-point scale and 10-point category ratio scale.

1.2.1.2 Ratio Scales

Ratio scaling methods, magnitude estimation and magnitude production, are another common and valid procedure used in perceived exertion experiments involving both static and dynamic exercise (Stevens and Mack, 1959; Pandolf 1983). Magnitude estimation requires the individual to subjectively rate the level of effort with a verbal or number score, whereas with magnitude production, the individual is required to exercise to intensities determined by their sense of perceived exertion. While ratio scaling methods allow one to examine the growth function of perceived exertion and for comparison between different levels of perceived exertion, it is suggested to not be the most suitable technique to use to compare different individuals or exercise tasks (Borg, 1973).

1.2.2 Physiological Mediators of Perceived Exertion

The literature reveals that a number of peripheral and cardiopulmonary variables, as well as neuromuscular parameters, contribute to overall perceived exertion during exercise of varying durations, intensities, modes and experimental conditions (Table I). However, it is not clear from the contradicting literature in this area, the manner in which the brain integrates the afferent information to induce a perceptual response and the extent to which these variables influence effort perception during exercise.

Table I: Sources of sensory information that contributes to perception of effort

Cardiopulmonary	Peripheral / Metabolic	Neuromuscular
Heart rate	Catecholamine concentrations	Mechanical strain
Oxygen consumption	Carbohydrate availability	Muscle damage
Respiratory rate	Skin temperature	
Ventilation rate	Core temperature	
	Blood and muscle pH	
	Blood lactate level	

1.2.2.1 Heart Rate Changes and RPE

Since Borg originally found a high correlation (r = 0.85) between RPE and the heart rate response during a cycling task with increasing exercise intensity, heart rate has become a convenient marker of perceived exertion (Borg, 1973). A number of studies have corroborated this relationship across genders (Skinner et al, 1969, Skinner et al, 1973; Stamford, 1976), irrespective of training status (Michael and Eckhardt, 1972; Bar-Or et al, 1972; Skinner et al, 1973), exercise modality (Borg, 1973; Skinner et al, 1973; Sargeant and Davies, 1973), or continuous and intermittent exercise protocols (Edwards et al, 1972). However, as maximal exercising heart rate decreases with age, studies have consistently shown that at a given heart rate, RPE is higher with increasing age (Borg and Linderholm, 1967; Bar-Or, 1977).

Whilst the relationship between heart rate and RPE may be highly correlated in these studies, the extent to which they are causally related has not been determined. If heart rate is a dominant mediator of perceived exertion, alterations in heart rate must be associated with concomitant changes in RPE. Several investigators have therefore attempted to examine the heart rate/RPE relationship during exercise performed under unusual conditions, such as altering the heart rate with pharmacological intervention and environmental manipulation. Changes in the heart rate response following the use of parasympathetic and sympathetic blocking agents did not affect RPE at a given exercise intensity (Ekblom and

Goldbarg, 1971; Davies and Sargeant, 1979). Similarly, in hot environmental conditions, heart rate increases were not associated with elevations in the perceptual response (Pandolf et al, 1972; Kamon et al, 1974). However, Skinner et al (1973) found contrasting results where RPE was directly related to heart rate in both neutral and hot environments.

Furthermore, the heart rate/RPE relationship has been challenged with various other experimental protocols. Pandolf and Noble (1973) have reported a higher RPE and lower heart rate response at a cycling cadence of 40 rpm compared to 60 and 80 rpm at the same power output. At similar heart rates, eccentric exercise induced a higher RPE than concentric exercise (Pandolf et al, 1978). The training status of individuals has also been shown to alter the relationship between heart rate and RPE, where reduced heart rate in response to training at a given workload is associated with an unchanged RPE (Sidney and Shephard, 1977; Lewis et al, 1980). In addition, cycling exercise has evoked a higher RPE response than treadmill running at the same heart rate (Hassmen, 1990).

Therefore, although there appears to be a strong correlation between heart rate and RPE during progressive exercise under neutral conditions, it is clear that under certain conditions these two variables can be dissociated from each other. Thus, it may be possible that heart rate is related to some other variables that invokes the perception of effort associated with increasing exercise intensity Alternatively, heart rate may not be a major sensory cue for perceived exertion, but rather only one of several cues that mediates perceived exertion (Hampson et al, 2001).

1.2.2.2 Oxygen Consumption and RPE

Studies have examined differences in perceived exertion with respect to absolute and relative oxygen requirements. Differences in RPE at absolute exercise intensities in lean and obese (Skinner et al, 1973), sedentary and active (Skinner et al, 1969), and trained and untrained individuals (Ekblom and Goldbarg, 1971)

are often eliminated when relative exercise intensities are used (% of VO_{2max}) (Skinner et al, 1973). While RPE can be compared across conditions at similar metabolic demands, relative exercise intensities based on a percentage of maximal oxygen consumption do not necessarily compare to other physiological responses such as lactate production, ventilatory hyperpnoea and increased catecholamines, which change with training and fitness, and may also have an effect on RPE at higher exercise intensities (Astrand and Rodahl, 1977). It has been suggested that VO_2 is indirectly associated to perceived exertion since it cannot be directly monitored by the individual perceptually during exercise (Mihevic, 1981; Carton and Rhodes, 1988). Therefore, VO_2 may be indirectly related to perceived exertion because it is one of a variety of physiological variables that increases with an increase in exercise intensity.

1.2.2.3 Ventilation and Respiratory Rate and RPE

The role and impact of ventilation and/or the respiratory rate upon perceived exertion has been extensively investigated. The association between RPE and both ventilation and respiratory rate have reported correlation coefficients ranging from r = 0.61-0.94 (Edwards et al, 1972; Kamon et al, 1974; Noble et al, 1973; Pandolf et al, 1972; Sargeant and Davies, 1973; Robertson, 1982). Edwards et al (1972) have observed that alterations in ventilation rate, which can be consciously monitored, induces afferent nervous system input that is distinguished from other proposed physiological inputs for perceived exertion, such as heart rate or VO_2.

Noble et al (1973) found that during exercise in either hot or neutral environmental conditions, ventilation and respiratory rate were the best predictors of perceived exertion among a number of physiological variables. In contrast, Horstman (1977) suggested an independent relationship between ventilation and RPE in a cold environment. However, these results were not presented in terms of statistical significance.

Several studies have attempted to experimentally manipulate the normal ventilatory response during exercise and examine the corresponding changes in RPE. These experimental treatments have included hypnosis (Morgan, 1973; Morgan et al, 1976), induced erythrocythemia (Robertson et al, 1979c), and hypoxia and hyperoxia (Pederson and Welch, 1977; Robertson et al, 1979c; Cafarelli and Noble, 1976). Morgan et al (1976), under hypnotic suggestion conditions, demonstrated parallel ventilation and RPE responses when subjects were told they were ascending a hill, when instead, they were cycling at a constant power output. Robertson et al (1979c) examined changes associated with erythrocythaemia in subjects after red blood cell reinfusion and showed that ventilation was reduced at both 45% and 70% VO_{2peak} exercise intensities, however, RPE was only altered at 70% VO_{2peak}. This result suggests that the onset of the ventilatory signal to perceived exertion is related to the relative exercise intensity and may be an important sensory cue at high exercise intensities. This observation has also been found by Cafarelli and Noble (1976) and Robertson et al (1979c), who showed a relationship between minute ventilation and RPE only at high intensities. Cafarelli and Noble (1976) examined subjects who had inspired room air enriched with carbon dioxide and exercised at 54 and 71% VO_{2max} and found that minute ventilation was higher at all submaximal exercise intensities, while increases in RPE only paralleled ventilation at the higher exercise intensity. Similarly, Robertson et al (1979c) found that RPE and ventilation increased concomitantly during hypoxic conditions, although RPE elevations only become apparent at higher exercise intensities. Pederson and Welch (1977) demonstrated that RPE and ventilation were significantly reduced when subjects inspired gas mixtures of 50% and 80% O_2 during progressive exercise, however, Allen and Pandolf (1977) showed that decreases in RPE were not matched with similar decreases in ventilation during hyperoxic conditions.

Although several studies have therefore supported a relationship between ventilation and respiration rate with RPE, the significance of ventilation as a perceptual cue during hyperoxic experimental studies remains unclear. In addition, the ventilatory threshold and lactate threshold occur at nearly the same exercise intensity, and therefore it is difficult to conclude whether the ventilation

and respiratory rate may have a direct or indirect role in perceived exertion. The ventilation and respiratory rate, however, are two cardiopulmonary variables that can be readily monitored during exercise and may possibly contribute a source of sensory information for perceived exertion during exercise.

1.2.2.4 Catecholamine Concentrations and RPE

Although catecholamine concentrations have been regarded as a hormonal mediator of perceived exertion during exercise, evidence supporting this suggestion is inconsistent. RPE has been shown to positively correlate with circulating levels of epinephrine and norepinephrine during incremental treadmill testing (Skrinar et al, 1983), however, other studies have demonstrated that RPE is independent of catecholamine levels (Felig et al, 1982; Womack et al, 1998). Since the catecholamine response is only prominent at higher exercise intensities (50 – 65% VO_{2peak}), it is possible they may only act as a perceptual cue at higher metabolic demands (Mihevic, 1981), reflecting increased levels of sympathetic activation, or as a consequence of accompanying sequelae, such as increasing heart rate, VO_2, etc.

1.2.2.5 Carbohydrate Availability and RPE

A number of studies have examined the effects of carbohydrate substrate availability on RPE during prolonged exercise and found an association between RPE and decreases in blood glucose and carbohydrate oxidation (Burgess et al, 1991; Coggan and Coyle, 1987; Utter et al, 1999; Utter et al, 1997). This relationship between perceived exertion and energy substrate changes may be mediated by sensory input from the muscle due to depleted carbohydrate energy levels. Kang et al (1996) showed increased leg and overall RPE values in the later stages of a prolonged cycling trial (70% VO_{2peak} till exhaustion) in individuals ingesting a placebo compared to carbohydrate solution. The RPE values at exhaustion, however, were not different between groups and the authors

concluded that physiological processes other than carbohydrate availability may influence perceptual intensity and be more important perceptual cues after endurance exercise. More recently, Utter et al (2003) examined RPE and their relation to certain physiological mediators during endurance exercise, specifically an ultramarathon race of 68 km, with carbohydrate ingestion. The results show that an increase in RPE was not associated with a decrease in blood glucose, and the authors concluded that perceptual responses during self-paced exercise may be mediated through other neurological and physiological mechanisms, rather than through glucose or carbohydrate mediated pathways.

1.2.2.6 Skin and Core Temperature and RPE

Along with ventilation and respiratory rate, skin temperature may be actively monitored by the individual during exercise. The effect of skin and core temperature on perceived exertion has been examined by comparing responses during heated and thermo-neutral environmental conditions. Nybo and Nielsen (2001) have reported a high correlation ($r = 0.98$) between RPE and increases in body temperature as a result of cycling in a hot environment (40°C). Exercise endurance has been shown to be reduced in the heat (~30°C) and was accompanied by a higher RPE during cycling trials to exhaustion when compared to exercise in cooler conditions (Galloway and Maughan, 1997; Pitsiladis and Maughan, 1999). More recently, Armada-da-Silva et al (2004) demonstrated that during short submaximal exercise, RPE increased when body temperature was increased by previous passive heating (rectal temperature of ~38.5°C). The increased RPE associated with hyperthermia was not associated with increased plasma lactate concentrations or with changes in perceived thermal comfort, and therefore, the authors suggested that muscle metabolic changes and sensations of displeasure did not contribute to changes in RPE with an elevated body temperature. However, not all studies have shown that exercise in the heat is associated with increased RPE (Glass et al, 1994; Tucker et al, 2004).

Although there are studies that highlight the importance of skin and core temperature on perceived exertion, there is also evidence that does not support this relationship. Therefore, it is difficult to establish whether a causal relationship exists between increases in RPE and body temperature during exercise, or whether it may just be coincidental as exercise itself produces various responses that may also influence perceived exertion.

1.2.2.7 Blood Lactate and pH Changes and RPE

Since lactate and RPE both increase exponentially with increases in exercise intensity, blood lactate has been extensively examined as a sensory cue that contributes to perceived exertion. Several studies have found strong correlations between RPE and blood lactate using a variety of exercise modalities, intensities, continuous or intermittent exercise protocols, environmental conditions and fitness levels (Ekblom and Golgbarg, 1971; Gamberale, 1972; Horstman et al, 1979a; Edwards et al, 1972; Horstman, 1977).

In a study by Gamberale (1972), RPE and lactate responses were examined during a variety of work tasks, namely cycling, weight lifting and wheelbarrow pushing. A direct relationship was found for each exercise modality between increases in lactate and RPE with increased exercise intensity. Edwards et al (1972) reported correlations of r = 0.77 and 0.63 between lactate and RPE for continuous and intermittent cycling, respectively, however, the small subject number (n=3) may limit the validity of the results.

More recently, Hetzler et al (1991) investigated measures of heart rate, VO_2 and RPE in untrained subjects at exercise intensities corresponding to the lactate threshold, blood lactate levels of 2.0, 2.5 and 4.0 mmol/L and maximal exercise, during a VO_{2max}/lactate threshold cycling and running exercise protocol. RPE was not significantly different, despite differences in heart rate and VO_2 at each blood lactate level between cycling and running. The authors concluded that lactate functioned as a physiological mediator for perceived exertion during exercise.

The RPE/lactate relationship may be potentially altered as a result of exercise training, since training has previously been shown to increase the percentage of VO_{2max} at the lactate threshold. Haskvitz et al (1992) explored this by monitoring untrained subjects while they completed a year long training programme. RPE was similar at each lactate concentration, despite increases in VO_2 at the lactate threshold and fixed blood lactate concentrations after training. Similarly, Boutcher et al (1989) investigated the effects of cycle and run training (10 week period) on changes in the lactate threshold and RPE. While both exercise modalities caused increases in VO_2 and work rate at the lactate threshold, the RPE at the lactate threshold remained the same.

Studies using experimental manipulations of lactate concentration have reported inconsistent results implicating blood lactate concentrations as a factor influencing perceived exertion. Pederson and Welch (1977) showed concomitant reductions in RPE and blood lactate but of different magnitudes during progressive cycling exercise while breathing 21%, 50% and 80% O_2. In contrast, Allen and Pandolf (1977) demonstrated that treadmill exercise at 50% and 80% of maximal aerobic power while breathing 80% O_2 resulted in similar reductions in blood lactate concentrations and RPE.

Not all previous studies have shown a correlation between RPE and blood lactate. In a study by Stamford and Noble (1974), subjects performed continuous and intermittent cycling exercise at 40, 60 and 80 rpm and results showed that RPE was higher at 40 rpm compared with 60 rpm, despite similar blood lactate concentrations.

Lactate does not increase appreciably below an exercise intensity of approximately 65% VO_{2peak}, and therefore, it is unlikely to have much contribution on perceived exertion at lower exercise intensities (Mihevic, 1981). Although blood lactate concentrations have been related to perceived exertion, the mechanism by which this relationship is mediated has not been identified. It has been suggested that lactate may indirectly modulate perceived exertion through

changes in pH (metabolic acidosis) associated with elevations in muscle lactate concentrations (Stamford and Noble, 1974; Pandolf, 1978). However, results from several studies do not support this hypothesis. Poulus et al (1974) demonstrated that increased pH from sodium bicarbonate infusion did not effect subjective ratings of fatigue during cycling at progressively increasing exercise intensities to exhaustion. In a study by Kostka and Cafarelli (1982), induced acidosis or alkalosis had no effect on perceived exertion during moderate-intensity exercise (50% VO_{2peak}), but acidosis increased effort perception during high-intensity exercise (80% VO_{2peak}). Robertson et al (1986) showed that induced alkalosis was associated with decreases in overall and localised RPE during high-intensity exercise (80% VO_{2peak}), but not at lower exercise intensities. Similarly, Swank and Robertson (1989) found that inducing alkalosis attenuated overall and localised RPE during intermittent high-intensity exercise (90% VO_{2peak}).

Thus, it seems that correlational evidence indicating that lactate concentrations may act as a perceptual cue is mostly consistent, however, under certain experimental conditions and with various tasks, the relationship between lactate concentrations and RPE remains unclear. Although lactate concentrations do not accumulate until an exercise intensity of about 65% VO_{2peak} is reached in most individuals, it appears that blood lactate concentrations may influence RPE at higher exercise intensities by an unidentified mechanism. Further research is required to explore the relationships between RPE and lactate and pH to establish whether the change in perceived exertion is a result of lactate or pH changes following lactic acid production.

1.2.2.8 Sensations of Strain in the Exercising Limb and RPE

Another local sensory cue that may directly mediate perceived exertion is the sensation of exertion and fatigue arising from the exercising limb. Many studies attempting to link the perceptual response with various kinaesthetic cues have compared different exercise modalities on the perceptual response or examined the effect of a variety of pedalling speeds during cycling on RPE. It has been

proposed that these muscle sensations arise from mechanoreceptor and proprioceptor feedback and golgi tendon activity (Mihevic, 1981). This hypothesis, however, remains speculative since the golgi tendon activity and muscle spindle feedback underlying these sensations cannot be quantified.

Pandolf et al (1978) compared concentric and eccentric exercise on a motor driven laddermill and found that RPE was higher during eccentric exercise at the same VO_2 and heart rate as concentric exercise, suggesting eccentric exercise causes greater localised muscular strain which influenced overall RPE. Ekblom and Goldbarg (1971) reported higher RPE during cycling compared to treadmill running at given submaximal levels of oxygen consumption and concluded that a greater physiological strain was associated with exercise requiring the use of small muscle groups. Banister (1979) also found that RPE increased exponentially when increased force was applied to the adductor pollicis and quadriceps muscle, but that the smaller adductor pollicis muscle had a greater RPE than the quadriceps muscle even though the same amount of force was applied to both muscle groups. Gearhart et al (2005) demonstrated increased RPE during high intensity cycle exercise with increased resistance and force production. The results from these studies suggest that the magnitude of localised force or strain on the muscle is a significant contributor to sense of effort, but the relationship depends on the muscle and contraction type being examined.

Several studies have used various pedalling speeds during cycling exercise to investigate muscular strain on RPE. The RPE measured at a cadence of 40 rpm was significantly greater than that measured at 80 rpm, at the same power output, VO_2 and heart rate values (Pandolf and Noble, 1973). Similarly, Stamford and Noble (1974) showed a greater RPE at 40 rpm than at 60 rpm during continuous and intermittent cycle exercise at equivalent power outputs that were not related to lactate and ventilation differences. Cafarelli (1977) also found a greater RPE during cycling at a lower cadence (30 vs 60 rpm), despite similar ventilation, VO_2 and integrated electromyography (IEMG). Furthermore, Lollgen et al (1977) demonstrated that RPE decreased with increasing pedal frequency (increasing cadence) during cycling at a constant workload at 40, 60, 80 and 100 rpm. The

findings from these studies suggest that mechanical strain in the exercising limbs is greater at lower cadences and is associated with increased RPE, despite comparable power outputs, metabolic and cardiac responses. Therefore, sensations of muscular strain and fatigue appear to be another important perceptual cue that may mediate RPE during cycling exercise.

1.2.3 Multiple Sensory Input Integration and Exercise

Several of the earlier studies have attempted to identify a primary cue underlying RPE. This research has been guided by a two-factor model of perceived exertion, first formally proposed by Ekblom and Goldbarg (1971), which was based on a local factor (feelings of strain in the exercising muscle/joint) and a central factor (feelings associated with cardiopulmonary systems). However, as previously mentioned in the Introduction, the gestalt nature of Borg's original model of perceived exertion proposes that RPE is determined by the integration of various sensory cues that have different perceptual weightings according to their importance during various types of exercise (Borg, 1977). In addition, Umer's teleoanticipation and perceived exertion model proposed that exercise performance may be accurately regulated through a process of teleoanticipation and the interpretation of afferent feedback against an expected outcome which results in the conscious perception of effort (Ulmer, 1996; Hampson et al, 2001). Therefore, as described in the previous section, the search for a primary perceptual cue is likely to be simplistic and ineffective, and examining the perceptual response during exercise in terms of various modifying variables should also be taken into account. These variables include exercise intensity and duration, steady-state vs self-paced exercise, closed vs open loop exercise, prior experience or knowledge regarding exercise duration and intensity, feedback vs no feedback during exercise, as well as training and the fitness level of the individual.

1.2.3.1 Exercise Duration and Intensity

It is possible that the duration of exercise may influence the efficacy of the perceptual cues and how they are integrated, since, at the onset of steady-state exercise, RPE increases progressively over time while metabolic variables rise rapidly followed by a plateau within 5-10 min into exercise. Previous studies have proposed that the perceptual response during short duration exercise is determined by peripheral and neuromuscular variables, whereas cardiopulmonary variables become dominant as exercise duration increases (Cafarelli, 1977). In addition, Edwards et al (1972) showed that during intermittent exercise of the same exercise intensity, RPE increased progressively as the exercise intervals increased from 15 s to an eventual 120 s, and suggested that the amount of work completed was less influential on RPE than exercise duration itself.

At low-to-moderate intensity exercise which does not significantly stress the cardiopulmonary and metabolic responses, neuromuscular variables such as muscular strain contribute the primary perceptual input, however, as exercise intensity increases, the dominance of cardiopulmonary and metabolic responses serving as sensory input increases (Cafarelli et al, 1977; Horstman et al, 1979b). Furthermore, considerable variations in exercise intensity, seem more likely to be influenced by neurogenically mediated cues such as proprioceptive cues and mechanoreceptors, than those cues depending on biochemical changes (Astrand and Rodahl, 1977).

Therefore, it seems that both exercise duration and the magnitude and rate of change in exercise intensity play significant roles in the manner in which various perceptual cues are weighted and integrated by the individual when determining RPE (Mihevic, 1981).

1.2.3.2 Steady-state and Self-paced Exercise

During exercise at a constant power output, parameters such as heart rate, VO_2 and ventilation increase at the onset of exercise but then plateau and remain relatively constant as a steady state is reached. Since these variables of metabolic demand have been shown to have high correlations with RPE, it would be expected that RPE displays a similar response. However, several studies have reported that while many physiological variables attain a steady state during constant power output exercise, RPE continues to rise with increased duration of an exercise bout (Noble et al, 1973; Kamon et al, 1974; Pandolf et al, 1972). Pandolf et al (1972) demonstrated that during cycling exercise at three submaximal intensities, oxygen consumption and ventilation reached a steady state after 10 min but RPE continued to rise over a 30 min interval. Heart rate attained steady state during the lowest exercise intensity but continued to increase over time during the other two intensities, however, the magnitude of increase in heart rate was substantially less that the increase in RPE. Similarly, Noble et al (1979) also reported a dissociation between the heart rate response and RPE during steady-state treadmill exercise.

Previous studies examining RPE at a constant work rate during cycling have shown that manipulation of cadence has resulted in alterations in RPE (Pandolf and Noble, 1973; Lollgen et al, 1975; Marsh and Martin, 1993, 1998). Reduced or slower cadences have reported increased RPE values which can be attributed to increased local rather than central sensations, as greater force is required to turn the pedals at the same power output. This suggests that sensations of strain in the leg muscle produced by variations in the contraction force contribute to changes in RPE and the perceptual response.

Therefore, during self-paced exercise, where work rate is free to vary, it is possible that RPE may regulate exercise intensity based on perceptual preference or comfort. In a study by Kay et al (2001), RPE and heart rate increased over time during cycle sprints of a 60 min self-paced TT in warm, humid conditions, whilst EMG activity was systematically reduced. During the final sprint, however, EMG

activity was restored close to initial values. Similarly, Tucker et al (2004) demonstrated that during a self-paced 20 km TT in the heat, power output and EMG activity were both reduced while RPE and heart rate increased over time. Both studies show that RPE did not track changes in power output and EMG activity but was rather related to a central process whereby the maintenance of thermal homeostasis was a necessity. In order to maintain thermal homeostasis, the central processes reset power output and muscle activation with a corresponding increase in RPE to prevent conscious overriding of this subconscious control, and therefore, the development of homeostatic failure (Tucker et al, 2004). These findings support Ulmer's model of teleoanticipation, whereby a "central programmer" regulates exercise intensity through the integration of afferent and efferent feedback so as not to exceed the limits of the body and therefore prevent bodily damage (Ulmer, 1996).

1.2.3.3 Teleoanticipation and RPE

The model of teleoanticipation proposes that exercise performance may be regulated by incorporating afferent feedback into a performance algorithm from the periphery and external environment with the end point of the exercise task (particular distance or duration), as well as knowledge gained for prior exercise tasks (Ulmer, 1996). Furthermore, the model suggests that RPE may be set by a feedforward system and is regulated by afferent feedback from various areas of the body. In addition, antecedent exposures or specific feedback could change the interpretation of RPE and may therefore reset the teleoanticipatory set points (Hampson et al, 2004).

Ulmer (1996) investigated this model by instructing subjects to exercise maximally or submaximally corresponding to a specific RPE value on the 15-point Borg scale, during short (400m run) and long (1500m run) duration exercise. The performance or running velocity during the short duration activity increased initially, followed by a steady decrease throughout the rest of the exercise bout. In contrast, during the longer duration activity, performance or running velocity at

each RPE level decreased rapidly, followed by a long plateau between the distances of 500m and 1500m. These results suggest that exertion and RPE is preset and may be subsequently altered over longer duration exercise in order to reach an end point, whereas feedback may be too late during short duration exercise to adjust exercise intensity and RPE.

A study by Nybo and Nielson (2001), examining perceived exertion during exercise with hyperthermia, provides further evidence for the teleoanticipation model. The study tested the relationship between cerebral changes (EEG), muscle activation (EMG) and RPE during submaximal exercise in normal and hot conditions. The results showed that RPE and EEG changes were highly correlated with increases in core temperature during exercise. In contrast, RPE was not associated with any of the EMG parameters, which were unchanged in the heat. Therefore, this hyperthermia-induced fatigue appeared to be related to altered activity in the central nervous system, which was associated with increased RPE, despite no changes in power output or muscle contractility, during prolonged exercise in the hot environment. It is possible that awareness of rate of change in core temperature, heat storage or various other metabolic variables may have activated this change in central nervous system activity.

Hampson et al (2004) investigated RPE during repeated bouts of high-intensity running. In this study, subjects were asked to complete three 1680 m running bouts at 80%, 83% and 86% peak treadmill running speed. The actual running speed was altered, such that subjects were deceived so that they were running at either higher or lower intensities than expected. There were no differences in overall RPE between deceived and control groups but deceived groups had consistently higher leg and chest exertion scores than head and other areas. There was also a tendency for overall RPE to be attenuated in the group who believed that they were running at a higher intensity compared to the control group who were honestly informed of increased intensity. The authors suggest that a precise system of afferent feedback exists that mediates the response of overall perceived exertion during high-intensity running.

Similarly, Albertus et al (2005) showed that providing incorrect distance feedback during 20-km cycling time-trials did not alter RPE, exercise performance nor the subjects' pacing strategies, and concluded that a pacing strategy exists that is set prior to exercise and may be unaffected by external distance feedback. These studies provide some evidence that external feedback inconsistent with afferent biophysiological feedback may be overridden in determining perceived exertion, during exercise of varying duration and intensities.

In another model of distance deception, Paterson and Marino (2004) examined three groups of cyclists who completed three self-paced time trials. Trials 1 and 3 were each 30-km, however trial 2 was either a longer distance of 36-km, a shorter distance of 24-km or a control distance (30-km). Subjects were told that trial 2 was 30-km (deception). Following the trial 2 deception, the performance time of trial 3 was decreased for the longer distance group, increased for the shorter distance group and remained unchanged for the control group. These results suggest that, in this instance, and in the absence of external feedback, exercise performance was altered on the basis of prior or antecedent exposure. The reason for the differences between this study and those cited previously are unclear, which further highlights the complexity of these relationships between RPE, performance, muscle activation and metabolic activity.

1.2.3.4 Closed and Open Loop Exercise and RPE

A closed loop exercise model may be described as exercise with a defined end point, that is a defined distance or duration, whereas an open loop exercise model comprises of exercise with an undefined end point (exercise until exhaustion). Most of the perceived exertion research has utilized exercise models with prescribed exercise intensities and defined end-points and thus, physiological mediators of RPE during closed loop exercise may, in addition to knowledge of the end point, contribute afferent input to a teleoanticipatory system enabling an exercise task to be completed within the limits of the body. RPE during exercise with an undefined end-point (open loop exercise) has not been fully studied, and it

is possible, that this exercise model may cause a differential perceptual response. Therefore, the relationship between RPE and the regulation of exercise duration cannot be established at present.

1.2.4 Perceptually Guided Exercise

Much of the literature examining perceived exertion in both research and clinical settings have used category scaling or magnitude estimation, a method in which individuals are asked to subjectively judge their level of effort with either a number or verbal rating. A method less frequently used during exercise is magnitude production, where individuals are required to exercise at intensities or by producing force determined by their perceptual feelings of exertion.

1.2.4.1 Accuracy of Magnitude Production Efforts

Using RPE to gauge exercise intensity has been generally accepted as a physiologically valid technique (Eston et al, 1987; Eston and Williams, 1988; Eston et al, 2005; Eston et al, 2006; Kang et al, 1998; Kang et al, 2003). Recent evidence has shown a generally close correlation between submaximal isometric contractions and RPE measured via the Borg CR-10 scale using magnitude estimation (Pincivero et al. 2000a). However, few studies have demonstrated such a relationship using magnitude production efforts.

Cooper et al (1979) studied static contractions in the adductor pollicis and both dynamic and static contractions in the quadriceps muscle, using estimation and production methods. The results from this study suggest that the level of force output during an isometric or a dynamic contraction using estimation and production methods, may in some instances be perceived with precision by both large and small muscle groups. Kumar and Simmonds (1994) measured the accuracy of magnitude production using a pinch grip, power grip and stoop lifting activity. In this study, however, there was a systematic bias in perception during

the three activities (precision, power and gross activity), at all submaximal force levels except at 40% of MVC. The perceived effort at 60% and 80% MVC was lower than the desired effort based on the MVC, higher at 20% MVC and equal at 40% MVC. A study done by Jackson and Dishman (2000) measured perceived submaximal force production using a chest press in the order of 25%, 50%, 75% of maximum and a final MVC. Subjects produced actual forces that were significantly correlated to the desired forces, however subjects overproduced force at the 25% workload and under produced forces at both 50% and 75% workloads when compared to the desired force production. The workloads in this study were presented to the subjects in a linear manner and produced a linear relationship. Pincivero et al (2003a) examined knee extensor torque at perceived voluntary contraction efforts using magnitude production (modified category ratio scale (CR-10)). The results demonstrated that peak torque output was significantly lower than equivalent percent values at higher RPE levels (6-9).

Taken collectively, the results from these studies show that the ability of individuals to accurately match voluntary muscle force to target contraction intensities in the absence of external feedback is inconsistent, especially at high perceived intensities where force output tends to be under produced (Cooper et al. 1979;Jackson and Dishman 2000; Kumar and Simmonds 1994; Pincivero et al. 2003a).

1.2.4.2 Use of Perceptual Anchors

The category scaling methods used during dynamic exercise are based on the rationale that as exercise intensity increases from low to maximal, there is a corresponding and equal increase in perceptual intensity. This range model of sensory responses to external physical stimuli helps transpose feelings of exertion into numerical ratings, as well as subjectively match minimal and maximal perceptual intensities between individuals (Robertson and Noble 1997). It also defines a measure to establish low and high perceptual scale anchors. This anchoring process allows individuals to cognitively establish an association

between the stimulus (i.e. exercise intensity) and the response (i.e. perceived exertion).

During dynamic exercise, using RPE to regulate exercise intensity has been shown to be physiologically valid, especially when perception during initial exercise testing is carefully anchored (Kang et al, 2003). Several of the magnitude production experimental designs used during isometric exercise have allowed individuals to experience a maximal voluntary contraction or submaximal contractions prior to the testing protocol, thereby enabling the individual to cognitively establish a perceptual response range (Kumar and Simmonds 1994; Jackson and Dishman, 2000; Pincivero et al. 2001; Pincivero et al. 2003a; Pincivero et al. 2004). Studies have shown that previous exercise experience, to some extent, may be necessary to accurately gauge RPE (Horstman et al, 1979c), however, others have shown that effort perception may be significantly influenced by a maximal contraction being performed immediately prior a submaximal contraction resulting in perceptual underestimation (Hutton et al. 1984). Thus, prior experience of an MVC may in some models, provide anchors for the perceptual range of effort, or alternatively may actually alter the perceptual response range.

1.2.5 Summary

In summary, previous research has shown that perceived exertion may not be dependent on a single physiological variable during exercise, but rather on a multitude of sensory cues, thus emphasizing the complex nature of perceived exertion.

Ventilation and blood lactate concentrations, however, have been identified as primary perceptual cues at high intensities which suggests that a physiological response needs to be highly activated and dominate awareness to have a significant impact as a perceptual cue. Although both have a strong correlation with perceived exertion, it is at present impossible to propose they are causally

related. Furthermore, an additional perceptual cue that appears to have a significant association with perceived exertion is the sensation of strain in the exercising limbs, especially during exercise modalities that necessitate large amounts of localised force production. This suggests a relationship between perceived exertion and the degree of strain being experienced in the active musculature.

The consideration of modifying variables (i.e. exercise intensity, exercise duration, exercise modality, environment) during exercise suggests that multiple physiological inputs are integrated and weighted in order to reach an overall perceived exertion. Further research, however, is required to investigate the nature and location of the afferent input associated with increased RPE during different exercise protocols.

Furthermore, the relationship between regulation of exercise intensity and duration and RPE is unclear at present. Many studies examining RPE have used prescribed exercise intensities and end points which may result in a perceptual response that is different from self selected exercise intensities and exercise with an undefined end point. Therefore, methodology examining the perceived exertion response during self-paced exercise and exercise with an undefined end point should be considered in future studies.

In addition, it appears that with the use of category scaling methods, an anchoring process is necessary to establish a perceptual range that is the same as the stimulus range during dynamic exercise. However, it is unclear how important the role of perceptual anchors are during isometric exercise using a magnitude production model. Furthermore, previous studies using magnitude production to examine the ability of individuals to accurately match voluntary force production to target contraction intensities based on a perceptual score or feeling during isometric exercise, in the absence of external feedback, have produced inconsistent results.

Therefore, it may be concluded that the "gestalt" of perceived exertion is determined by the integration of multiple sources of sensory information from a variety of perceptual cues, although the underlying mechanism by which the various physiological variables invoke perceptual responses, remains undefined. In addition, the extent to which perceived exertion modifies subsequent performance and fatigue also remains to de defined.

1.3 NEUROMUSCULAR CONTROL MECHANISMS DURING FATIGUING EXERCISE

The neuromuscular system is of fundamental importance during exercise in the control of movement and in the ability to generate and sustain power output. The functional unit of the neuromuscular system is the motor unit, which is comprised of a cell body and an alpha motor neuron together with the muscle fibers it innervates (Enoka, 1995). Gandevia (2001) has proposed, that, if muscle is analogous to a motor, then the muscles behavior is dependent not only on its intrinsic properties but also on the manner in which it is "driven" (central nervous system), and the means by which the feedback systems maintain its output. Voluntary and reflex movements are produced by appropriate neural circuits located in the spinal cord. However, during more complex movement patterns, higher brain centres control and influence the circuits in the spinal cord (Brooks et al, 2000). While action potential signals sent by the motor neuron cause muscle fiber activation and thus contraction of muscles, sensory receptors provide feedback to the central nervous system regarding movement status. The central nervous system uses this feedback to constantly adjust movement in progress, in addition to initiating new movements (Brooks et al, 2000).

1.3.1 Muscle Force Production and Motor Unit Activation/Recruitment Strategies

The force produced during a voluntary muscle contraction is controlled through two mechanisms, the activation (recruitment) of motor units and the change in firing rates driving the active motor units, termed rate coding (Enoka, 1995; Conwit et al, 1999). Alterations in force production therefore occur through changes in the number of active motor units, the type of recruited muscle fibers and motor unit firing rates (Kamen and Caldwell, 1996). The recruitment and the rate coding of the motor units are related and occur in response to a common excitatory drive. Motor unit recruitment follows an orderly sequence according to the size principle (Henneman et al, 1965), where the smaller type I units (slow twitch, fatigue resistant fibers) are recruited at lower force levels before the larger type II units (fast twitch, fatigue nonresistant fibers) are activated at the higher levels of force. The force of a muscle contraction therefore increases as more motor units are progressively recruited within a muscle.

It is has been shown that the full recruitment of new motor units occurs at 50-80% of MVC depending on the muscle, after which additional force is attained only by increases in the motor unit firing rate (Figure 3) (Milner-Brown et al, 1973; Moritani and Muro, 1987; Bernadi et al, 1999). However, the relationship between these two mechanisms (recruitment and rate coding) differs depending on the muscle size and the type of contraction (Conwit et al, 1999). For example, large muscle groups (biceps brachii) have demonstrated that recruitment of motor units continues over the entire range of force, but that rate coding only contributes significantly towards the high end of the force range. In contrast, in smaller muscles (first dorsal interosseous), motor unit recruitment during the muscle contraction is normally complete by 30% of the MVC, after which rate coding mediates any further increases in force (Kukulka et al, 1981; De Luca et al, 1996). Furthermore, maximal motor unit recruitment has been shown to be lower during dynamic compared to isometric activity (Tax et al, 1989; Ivanova et al, 1998; Linnamo et al, 2003).

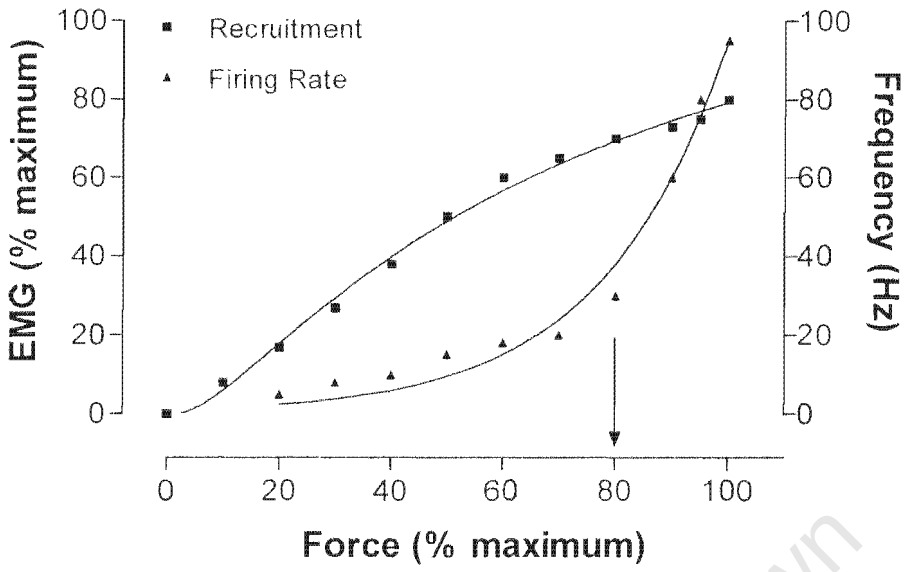


Figure 3: Schematic diagram of the relation between muscle force and the motor unit involving both the recruitment and firing rate.

In addition, an association exists between the state of energy supply and the recruited muscle fiber type, therefore, it may be suggested that the availability of oxygen and blood-borne substrates possibly play a part in regulating motor unit recruitment and firing rate (Moritani and Yoshitake, 1981; Moritani et al, 1986). Taking this into account, the motor unit activation strategy during fatigue development may be altered due to the availability of these substrates and oxygen, as well as metabolic by-products. Enoka et al (1989) showed that fatigue changed the motor unit recruitment, derecruitment and firing rate with increases in the variability of recruitment order in comparison to the non-fatigued state.

During maximal exercise activity, the individual is not able to increase motor unit recruitment, and therefore, alterations in the motor unit firing rate are needed to counteract the reduction in force output (St Clair Gibson et al, 2001a). This concurrent decline in force, the relaxation rate and the motor neuron discharge rate are collectively known as "muscle wisdom" (Enoka and Stuart, 1992). The theory of muscle wisdom proposes that the motor neuron firing rates decrease to match muscles contractile speed (Gandevia, 2001), and as a result, causes force output optimisation and economical activation of the fatiguing muscle by the central nervous system (Enoka and Stuart, 1992).

1.3.2 Muscle Activation and Fatigue

The manifestations of fatigue, as shown by the reduced ability to produce a required force or power, are clearly evident soon after the initiation of high intensity exercise. Although fatigue has been extensively researched, its interpretation is complicated by the variety of definitions and the multiplicity of paradigms used to examine it, and therefore, fatigue development during exercise and its association with underlying central and peripheral mechanisms remain controversial. In general, as described previously, fatigue can be defined as an impairment in performance that includes both an exercise-induced reduction in the muscles' ability to generate force or power, and an increase in effort perception necessary to produce the desired force (Gandevia, 2001; Enoka and Stuart, 1992). The mechanisms of fatigue have been classified as either 'peripheral' or 'central' in origin and the relative contribution of these factors appears to be task dependent (Enoka and Stuart, 1992).

Traditionally, fatigue during prolonged exercise has been associated with dysfunction of the contractile process. The peripheral model of fatigue focuses on metabolic changes in the skeletal muscle which are independent of the central nervous system, and implies a reduced ability of the muscle to produce force (Taylor et al, 1997; Nordlund et al, 2004). These peripheral sites and processes include the motor neuron, impairments in the neuromuscular transmission and propagation down the sarcolemma, excitation/contraction coupling failure, the accumulation of metabolites and the availability of metabolic substrates (Kirkendall, 1990; Enoka and Stuart, 1992; Fitts, 1994).

The central fatigue model, however, is associated with changes in the central nervous system that cannot be explained by the dysfunction in the muscle itself (Davis and Bailey, 1996). Central fatigue may be characterised by reduced neural drive or motor command failing to maintain voluntary muscle activation, resulting in a decrease in force production (Bigland-Ritchie, 1984a; Enoka and Stuart,

1992; Nordlund et al, 2004). Possible mechanisms contributing to central fatigue include decreased facilitation from muscle spindles, suboptimal facilitation from the motor cortex, desensitisation of the motor neurons, and increased inhibition from the group III and IV afferents (Nordlund et al, 2004).

A reduction in central drive to the muscle may, in fact, act as a safety mechanism, mediated by afferent feedback from the muscle and protecting the muscle metabolic activity from entering into a catastrophic state from which recovery is delayed or even impossible (Davis and Bailey, 1996; St Clair Gibson et al, 2001b; Gandevia, 2001). In addition, central fatigue may also protect vital homeostatic mechanisms such as ventilation, blood pressure and temperature (Gandevia, 2001). Similarly, evidence demonstrating reduced cardiac output and muscle recruitment during exhaustive dynamic exercise at high altitude (Kayser et al, 1994), as well as muscle recruitment regulation in an attempt to prevent progressive myocardial ischemia before muscle rigor development during maximal exercise (Noakes, 1998), may be reflective of the central fatigue model acting as a protective mechanism.

Changes in activation levels in the working muscle (motor unit recruitment and rate coding) and the relative contributions of the central and peripheral components of fatigue can be assessed to some degree by surface or invasive electromyographic (EMG) techniques during submaximal and maximal activity.

1.3.3 Electromyography (EMG)

Electromyography (EMG) is a technique used to measure muscle electrical activity. This technique identifies the recruitment of motor units in skeletal muscle where both the amplitude and frequency of the electrical activity can be measured. The EMG signal offers an understanding on the relationship between muscle force and muscle electrical activity, the manner in which groups of muscles are coactivated around a joint, and neuromuscular adaptations associated with exercise (Kamen and Caldwell, 1996), in addition to information

concerning the histological, anatomical and physiological structure of the muscle (Merletti and Lo Conte, 1997). The electromyogram however, is not a measurement tool without its weaknesses, and interpretation of the EMG signal should be conducted with caution. There are also many techniques used to measure the EMG signal and the strengths and weaknesses of these methods are still being challenged.

1.3.3.1 Methods of Measurement

There have been many different electrophysiological techniques developed over the years in an attempt to study the motor unit properties and its behavior. The measurement clearly needs to be appropriate for the task and therefore, the type of electrode employed depends on the information required and the muscle being studied. The intramuscular and intraneural (wire and needle) electrodes are an invasive technique used to measure activity during static activity from small peripheral muscles or muscle located deep within the body, obtaining information mainly about the recruitment and firing behaviour of single motor units (Moritani et al, 1986). Surface EMG is a technique that provides a non-invasive, objective method of measuring muscle function (superficial muscle activity), and is a widely accepted method of quantifying muscle fatigue (Giannesini et al, 2003; De Luca and Merletti, 1988, De Luca, 1997; Merletti et al, 1991, Merletti and Roy, 1996; Kadaba et al, 1985). We chose surface EMG as the method to assess muscle activation levels in the various studies of this thesis due to its non-invasive nature and because of the dynamic activities used in the exercise protocols.

1.3.3.1.1 Surface EMG – Amplitude and Frequency

The surface EMG is composed of electrical contributions made by the active motor units (MU) obtained from electrodes placed on the skin overlying the muscle, and is represented by two fundamental variables; namely the EMG amplitude and frequency (Farina et al, 2004). These characteristics of the surface

EMG signal depend on the membrane properties of the contributing muscle fibers, their firing patterns, as well as their interdependence (Stegeman et al, 2000). Surface EMG, therefore, reflects both peripheral and central properties of the neuromuscular system and can be used to assess peripheral and central contributions to changes in muscle recruitment and force output during different exercise protocols.

The surface EMG signal, both the amplitude and frequency variables, observed during assessment of muscle function is a realisation of a non-stationary stochastic process (Farina and Merletti, 2000). Any variable of the EMG signal is an estimate with an associated variance and bias which depends on the estimator used and the window length sampled. A feature of the signal may be expressed by a number of different estimators. For example, the root mean square value or the average rectified value are commonly used to describe the amplitude of the EMG signal, and the EMG frequency may be indicated by the mean frequency (MNF) or median frequency (MDF) (Farina and Merletti, 2000). Many processing techniques have been adopted for estimating amplitude and frequency variables of the surface EMG signal, and each approach used has strengths and weaknesses in the description of the EMG signal.

The surface EMG amplitude is related to both the recruitment and firing rates of active motor units (Farina et al, 2004), and has been shown to represent the underlying motor unit activity during sustained muscle activity (Moritani et al, 1986). An increase in EMG amplitude is associated with an increase in force output, reflecting the recruitment of new motor units and an increase in the motor unit firing rate (Viitasalo and Komi, 1977; Bigland-Ritchie, 1984a; Karlsson and Gerdle, 2001; Finsterer, 2001; Suzuki et al, 2002). The EMG amplitude, however, is influenced by factors that include electrode position, thickness of subcutaneous tissues, distribution of motor unit conduction velocity, and the system used to record EMG (Farina et al, 2004), although with awareness and appropriate electrode placement these effects can be reduced (Jensen et al, 1993).

The EMG frequency content is determined by the underlying physiological processes associated with motor unit excitation, and is reflected in changes in motor unit recruitment and rate coding patterns (Moritani et al, 1986; Solomonow et al, 1990; Bernadi et al, 1999). Frequency analysis has also been used to estimate activation of type I and II muscle fibers since muscle fiber diameter and conduction velocities of motor units vary systematically with the type of motor unit. For example, surface EMG measured from a muscle with more type II fibers should have a higher frequency due to their higher conduction velocity compared to a muscle with purely type I fibers (Bilodeau et al, 1990). The EMG frequency has been shown to increase, remain constant, or decrease in the unfatigued state using different protocols (Viitsalo and Komi, 1977; Potvin, 1997), as well as during fatigue development (Moritani and Muro, 1987; Tesch et al, 1990; Masuda et al, 1999). These inconsistent results may be due to the techniques used to record and analyse the frequency content, difference in muscle morphology, gender difference, electrode differences and the thickness of the skinfold underlying the EMG electrode (Karlsson et al, 2003). Therefore, the interpretation of the frequency analysis of the EMG signal during dynamic exercise remains a debatable issue.

1.3.3.2 Factors affecting the EMG Signal

The amplitude and frequency of surface EMG are sensitive to many intrinsic and extrinsic factors (De Luca, 1997). Some of the intrinsic factors which cannot be controlled include muscle fibre type, diameter, the depth and location with respect to electrodes, and the amount of tissue between the electrode and muscle. Extrinsic factors include the location, orientation, and the shape and area of the electrodes and can be influenced by the experimental tester (Mathiassen et al, 1995). Another issue that potentially affects the EMG signal is the manner in which the data is acquired and filtered (Kamen and Caldwell, 1996). To minimise variability and misinterpretation of the EMG signal, during all studies of this thesis, the same individual positioned the electrode at the appropriate anatomical

landmark (vastus lateralis) (Rainoldi et al, 2000; Farina et al, 2002; Rainoldi et al, 2004) and measured EMG, as well as analysed and interpreted the EMG data.

1.3.3.3 Normalisation of the EMG Signal

The characteristics of the surface EMG signal are, as discussed above/previously, sensitive to many factors. To enable the investigator to compare and assess the EMG data collected from different muscles, over time, and between individuals, the EMG needs to be normalised, that is the data needs to be compared to a reference value recorded during standardised and reproducible conditions and then expressed as a percentage of the reference value. The normalising method not only needs to be reliable, but should also be relevant to the exercise task. Researchers examining the different methods of EMG normalisation acknowledge its importance, however numerous studies have reported conflicting results and therefore normalisation methods remain contentious (Yang and Winter, 1984; Mirka 1991; Burden and Bartlett, 1999; Burden et al, 2003; Hunter et al, 2002a).

Earlier studies have used an isometric maximal voluntary contraction (MVC) to normalise EMG, however, it is recognised that the isometric MVC EMG is less reliable than EMG from an isometric submaximal contraction (Yang and Winter, 1983), as an MVC might not represent maximum activation capacity of the muscle. However, the isometric MVC method is the only method that aims to express the percentage of the maximum activation capacity of the task specific muscle (Yang and Winter, 1984). Knuston et al (1994) compared an isometric MVC to dynamic values and showed a higher intra-class correlation coefficient between trials with the isometric MVC, thereby improving the reproducibility of the data. Burden and Bartlett (1999) compared four different normalising methods using the biceps brachii, and concluded that the isometric and isokinetic MVC methods rather than isotonic contractions (dynamic peak and mean methods) should be used to normalise EMG if comparing data between muscles, tasks and individuals. More recently, it was found that the isokinetic MVC method had a

greater intra-individual variability in the EMG measurement and was therefore, less reliable than the isometric MVC method (Burden et al, 2003).

Some studies have further questioned the validity of using the isometric MVC method to normalise EMG for non-isometric tasks (Mirka, 1991; Knudson and Johnston, 1993; Kellis and Baltzopoulos, 1996). However, Hunter et al (2002a) investigated the most effective normalisation method for cycling fatigue protocols and showed that the isometric MVC recorded the highest EMG compared to fixed cycle pedal contractions at knee joint angles of 60° and 108° and a dynamic single maximal revolution of a cycle pedal. These authors therefore concluded that the isometric MVC is an effective normalising method as it represents more muscle recruitment activity (Hunter et al, 2002a).

1.3.3.4 EMG changes during Static and Dynamic Exercise

The characteristics of the EMG signal are affected differently depending on the type of contraction being performed by the measured muscle. The EMG amplitude and frequency has been shown to differ between a static and dynamic fatiguing contraction (Masuda et al, 1999). Much of the earlier EMG research have examined static exercise to evaluate changes in EMG parameters during muscle activity and fatigue, however more recently, dynamic exercise, which is more representative of muscle activity and fatigue development during actual work or exercise, is being explored using EMG techniques. Dynamic contractions, however, introduce additional factors which either differ from or are absent during static contractions, and therefore, the EMG data needs to be interpreted with caution.

Firstly, during dynamic exercise the muscles shift during movement with respect to the skin and electrodes, and therefore, the EMG amplitude can be altered as a result of geometrical artefacts and not only due to changes in muscle activation (Masuda et al, 1999; Rainoldi et al, 2000). Secondly, changes in EMG amplitude and frequency during sustained isometric contractions have largely been

attributed to ischaemia caused by increased intramuscular pressure and thus, an accumulation of metabolites associated with fatigue (Hagberg, 1981; Masuda et al, 1999; Kay et al, 2000), whereas during dynamic exercise, there is enhanced blood flow which facilitates the removal of contraction-inhibiting metabolites. Lastly, there is the issue of stationarity which remains controversial. It is necessary for the EMG signal to be stationary when analysing EMG frequency, however most studies performing spectral analysis of the EMG signal seldom test for it and rather just assume stationarity (Bilodeau et al, 1997). The signal during sustained isometric contractions is thought to be stationary during short time periods (0.5 to 2 seconds), and therefore, spectral analysis of the EMG signal (based on Fourier Transform) may be applied. This method, however, is problematic in cases such as dynamic exercise.

The exercise protocols designed for this thesis include dynamic exercise. Therefore, due to these technical and methodological limitations of spectral analysis during dynamic contractions, this thesis does not detail and report the frequency content of the EMG signal.

1.3.3.5 EMG Signal Changes associated with Fatigue

As described previously, fatigue is associated with decrements in muscular performance and function, and hence, fatigue resistance may be a component of neuromuscular activity changes during exercise. The characteristics of the EMG signal may therefore provide insight into the underlying mechanisms of muscle activity changes associated with fatigue development during exercise. Since both the central nervous system and peripheral muscular sites are involved during sustained muscular activity (Bigland-Ritchie, 1981a), the EMG signal is affected by both nervous and muscular processes of the neuromuscular system. For example, changes in motor command by the central nervous system during sustained muscular activity will alter muscle activation, and therefore, affect the EMG signal (Bigland-Ritchie, 1984a). Similarly, prolonged muscle activity causes changes in metabolite concentrations, which in turn affects the muscle excitation-

contraction coupling, including the muscle membrane properties and action potential propagation, resulting in EMG signal changes (Bigland-Ritchie, 1981a; Moritani et al, 1982; Moritani and Yoshitake, 1998). The EMG parameters during muscle activity are also affected differently, depending on the type of contraction being performed (as described previously), as well as the intensity of the contraction.

1.3.3.5.1 Changes in the EMG signal associated with fatiguing submaximal contractions

The EMG amplitude increases progressively, accompanied by a decrease in EMG frequency during an endurance submaximal contraction held at a constant force or when regularly repeated at the same intensity, (Bigland-Ritchie, 1984a; Moritani et al, 1986; Fuglevand et al, 1993). This increased EMG amplitude has been attributed to the recruitment of additional motor units and increased firing rate of motor units already active in an attempt to maintain force output. It has been suggested that the additional motor unit recruitment compensates for the consequences of decreased contractility of the fatigued motor units. Further possible mechanisms that could contribute to the increased EMG amplitude include increased synchronization of already active motor units, alterations in the electrical characteristics of active motor units, and changing action potential propagation (Suzuki et al, 2002).

Sustained isometric contractions at different force levels, as well as dynamic fatiguing contractions, measured in various muscles, have demonstrated increased EMG amplitude (Potvin, 1997; Conwit et al, 2000; Mullany et al, 2002; Suzuki et al, 2002; Hunter and Enoka, 2003). Furthermore, higher EMG amplitude has been reported during dynamic compared to static knee extension exercise until exhaustion (Masuda et al, 1999).

1.3.3.5.2 Changes in the EMG signal associated with fatiguing maximal contractions

Prior to discussing changes in the EMG signal during a maximal contraction, it is important to note that maximum voluntary force exerted by an individual has been shown to be less than the maximal evocable force or true force (Gandevia, 2001), but rather the maximal effort the individual is willing to produce. In contrast to a sustained submaximal contraction, the EMG amplitude decreases progressively during a sustained maximum voluntary contraction, together with a decrease in EMG frequency (Bigland-Ritchie, 1981b; Bigland-Ritchie and Woods, 1984b; Moritani et al, 1985; Kay et al, 2000; Suzuki et al, 2002; Mullany et al, 2002). The change in EMG amplitude during an MVC is associated with a gradual decline in motor unit excitation rates (Bigland-Ritchie et al, 1983a) and de-recruitment of motor units, which may be a process linked to the central nervous system response to extreme muscle fatigue and ischaemia (Mullany et al, 2002). The extent of the reduced motor unit activation seems to be dependant on the muscle fiber type, with more pronounced reductions in motor unit activity in muscles with higher proportions of type II fibers (Moritani et al, 1985).

Contrary to similar EMG amplitude changes during both static and dynamic sustained submaximal exercise, studies examining maximal voluntary contractions during both types of exercise have produced inconsistent findings. Kay et al (2000) have reported decreased EMG amplitude and force output during a 100 s isometric maximal voluntary contraction (MVC). The authors suggest that this result, although speculative, may either be a response to changes in afferent input due to metabolic perturbations in the peripheral muscles or initiated by a pre-programmed central nervous system activity. However, during 100 s concentric and eccentric maximal voluntary contractions, EMG amplitude was maintained or increased, while force output progressively decreased indicating that neural drive to the peripheral muscle was maintained (Tesch et al, 1990; Kay et al, 2000).

In contrast, Hunter et al (2003a) found no changes in EMG amplitude during a 30 s supramaximal cycling bout (Wingate anaerobic test), despite a significant decrease in the mean power frequency spectrum which was suggested to be a result of an accumulation of metabolites in the periphery. The authors proposed that the duration of time required for feedback from intramuscular metabolism to the central nervous system to occur may be unable to alter the neural recruitment strategy within the 30 s exercise period.

1.3.3.6 EMG changes during Closed Loop and Open Loop Exercise

As described previously, a closed loop exercise model has a defined end point and individuals are aware of the nature of the exercise trial to be performed. Due to the known duration or distance of a self-paced exercise activity, a feedforward and feedback system may induce alterations in the motor unit recruitment in order to changer power output or speed, enabling exercise performance to be regulated in accordance with the time necessary to complete the exercise activity. This "resetting" of power output and speed through a central "programmer", at a subconscious level, may possibly permit the completion of an exercise task without incurring muscle damage or substrate depletion (Ulmer, 1996).

Using a closed loop exercise model, St Clair Gibson et al (2001c) examined neuromuscular activity during a self-paced 100-km time trial, interspersed with high intensity 1-and 4-km sprints, with carbohydrate ingestion and a placebo. Although subjects were instructed to exercise at a maximal effort during the sprints, the results showed that mean power output decreased progressively during the 1-km sprints, which was paralleled with a decrease in IEMG activity in both trials. This finding suggests there was a possible downregulation of efferent signals indicating that the central nervous system reduced force output by decreasing neural drive to the peripheral musculature, despite conscious efforts by the subjects to exercise at a maximal effort which may have been a subconscious protective response.

Furthermore, Kay et al (2001) demonstrated that during a self-paced 60 min closed loop cycling time trial interspersed with six maximal sprints during warm, humid conditions, mean power output and IEMG activity were reduced from the second to the fifth sprint. However, both power output and IEMG activity increased during the sixth sprint which was performed in the last minute of the trial. Again, this result suggests there my have been a downregulation of efferent signals, but also shows the possible existence of a motor unit activation reserve which is subconsciously controlled, thus enabling an increase in muscle activation during the last sprint.

Tucker et al (2004) showed reduced power output and IEMG activity during a closed loop self-paced 20-km time trial in hot compared to cool conditions. It was suggested that this impaired performance was the result of central regulation of skeletal muscle recruitment, thus reducing heat production in the body, and thereby ensuring thermal homeostasis was maintained during exercise in the heat.

Collectively, the findings from all these studies support a centrally controlled neural mechanism during self-paced closed loop exercise. The neural command changes occur irrespective of physiological alterations, or in some instances, physiological changes are anticipated, and skeletal muscle recruitment altered so that exercise can be completed while physiological homeostasis is maintained (Marino, 2004).

An open loop exercise model, as described previously, is an exercise activity with an undefined end point. This type of exercise is a bit more complex from a control perspective than the closed loop exercise. Subjects have shown volitional fatigue, particularly during low-intensity exercise, with no metabolic or physiological explanation (Jones, 1996). Mental functioning such as the ability to concentrate on the exercise task or motivational capacity may also contribute to exercise performance during this type of exercise model (St Clair Gibson et al, 2001a).

In a previous study by Maisetti et al (2002), surface EMG changes over a 30 s isometric contraction at 50% MVC, where subjects knew the duration before they started the exercise bout, were compared to those measured during the first 30 s of a prolonged open loop isometric contraction until exhaustion at 50% MVC. The results showed that the surface EMG parameters changed similarly during both exercise bouts, and the authors concluded that prior knowledge of the duration of isometric exercise does not influence the motor unit recruitment strategy. However, the exercise protocol involved only isometric exercise at 50% MVC, and therefore, future research needs to be done to examine whether the influence of prior knowledge of exercise duration on the muscle recruitment strategy is dependent on the force level, exercise duration, and/or the type of exercise being performed.

1.3.4 Summary

In summary, exercise performance is reliant on the neuromuscular system for control of movement and the ability to generate and sustain force output. Force production is influenced by both neural and muscular processes, and alterations in force production can be attributed to changes in the type of muscle fibers recruited, the number of active motor units and the motor unit firing rate. Therefore, the mechanisms that result in fatigue-induced decrements in exercise performance involve alterations in the peripheral contractile processes and/or a reduction or failure of muscle activation by the central nervous system. However, it remains debatable how the neuromuscular fatigue response may be controlled in the path from the central nervous system to the peripheral contractile mechanism during exercise.

The measurement of skeletal muscle recruitment/activation during exercise can be assessed using electromyography. The EMG signal provides insight into muscle activity and an understanding on the relationship between muscle force production and muscle electrical activity, and neuromuscular adaptations associated with exercise. Analysis of the EMG signal necessitates the calculation

of the amplitude and frequency content of the electromyogram. These characteristics of the surface EMG signal are dependent on the number and membrane properties of the contributing muscle fibers, as well as their firing patterns. The EMG amplitude and frequency are affected differently depending on the type of contraction being performed by the measured muscle and the intensity of the contraction.

Therefore, the measurement of EMG activity during different exercise protocols may offer us some understanding on how skeletal muscle activation is controlled within an integrative regulatory system, in response to changes in physiological systems causing alterations to muscle force production.

1.4 SYMPATHETIC NERVOUS SYSTEM ACTIVITY DURING EXERCISE AND REST

The autonomic nervous system (ANS) is predominately an efferent control system which regulates individual organ function and homeostasis. It sends impulses from the central nervous system (CNS) to peripheral organ systems and operates independently of voluntary control under most conditions. The ANS is composed of the sympathetic and parasympathetic nervous systems. These systems work together and the state of the body at any given time represents a balance between the two systems (Brookes et al, 2000).

The general function of the parasympathetic nervous system (PNS) is to control resting metabolism and this parasympathetic function is regulated by the vagus, or tenth cranial nerve. The "fight or flight" response, also known as the sympatho-adrenal response of the body, is controlled by the sympathetic nervous system (SNS). The SNS is primarily related to processes involving expenditure of energy. This response is mediated directly by impulses that are sent through the SNS and indirectly by the secretion of catecholamines from the adrenal medulla. The most common and abundant catecholamines are epinephrine and norepinephrine. Epinephrine acts as a hormone in the blood circulation and fills a neurotransmitter

role in the CNS. Norepinephrine is also present in the blood but is mainly a neurotransmitter of the peripheral SNS. The adrenal secretion of epinephrine to norepinephrine is 4:1, however, circulating norepinephrine levels are typically fivefold higher than that of epinephrine levels through sympathetic release or "spillover" (Brookes et al, 2000).

The physiological effects of epinephrine and norepinephrine are initiated by their interaction with adrenergic receptors, which are a class of G protein-coupled receptors, on the surface of target cells. These two main groups of adrenergic receptors are referred to as alpha (α) and beta (β) receptors and are situated on visceral effectors (muscles and glands) innervated by most sympathetic postganglionic axons. Both receptors can be further divided into α_1, α_2, β_1 and β_2. Epinephrine binds with both the α and β receptors, whereas norpepinephrine has a higher affinity with the α receptors. The receptors are characterized by the specific responses they produce, for example, the α receptors are known to be excitatory whereas the β receptors are either excitatory or inhibitory (Tortora and Grabowski, 1993).

Previously, the adrenal medulla and the sympathetic nerves have been recognised as a well-defined neuroendocrine unit, however, evidence has described a dissociation of SNS and adrenal medullary responses under certain conditions such as thermogenesis, hypoglycaemia, hypoxia and injury (Young et al, 1984). There may be two patterns of alterations in the sympathoadrenal system that work in a reciprocal manner. Firstly, the adrenal medullary responses to a variety of stimuli are increased when the SNS activity is suppressed. Secondly, the SNS response to certain stimuli is biphasic where it is initially suppressed followed by stimulation. During this initial suppression phase, there is a marked increase in the adrenal medullary secretion. The authors therefore concluded that under SNS suppressed conditions, the physiological contribution of the adrenal medulla is especially important (Young et al, 1984).

Physical exercise stimulates the ANS, decreasing the PSN and enhancing the SNS (Thomas and Segal, 2004). The plasma epinephrine and norepinephrine concentrations are increased with exercise intensity and duration, especially at high-intensity exercise (50 – 60% VO_{2max}), and are primarily derived through feedforward stimulation from motor centers in the brain, and importantly, via afferent impulses from working muscles (Kjaer et al, 1987). The sympathoadrenal activity is important for exercise capacity as it has a dominant effect on blood glucose and carbohydrate metabolism, as well as playing a role in regulating lipolysis (Brookes et al, 2000). Physiological perturbations, such as high-fat feeding and hypoglycaemia, have previously been shown to alter the SNS response during subsequent exercise resulting in increased or suppressed SNS activity, respectively (Jansson et al, 1982; Sasaki et al, 1991; Helge et al, 1996; Davis et al, 2000a).

Furthermore, *in vitro* research has shown that decreased membrane excitability is attenuated by exposing muscle fibers to catecholamines and therefore, catecholamines are important for the expression and/or maintenance of muscle excitability and contractile force generation (Mikkelsen et al, 2005). However, few data exist demonstrating the relationship between catecholamines and muscle force production *in vivo* during dynamic exercise.

Recently, French et al (2006) showed that catecholamine concentrations measured before an acute heavy resistance exercise protocol were increased compared to those in the control protocol which consisted of only rest. The catecholamines continued to increase during exercise while peak and mean force decreased over the course of the exercise protocol. Interestingly, post-hoc analysis showed that the subjects who managed to maintain muscular performance during exercise had greater integrated area under the curve for epinephrine and norepinpehrine than the subjects who reported significant reductions in peak and mean force. The authors concluded that an anticipatory rise in catecholamines existed and that this may be necessary for optimal force production at the onset of exercise. Furthermore, they concluded that the relationship between circulating catecholamines and muscle force may be specific

to the individual (French et al, 2006). Therefore, it appears there is an association between circulating catecholamines and muscle force performance.

Plasma levels of catecholamines are dependent, somewhat, on both relative and absolute exercise intensity, as well as training state or fitness levels (Kjaer and Galbo, 1988; Kjaer, 1989; Kjaer, 1998). The downstream physiological sequelae of the sympathoadrenal response is determined to some extent by tissue responsiveness, populations of adrenergic receptors, and metabolic clearance (Kjaer et al, 1987). Therefore, the interpretation of plasma catecholamine concentrations may be limited to some extent by these mitigating factors.

An alternate technique for characterising sympathetic activation, at least peripherally, is microneurography. This technique evaluates the firing rates in the muscle sympathetic nerve activity (MSNA) and provides insight, for example, on sympathetic control of non-active skeletal muscle blood flow during exercise (Wallin and Fagius, 1988). A number of factors, such as exercise mode, intensity and duration, muscle fatigue, muscle mass, physical training and environment, all influence the direction, pattern and magnitude of the MSNA response to exercise. The MSNA has been shown to increase in response to several types of exercise (Saito et al, 1986; Victor et al, 1987; Carrasco et al, 1999), but the magnitude of increase in MSNA is greater during isometric compared to dynamic muscle contractions of similar duration and percentage of MVC (Saito et al, 1986). It has been demonstrated that the primary mechanism stimulating MSNA is the muscle metaboreflex during isometric exercise involving small muscle groups, as well as during dynamic moderate-intensity exercise (Victor et al, 1987; Carrasco et al, 1999). An association between the MSNA response and the onset and development of fatigue has been reported (Seals and Enoka, 1989), as well as a relationship with the perception of fatigue during sustained isometric contractions (Saito et al, 1989).

Although the SNS plays a contributing role in the regulation of arterial blood pressure and blood flow during exercise to the active muscle, there are limitations to the measurement of MSNA during exercise. Firstly, the measurement site must

remain completely relaxed during recording, and therefore, it is not possible to measure MSNA in contracting muscles. Secondly, discomfort and local soreness have been reported following MSNA experiments. Thirdly, it is more a measure of local sympathetic activity in non-active skeletal muscle than a systemic measure even though MSNA is assumed to be uniform throughout the body. This is supported by Saito and Mano (1991) who demonstrated that the MSNA response to exercise did not exclusively reflect whole body metabolism but was rather related to local metabolic changes.

The release of epinephrine and norepinephrine mediate the sympathetic influence of the heart, and therefore, heart rate variability (HRV) is an additional technique used to assess autonomic influences on the heart (Goldberger, 1999). HRV is the beat to beat alterations in heart rate (R-R interval). The duel contrasting effect of the SNS and PNS on the sinus node is called sympathovagal balance, which may be described by heart rate variability (HRV). Heart rate variability is measured by either a time-domain or spectral method. The more recent and common approach is the power spectral density (PSD) analysis which uses Fourier transforms and provides information of how power (variance) is distributed as a function of frequency (Ori et al, 1992). It contains two components, the low frequency (LF) component which is associated with increased SNS activity, and the high frequency (HF) component which reflects PSN modulation (Pichon et al, 2004). Indices of sympathovagal balance, such as the ratio of the low- (LF) to high-frequency (HF) power or the fractional LF power, are also used as a marker of autonomic interaction (Ori et al, 1992).

There are, however, contrasting views regarding sympathovagal balance and measures of HRV, especially during exercise (Perini et al, 1990). It has been shown that during exhaustive exercise, HRV and the usual indexes of sympathetic activity may not accurately reflect changes in autonomic modulation (Pichon et al, 2004). Furthermore, body position during dynamic exercise has demonstrated an effect on the power spectrum of HRV (Perini et al, 1993). Perini and Veicsteinas (2003) showed opposite trends for LF rhythm in supine and sitting exercises and

suggested that different readjustments may have occurred in relation to different muscular inputs in the two different positions.

Although the physiological correlates of HRV appear to be quite complex, and at times, an impractical measure under certain conditions (such as dynamic exhaustive exercise), HRV may provide an index of the sympathovagal balance with R-R interval being proposed as the most suitable index (Goldberger, 1999).

In summary, sympathoadrenal activity plays a significant role in metabolism and is important for exercise capacity. Although several methods have been used to measure SNS activity, these findings demonstrate a relationship between SNS activity and muscle force, fatigue development and the perception of fatigue during exercise. Physiological perturbations, such as high-fat feeding and hypoglycaemia, have also been shown to alter the SNS response. Therefore, the association between SNS activation and perceived exertion, muscle force and the development of fatigue during exercise may be relevant in response to antecedent exposures and physiological perturbations.

1.5 THE EFFECT OF ANTECEDENT EXPOSURES AND PHYSIOLOGICAL PERTURBATIONS ON THE REGULATORY RESPONSES DURING AN ACUTE BOUT OF EXERCISE

The complex system model of regulatory control proposes that the perception/sensation of fatigue during exercise is the result of many peripheral physiological systems interacting and acting as signallers to the brain (central nervous system) in a dynamic, nonlinear integrative process (St Clair Gibson and Noakes, 2004; Lambert et al, 2005). Exercise performance is therefore continually altered in response to the interaction of these physiological systems, which is regulated by feedforward and feedback control. The brain integrates the peripheral feedback, along with centrally located sensors, thus ensuring exercise

is performed optimally for prevailing conditions without pushing any one of the physiological systems beyond homeostasically acceptable limits.

The continuous interaction between afferent and efferent feedback resets, and thereby, directly or indirectly regulates metabolic and motor activity. However, the interpretation of the afferent feedback may be altered by factors such as the muscle metabolic rate, muscle reserve, training, physiological perturbations and prior or antecedent experiences (Ulmer, 1996).

This thesis attempts to examine the effects of antecedent exposures and physiological perturbations on perceived exertion, muscle activation levels and exercise performance. It is unclear how the different physiological systems change in response to antecedent exposures and physiological perturbations. In order to identify with the terminology, **antecedent** and **perturbation**, we need to clearly define them. Thus, antecedent can be defined as *an event that happens before another* or *prior; preceding in time or order*. Perturbation is defined as *the act of perturbing or the state of being perturbed* or *a cause of disturbance* or *a secondary influence on a system that modifies simple behaviour* (The Collins English Dictionary, 1986). Therefore, within the context of the present thesis, the terms antecedent exposure and physiological perturbation are indicative of prior exposure to an activity / prior experience of an activity, and changes / disturbances in a physiological system or variable, respectively. Specifically, this thesis has utilised antecedent fatiguing exercise, high-fat feeding and hypoglycaemia, as well as increased levels of epinephrine via infusion, during open and closed loop exercise at a self-paced or constant workload. In addition, antecedent MVC and fatiguing exercise during isometric exercise was also used.

1.5.1 Effect of Increased Exogenous Epinephrine Levels on Exercise Performance

Previous studies have attempted to differentiate the role of circulating epinephrine concentrations from that of general sympathoadrenal response in regulating the

metabolic sequelae during exercise of different intensities by perturbating the epinephrine levels. These studies have typically infused epinephrine to levels at least twice the normal range measured during exercise.

Several studies have demonstrated that epinephrine infusion increases the mobilization of fuels, resulting in elevated circulating glucose and lactate concentrations during dynamic exercise (Chesley et al, 1995; Febbraio et al, 1998; Howlett et al, 1999a; Kjaer et al, 2000; Watt et al, 2001; Wendling et al, 1996). These elevated plasma glucose levels associated with epinephrine infusion can be explained by an increase in hepatic glucose output (Howlett et al, 1999a; Howlett et al, 1999b) and a possible attenuation in the exercise-induced increase in peripheral glucose uptake (Howlett et al, 1999b; Watt et al, 2001). Watt et al. (2001) postulated that the reduction in glucose disposal associated with epinephrine infusion might be attributed to an increase in glycogenolysis (Febbraio et al, 1998; Jansson et al, 1986; Spriet et al, 1988; Watt et al, 2001), resulting in an accumulation of glucose-6-phosphate, with the consequent inhibition of hexokinase and glucose phosphorylation (Watt et al, 2001).

However, not all studies have demonstrated an increase in glycogenolysis with epinephrine infusion during moderate (65 % VO_{2max}) {124} and high-intensity exercise (80-85 % VO_{2max}) (Chesley et al, 1995; Kjaer et al, 2000), despite significant increases in glycogen phosphorylase activity (Kjaer et al, 2000). Discrepancy in the findings of these studies may relate to the epinephrine concentrations achieved with infusion, the intensity and duration of the exercise bout, and the subjects' health, training and nutritional status.

Nonetheless, when plasma epinephrine levels are increased above those typically associated with the corresponding exercise intensity, a mismatch between the mobilization and oxidation of fuels has been described. Kjaer et al (2000) found that, in parallel to the increase in glycogen phosphorylase activity stimulated by epinephrine infusion, an increase in skeletal muscle hormone sensitive lipase (HSL) activity was also found. However, there are no studies of which we are aware, that have measured the changes in intramuscular triglyceride (IMTG)

utilization in response to epinephrine infusion. Mora-Rodriguez et al (2000), using isotope techniques, demonstrated a mismatch between mobilization and oxidation of FFA's with epinephrine infusion. They found that whole-body lipolysis increased progressively throughout exercise with graded epinephrine infusions (0.96-3.44 nmol/l) during low-intensity (25% VO_{2max}) exercise, resulting in an increase in plasma FFA concentrations, with evidence of subsequent reesterification.

Kjaer et al (2000) postulated that epinephrine secretion during exercise is not entirely dependent on the metabolic demand of the muscle, but is the result of and acts as part of a feedforward control mechanism, mobilizing both intra- and extra-muscular fuel stores. Conversely, muscle activation levels and work rate in response to increasing exercise intensity, and the associated increase in plasma epinephrine concentrations with SNS activation, results in the mobilization of fuel stores to match oxidation (Mora-Rodriguez et al, 2000). However, to our knowledge, no previous studies have examined the effects of epinephrine infusion on muscle activation levels measured by EMG activity during a single bout of dynamic exercise.

Several previous studies have also reported significant increases in heart rate with epinephrine infusion (Febbraio et al, 1998; Kreisman et al, 2000; Watt and Hargreaves, 2002) during higher-intensity exercise ranging from 50 to 70% of VO_{2max} However, not all studies have showed that epinephrine infusion is associated with increased heart rate (Womack et al, 1998; Kreisman et al, 2003), but this may be attributed to the infused levels of epinephrine and/or the exercise intensity.

Ratings of perceived exertion have been related to plasma epinephrine concentrations, blood glucose and lactate concentrations, ventilation and heart rate during exercise (Mihevic, 1981). However, Womack et al (1998) examined the effect of epinephrine infusion on perceived exertion during a 20 min constant workload exercise test. The results showed that epinephrine infusion failed to increase RPE despite significant increases in epinephrine concentrations, blood glucose and lactat concentrations, and ventilation, and therefore, the authors

suggest that RPE is not causally related to changes in plasma epinephrine changes during exercise.

Therefore, epinephrine infusion has been associated with changes in metabolic activity and heart rate, however, the effects of increased epinephrine concentrations via infusion, on perceived exertion, muscle activation levels and overall performance during prolonged exercise are less well known and requires further investigation.

1.5.2 The Effect of Antecedent Fatiguing Exercise on Exercise Performance

It is well know that during an exercise bout, the development of fatigue results in an attenuation of exercise performance associated with substrate and metabolic alterations, as well as changes in neural drive or motor command to the muscle. Models that have been used experimentally to examine the effect of antecedent exercise on metabolism, physiological changes and the mechanism of fatigue are sequential muscle groups and/or single-muscle group exercise models. Examples include alternate arm and leg exercise (Karlsson et al, 1975) and single limb exercise (Saltin et al, 1976; Sargeant and Davies, 1977; Neary and Wenger, 1986; Ray, 1999; Ogita et al, 2000). These models are usually used to examine metabolic processes in an inactive muscle group during exercise with the other active muscle group (Ahlborg et al, 1975, Ahlborg, 1985; McDermott et al, 1987; McDermott et al, 1991; Megeney et al, 1992; Ray, 1993). With regard to the mechanism of fatigue, metabolic and physiological changes induced by fatiguing exercise in the first active muscle group can be examined as potential contributing factors for fatigue in the second muscle group.

Neary and Wenger (1986) compared the effct of one- and two-leg exercise on the lactate (LT) and ventilation (VT) threshold during submaximal and maximal exercise. They showed that oxygen consumption and ventilation ($l.min^{-1}$) were significantly higher at any given power output during one-leg exercise, which indicates reduced mechanical efficiency in one-leg compared to two-leg exercise.

However, no differences were found in VO_2 and V_E at both LT and VT between the two exercise protocols. Lactate concentrations were significantly different at LT between one- and two-leg exercise. The results suggest that it is VO_2 rather than muscle mass that may be the stimulus for the VT and LT. The VO_{2peak} for the one-leg exercise was ~ 80% of the two-leg value which implies that central circulation rather than peripheral muscle limits VO_{2peak} during two-leg exercise.

Ogita et al (2000) used two types of one- and two-leg exercise protocols (cycling and knee extension) to determine the key factors causing a differential VO_2 response between one- and two-leg exercise at submaximal intensities, and to establish whether VO_{2peak} increases in proportion to increases in active muscle mass. At submaximal intensities, the VO_2 responses were significantly higher in one-leg compared to two-leg exercise regardless of the exercise type. The VO_{2peak} values for one-leg exercise were 75-85% of the two-leg exercise values, which may be the result of a different circulatory response caused by the difference in limb(s) performing the exercise. These results suggest that the differential VO_2 response between one- and two-leg exercise may not only be due to force application differences throughout the exercise movement and a postural component effect but also attributed to the inhibited circulatory response as a result of multiple limb exercise. Furthermore, it was proposed that VO_{2peak} does not increase in proportion to the exercising muscle mass even during smaller muscle activity where cardiac pumping capacity has not been reached.

Klausen et al (1982) investigated one- and two-leg submaximal and maximal exercise before and after an 8-week one-leg training program with each leg. The measured variables included oxygen uptake, heart rate, mean arterial blood pressure, cardiac output, leg blood flow and iliac arteriovenous differences for oxygen and lactate. The results showed that the one-leg training response caused differential circulatory adaptations during one-leg and two-leg exercise.

Research has shown that a degree of glycogenolysis occurs in the non-exercising muscle during both exercise and after an exercise bout (Ahlborg et al, 1975; Ahlborg, 1985; McDermott et al, 1991). Ahlborg et al (1975) studied metabolic

alterations and EMG activity in the non-exercising leg during one-leg (40 min at 105W) and one-arm exercise (20 min at 65W). During the one-leg exercise bout, there was enhanced blood flow, a five fold increase in oxygen uptake, significant increases in glucose and lactate uptake, as well as a rise in EMG activity in the non-exercising leg supported by a sling at an angle of ~ 135° to the trunk. During the arm exercise, leg blood flow and oxygen uptake increased and a significant uptake of lactate occurred in the legs, despite minimal thigh muscle EMG activity. The authors concluded that substrate utilisation is altered towards a greater uptake of blood-borne carbohydrates substrates, particularly lactate, in the non-exercising muscle. Similarly, Ahlborg (1985) demonstrated increased glycogenolysis in non-exercising muscle during prolonged exercise which persisted during recovery. In a study by McDermott et al (1991), rat hindlimb muscles were examined during exercise on a treadmill. The study compared the muscles when the rats were exercising on all four limbs or whilst the hindlimbs were suspended above the treadmill. The results showed enhanced glycogen metabolism in the non-exercising muscle, and that the glycogen utilisation was not associated with increased energy demands/contractile activity of the muscle.

The enhanced glycogenolysis in the inactive muscle described in the studies above has been attributed to adrenal stimulation or sympathetic activation (Ahlborg, 1985; McDermott et al, 1987). McDermott et al (1987) studied rats, that were either adrenalectomized or had intact adrenals whilst performing forelimb exercise, and showed that there was no glycogen loss in the absence of epinephrine in the non-exercised muscles. The group who had increased epinephrine levels of sixfold above basal displayed significant glycogen depletion in all non-exercising muscles. The authors concluded that glycogenolysis in non-exercising muscle was probably a result of the epinephrine effects and was not dependent on muscle contractile activity.

Therefore, the role of catecholamines in metabolic mobilisation in these studies provides evidence for an association with central regulation of metabolic activity in the inactive musce/limb. This link is further highlighted when examining adaptations to single-limb training. Rube and Secher (1991) examined strength

and fatigue development during repeated one- and two-leg isometric MVC's before and after a 5-week leg training program with either the one- or two-leg exercise task. The training program increased one- and two-leg strength and reduced fatigue development. However, this fatigue effect was specific to the training exercise task such that the two-leg training decreased fatigue only during the two-leg MVC's and the one-leg training only during one-leg MVC's, despite both legs being trained This suggests a difference in the motor command strategy in response to the one-leg training to that of the two-leg training. Ray (1999) studied the effect of a 6-week single-leg exercise training program (40 min/day, 4 days/week) on the sympathetic nervous system response at rest and during single-leg exercise. They found that the resting muscle sympathetic nervous activity (MSNA) was not altered by endurance training, but that increases in MSNA during dynamic single-leg exercise in the non-exercised limb were attenuated. This supressed MSNA after training may be related to an attenuation of the muscle metaboreflex that subsequently prevents the sympathoinhibitory effect of the cardiopulmonary baroreflex from being overridden.

Although single-muscle/limb exercise models have been used to examine non-exercising muscle metabolism and substrate utilisation, physiological responses to single-limb exercise, as well as training responses and adaptations to single limb training, the effect of previous single-limb exercise on subsequent exercise performance capacity has not been sufficiently studied. A previous study by Klausen et al (1972) suggested that increased lactate concentrations from prior activity of other muscles resulted in decreased performance capacity of previously inactive muscle. Subsequently, Karlsson et al (1975) studied the effect of previous exhaustive exercise on the ability of previously inactive muscles to perform exercise, using alternate arm and leg exercise. Exhaustive exercise resulted in significant increases in lactate and decreases in ATP and CP in the first exercised muscle, and similar changes, especially in lactate concentrations, in the non-exercised muscle. Performance time was decreased in the second exercise bout compared to the initial exercise bout. It was concluded that the decreased performance capacity of the previously inactive muscle was associated with

metabolic changes (increased blood lactate and / or H^+ concentrations) caused by the muscle groups that were initially exercised to exhaustion.

Taken collectively, these studies show that there is increased hormonal and metabolic activity in the inactive limb, overall circulatory adaptations and a specific role for neural control in training for this type of single-limb submaximal exercise. EMG activity has been measured in previous studies in an attempt to quantify muscle activation in the non-active muscle, however, muscle activation in the exercising limb has not been examined. Antecedent fatiguing single-limb exercise, in addition to the humoral changes, may be associated with an altered central command, and therefore, investigating and comparing muscle activation levels between exercising limbs consecutively, may provide insight into neuromuscular strategies when exposed to a system perturbation. To our knowledge, there are no studies that have examined perceived exertion, muscle activation and performance capacity of the previously inactive limb using a single-limb exercise model to exhaustion in a sequential manner where prior exercise has caused a general humoral effect in the absence of active mechanical work in the inactive limb.

1.5.3 The Effect of Antecedent Dietary Manipulation on Exercise Performance

Research has been conducted examining the ability of fuel substrates to enhance exercise performance and attenuate fatigue development (Ivy, 1999). Various nutritional strategies have focused on increasing CHO reserves prior to exercise and/or maintaining or sparing CHO for oxidation during exercise. Much of the research has persistently investigated CHO loading as the optimal dietary strategy for endurance capacity, however, fat loading has previously been named "the next magic bullet" in optimizing performance during endurance sporting activities (Sherman and Leenders, 1995).

Both short and long term antecedent fat adaptation have been shown to induce a significantly higher rate of fat oxidation during exercise, and therefore, CHO oxidation is reduced. In addition, muscle glycogen concentrations are at best maintained, and in many cases, are lower with a fat-rich diet when compared to a high CHO diet (Helge et al, 1998; Phinney et al, 1983). This fat-diet-induced CHO sparing may, however, not be sufficient to compensate for the lower glycogen storage, and therefore, may affect exercise capacity negatively. Ingestion of a short term high-fat (1-3 days) is associated with reduced endurance capacity and performance during prolonged exercise, which may be attributed to a combination of early depletion of the lowered muscle glycogen stores and the absence of an increase in fat utilization to counteract the reduced available CHO fuel (Starling et al, 1997; Pitsiladis and Maughan, 1999; Burke and Hawley, 2002). In contrast, there is evidence that exposure to a high-fat diet over a longer duration (>7 days) results in metabolic adaptations which increase fat oxidation and may compensate for the reduction in available CHO (O'Keeffe et al, 1989; Lambert et al, 1994; Goedecke et al, 1999).

There is, however, inconsistent evidence supporting long duration antecedent high-fat feeding as a performance benefit. Phinney et al (1983) examined exercise performance to exhaustion during moderate-intensity exercise before and after a 4-week adaptation to a high-fat diet. The results showed that the group performance was skewed by one subject who displayed a significant performance improvement, although, little or no improvements were seen in the remaining subjects. However, in a study by Lambert and colleagues (1994), endurance exercise capacity, which was preceded by two separate high intensity exercise bouts, was increased during moderate-intensity exercise to exhaustion after 14 day adaptation to an antecedent high-fat diet compared to a high-CHO diet.

Other studies have found that antecedent fat adaptation fails to alter or essentially attenuates exercise capacity. O'Keeffe et al (1989) demonstrated a significant decrease in exercise performance at a submaximal exercise intensity in moderately trained female cyclists after adaptation to a 7 day antecedent high-fat diet. Using a rowing exercise model, Simonsen et al (1991) showed impaired

mean power output during three time trials after a 28 day adaptation to an antecedent high-fat diet. More recently, Goedecke et al (1999) examined metabolic adaptations in well trained cyclists after a 15 day antecedent high fat dietary treatment and found no performance benefits during a 40-km time trial, although ingestion of an antecedent high-fat diet significantly altered substrate utilization after just 5 days.

Another antecedent dietary strategy which has been studied, termed "dietary periodisation", involves 5-6 days of fat adaptation followed by 1 day of CHO restoration prior to an exercise event. This strategy is aimed at sparing muscle glycogen by enhancing fat oxidation during exercise, without compromising muscle glycogen stores prior to exercise (Hawley et al, 1998). Studies investigating this dietary treatment have demonstrated increased fat oxidation at rest and during exercise, increased muscle glycogen stores and reduced muscle glycogen utilization during exercise (Burke et al, 2000; Burke et al, 2002; Carey et al, 2001). The effects of this dietary strategy, however, have only been examined during time-trial performances (~25 min to 1hr) following prolonged submaximal steady-state exercise and has resulted in no overall improvements in exercise performance (Burke et al, 2000; Burke et al, 2002; Carey et al, 2001). While this particular dietary treatment optimizes muscle glycogen stores (predominant fuel for high-intensity exercise) and has a muscle glycogen sparing effect, it seems an ideal method to investigate endurance exercise, which includes high-intensity bouts (>85% VO_{2peak}).

However, there may be numerous factors, other than muscle glycogen content, that influence overall exercise performance following an antecedent high-fat diet. Studies have shown that ingestion of an antecedent high-fat diet (5-7 days) is associated with increased sympathetic activation during subsequent exercise (Jansson et al, 1982; Sasaki et al, 1991). Furthermore, Helge et al (1996) showed that long-term fat adaptation (7-wk dietary period) also induced an increased sympathetic activation, accompanied by an increase in the perception of effort and a reduction in exercise capacity (unpublished data, described in Helge, 2002), which may possibly be attributed to the increased sympathetic activation.

Whilst ingestion of a high-fat diet may result in an increased training capacity, studies have shown the cost of a high-fat diet to be an increased perception of effort. In a study by Stepto et al (2002), elite trained endurance athletes consumed either a 3 day high-CHO or high-fat diet in a randomised crossover design. On days 1 and 4 of each dietary treatment, two controlled high-intensity exercise sessions were completed. The authors concluded that the athletes were able to perform the intense interval exercise training, however, on day 4 the high-intensity exercise (~85% VO_{2peak}) was associated with increased RPE (16.0 ± 1.3 vs 13.8 ± 1.8) following exposure to a high-fat compared to a high-CHO diet. Helge et al. (1996) examined RPE during submaximal exercise to exhaustion before and after 7-wk dietary adaptation. The results showed no difference in RPE during the initial test, whereas after 7 weeks, there was increased RPE with the high-fat diet compared to the high-CHO diet during the exercise test, but interestingly, no difference at exhaustion (unpublished data, described in Helge, 2002).

Although the mechanism for the increased perceived exertion associated with antecedent high-fat feeding remains unclear, it is possible that an altered autonomic nervous system activity may play a role. Therefore, the relationship between the increase in sympathetic activation and RPE, muscle activation and performance during exercise with antecedent fat adaptation requires further investigation.

1.5.4 The Effect of Antecedent Hypoglycaemia on Exercise Performance

As described previously, CHO metabolism during exercise has been well studied in both the medical and scientific domain, specifically its association with exercise performance and fatigue development during exercise of long duration (Coyle et al, 1986; Brewer et al, 1988; el Sayed et al, 1997; Ivy, 1999). However, the importance of maintaining euglycaemia and the mechanisms by which performance is improved by fuel substrate perturbations are unclear.

In view of this, the effects of altering blood glucose concentrations on exercise performance are contradictory and remain under debate. Previous studies have shown that maintaining blood glucose concentrations either enhances performance or prolongs exercise time to fatigue (Coggan and Coyle, 1987; Bosch et al, 1993; Febbraio et al, 2000). In contrast, others have reported that the prevention, or reversal of hypoglycaemia (plasma glucose <2.5 mmol/L) during exercise failed to improve endurance capacity (Felig et al, 1982; Jentjens et al, 2003).

More recently, in a study done in our laboratory, perturbation of glucose levels via glucose infusion was shown to improve the endurance capacity of CHO-depleted subjects compared to a placebo infusion by ~ 28% (Claassen et al, 2005). This improvement in endurance capacity was, however, highly variable among subjects, who demonstrated either large or no improvements with glucose infusion. Furthermore, there was an inverse relationship between exercise duration and blood glucose concentrations at exhaustion during the placebo infusion trial, where subjects who became the most hypoglycaemic, exercised for the longest duration. These results suggest that the effects of hypoglycaemia during prolonged exercise vary among individuals.

The individual's ability to recognise symptoms of hypoglycaemia (~ 3mmol/L) are reduced as a result of recurrent episodes of hypoglycaemia (Bolli, 1999). This phenomenon has been termed "hypoglycaemia unawareness" and is commonly reported in type I diabetics (Davis et al, 1992), as well as being described in

normal healthy individuals (Heller and Cryer, 1991). These antecedent periods of hypoglycaemia have also been shown to reduce or blunt subsequent counter-regulatory responses to hypoglycaemia (Heller and Cryer, 1991; Davis et al, 1997). Several other antecedent stressors, sufficient to result in raised glucocorticoid concentrations, have been shown to have the ability to blunt neuroendocrine and autonomic nervous system responses during subsequent exposures (Davis et al, 1996; Komesaroff and Funder, 1994; Kvetnansky et al, 1993). Since hypoglycaemia and exercise can both result in elevations in glucocorticoid concentrations and elicit similar neuroendocrine and autonomic nervous system counter-regulatory responses, it is possible that both these forms of stress may similarly blunt their respective counter-regulatory responses (Galassetti et al, 2001a).

In a study by Davis et al (2000a), antecedent hypoglycaemia resulted in significant blunting of essential neuroendocrine and metabolic responses during a subsequent (next day) bout of steady-state exercise in healthy individuals. However, euglycaemia was maintained during the exercise trial via an exogenous glucose infusion. Subjects during the antecedent hypoglycaemic trial required a 10-fold higher rate of glucose infusion compared to the antecedent euglycaemic trial to maintain euglycaemia, and therefore, the results should be interpreted with caution. Subsequently, Galassetti et al (2001a) showed that two bouts of antecedent prolonged submaximal exercise under euglycaemic conditions resulted in blunted neuroendocrine and metabolic counter-regulatory responses to subsequent hypoglycaemia in normal healthy subjects. Glucose was again infused to maintain euglycaemia during the exercise bouts, which makes it difficult to interpret the blunted counter-regulatory response.

There has been much focus on the regulation of CHO metabolism to attenuate the fatigue process, however, muscle glycogen depletion does not exclusively contribute to fatigue during prolonged exercise (Fitts, 1994). It is possible that changes in neural drive to the muscle may also contribute to reduced exercise performance and fatigue development. During a closed-loop exercise model, Bangsbo et al (1992) demonstrated that deceased power output was not highly

correlated with any of the measured metabolic changes and suggested that other factors such as neural control mechanisms may be associated with fatigue during prolonged closed-loop exercise. St Clair Gibson et al (2001c) examined the effect of CHO manipulation on neuromuscular activity and force production during prolonged self-paced cycling and found that changes in neuromuscular activity preceded any metabolic changes during two 100-km TT despite dietary carbohydrate manipulation.

Nybo (2003) investigated the effect of prolonged cycling exercise with or without glucose supplementation on skeletal muscle CNS activation during a subsequent sustained MVC. The results show that exercise-induced hypoglycaemia (placebo trial without glucose supplementation) reduced average force production during the sustained maximal muscle contraction, which was associated with decreased CNS activation. The author concluded that hypoglycaemia may impair the ability to sustain a high neural drive to the muscles, however, this central fatigue is effectively counteracted when blood glucose homeostasis is maintained. In a study by Nikolopolous et al (2004), the ingestion of CHO during steady-state cycling to exhaustion significantly attenuated the increased in surface EMG activity, and the authors attributed this result to changes in afferent sensory input rather than altered CHO availability.

It may also be beneficial in understanding the integrative system and fatigue development more fully, to quantify the subjective sensations associated with exertion and carbohydrate availability. Carbohydrate ingestion has also been shown to lower overall RPE during prolonged running (Utter et al, 2004), as well as general overall and leg RPE during prolonged cycling (Kang et al, 1996). Kang et al (1996) suggested that carbohydrate availability during endurance exercise may be a sensory cue, however other physiological factors may also influence perceptual intensity at exhaustion.

Therefore, it is unclear how the altered counter-regulatory responses to antecedent hypoglycaemia may affect the integration of central and peripheral control mechanisms of fatigue. In addition, the interaction between the conscious

perception of fatigue, hypoglycaemia and performance is unknown, and to what it extent it varies between individuals. We are no aware of any previous studies that have investigated the effect of reduced neuroendocrine and metabolic responses after antecedent exposure to a physiological stress (eg. hypoglycaemia or exercise (Davis et al, 2000a; Galassetti et al, 2001a)) on overall exercise performance, neuromuscular activity such as muscle activation levels, and effort perception in well-trained healthy individuals.

1.5.5 Summary

In summary, antecedent exposures and physiological perturbations have demonstrated altered metabolic activity and physiological system changes, which illustrates the complexity of the integrative control system during exercise. What remains to be determined is whether a coherent model can be developed in which this integrated control can be linked to effort perception and resistance to fatigue.

Studies examining the effects of increased levels of epinephrine during exercise of different intensities have reported increases in the mobilization of fuels, resulting in elevated circulating glucose and lactate concentrations, variable changes in metabolic activity and increases in heart rate. However, the humoral effects of elevated levels of epinephrine, from the exercise response itself, and its association with perceived exertion, muscle activation levels and overall performance during prolonged exercise is less well known.

Previous studies examining single-muscle/limb exercise has showed increased hormonal and metabolic activity in the inactive limb, overall circulatory adaptations and a specific role for neural control in training for this type of single-limb submaximal exercise. Muscle groups performed in a sequential manner have demonstrated reduced performance capacity in the previously rested muscle as a result of increased metabolic activity elicited during the first exercised muscle group. However, the effects of antecedent fatiguing exercise on perceived exertion, muscle activation levels and performance capacity on the previously

inactive limb, using a single-limb exercise model to exhaustion in a sequential manner, where prior exercise has caused a general humoral effect in the absence of active mechanical work in the inactive limb requires further investigation.

Ingestion of an antecedent short term high-fat increases fat oxidation but has been associated with reduced endurance capacity and performance during prolonged exercise, however, there is contrasting evidence with regard to long duration antecedent high-fat feeding and exercise capacity and performance. An additional dietary strategy, "dietary periodisation", has demonstrated no overall improvements in exercise performance. However, this dietary strategy has only been examined during time-trial performances (~25 min to 1hr) following prolonged submaximal steady-state exercise, and seems an ideal method to investigate endurance exercise, which includes high-intensity bouts, due to its optimization of muscle glycogen stores and muscle glycogen sparing effect. Previous research has also shown that ingestion of an antecedent high-fat diet (5-7 days) is associated with increased sympathetic activation and RPE during subsequent exercise. The mechanism for the increased RPE remains unclear, but it is possible that an altered autonomic nervous system activity may play a role. Thus, the relationship between an increase in sympathetic activation and perception of effort, muscle activation and performance during exercise with antecedent fat adaptation requires further investigation.

There is contrasting evidence as to how fatigue development/exercise performance during prolonged exercise is affected by decreased CHO availability and the associated neuro-hormonal response. Antecedent periods of hypoglycaemia have been shown to reduce or blunt subsequent counter-regulatory responses to hypoglycaemia or exercise, however, the effects of hypoglycaemia are variable among individuals. Therefore, the effects of antecedent hypoglycaemia and the associated counter-regulatory responses on the integration of central and peripheral control mechanisms of fatigue remain unclear. Furthermore, the interaction between the conscious perception of fatigue, hypoglycaemia and performance is unknown, and to what it extent it varies between individuals. To our knowledge, no previous studies have investigated the

effect of reduced neuroendocrine and metabolic responses after antecedent exposure to a physiological stress (eg. hypoglycaemia or exercise (Davis et al, 2000; Galassetti et al, 2001)) on overall exercise performance, muscle activation and perceived exertion during subsequent exercise in well-trained healthy individuals.

Therefore, further work is needed to investigate how the integrative regulatory system governing exercise performance is altered in response to antecedent exposures and physiological perturbations such as increased epinephrine concentrations, prior fatigue, dietary manipulation and hypoglycaemia. In addition, the effects of these exposures and perturbations on the relationship between perceived exertion, muscle activation levels and exercise performance requires further examination.

AIMS AND OBJECTIVES

An integrative model, such as Borg's effort continua, represents the relationship between the physiological demands of exercise performance and the perception of effort associated with that performance. The model proposes that the variables in the perceptual, the performance, and the physiological continuum are not linearly related but the contribution of each continuum to fatigue complements each other and may be 'weighted' differently in each individual for a given exercise task. Another integrative exercise model, based on Ulmer's theory of teleoanticipation, centers on a feedforward/feedback system that integrates both afferent and efferent information during exercise, as well as antecedent experiences, training, metabolic reserves and actual metabolic rates, and the time necessary to complete the exercise task. A "central programmer" incorporates this feedback and thus exercise performance is regulated as a result of changes in the metabolic milieu and efferent motor command. Therefore, it appears there is a complex interplay between afferent and efferent processes in regulating exercise performance where all physiological systems interact continuously to maintain homeostasis and prevent bodily harm.

Based on the findings and previous work described in the Literature Review, the aim of this thesis was to examine the effect of various antecedent exposures and physiological perturbations specifically designed to challenge components of the integrative regulatory system during exercise, particularly on the 'sensation of fatigue' (perception of effort), muscle activation levels (EMG activity) and exercise performance during both open and closed loop exercise at either a self-paced or constant workload. The specific objectives of this thesis were to:

1. Determine whether antecedent fatiguing exercise during sequential single-leg cycling (Leg 1) alters perceived exertion and muscle activation levels thus influencing exercise performance in the previously rested leg (Leg 2) during an open-loop exercise protocol to exhaustion.

2. Evaluate the humoral role of raised epinephrine concentrations, similar to those found during moderate-intensity exercise, on muscle activation levels, and perceived exertion, as well as circulating substrate availability and intramuscular substrate utilization, during prolonged, low-intensity exercise.

3. Examine the effect of antecedent high-fat feeding, followed by 1 day of CHO-loading, on heart rate variability as a proxy measure of sympathetic activation, perceived exertion, muscle activation levels and performance during a 100-km cycling time-trial including high-intensity sprints that simulates actual race conditions.

4. Determine the effect of antecedent hypoglycaemia on perceived exertion, muscle activation levels and performance during a subsequent (next day) bout of steady-state and self-paced exercise.

5. Investigate, using a magnitude production model, the perceptual response during submaximal isometric contractions prior to the performance of an MVC as a perceptual anchor and the effect of antecedent fatiguing isometric exercise on the subsequent perceptual response during isometric contractions.

We hypothesized that the antecedent exposures and physiological perturbations would alter the perceptual and physiological milieu and, as a result, impact on perceived exertion, muscle activation levels and overall exercise performance. A further hypothesis tested was that the variables in the perceptual, the physiological and the performance continuum of Borg's effort continua would not be linearly related but rather "weighted" differently during each specific exercise protocol.

CHAPTER 2

THE EFFECT OF ANTECEDENT FATIGUING EXERCISE ON PERCEIVED EXERTION, MUSCLE ACTIVATION AND PERFORMANCE DURING SINGLE-LIMB EXERCISE

2.1 INTRODUCTION

As described in Chapter 1 of the present thesis, a number of studies have attempted to identify the mechanism affecting exercise performance/fatigue development during prolonged exercise. However, no single mechanism has been established. Rather, evidence suggests that multiple factors may be involved (Fitts, 1994). These factors, all contributing to fatigue, comprise of perceptual, physiological and performance variables and may not be linearly related to one another but rather complement each other during exercise (Borg, 1977). In spite of this, reduced substrate availability, particularly CHO during exercise, and the development of hypoglycaemia and/or muscle glycogen depletion, has commonly been linked to fatigue development during prolonged exercise (Ivy, 1999).

Models that have been used experimentally to examine metabolism, physiological changes and the mechanism of fatigue are sequential muscle group and single-muscle group exercise models. Examples include alternate arm and leg exercise (Karlsson et al, 1975) and single-limb exercise (Saltin et al, 1976; Sargeant and Davies, 1977; Neary and Wenger, 1986; Ray, 1999; Ogita et al, 2000). These models are usually used to examine metabolic processes in an inactive muscle group during exercise with the other active muscle group (Ahlborg et al, 1975, Ahlborg, 1985; McDermott et al, 1987; McDermott et al, 1991; Megeney et al, 1992; Ray, 1993). With regard to the mechanism of fatigue, antecedent metabolic and physiological changes induced by fatiguing exercise in the first active muscle group can be examined as potential contributing factors for fatigue in the second muscle group.

Karlsson et al (1975) examined the effect of antecedent exhaustive exercise on the ability of previously rested muscles to perform exercise, using a sequential arm-leg exercise model. Performance time was reduced in the second exercise bout and the authors suggested that this may be the result of metabolic changes (increased blood lactate and/or H^+ concentrations) elicited during previous exercise to exhaustion of other muscle groups. Recently, research from our

laboratory (Bosch et al, unpublished observations) used a sequential single-limb exercise protocol to examine the relationship between muscle glycogen depletion and exercise performance of each limb. The results demonstrated that time to fatigue was significantly reduced in the second leg (173 ± 70 vs 56 ± 35 min) (n=8) when the non-exercising leg was passively rotated, despite similar muscle glycogen levels at exhaustion.

Therefore, changes in metabolism do not readily explain fatigue development during prolonged exercise, and other potential mechanisms must be considered. One possible mechanism that may contribute to fatigue development is neural control processes, since they regulate muscle contractions, thereby altering force production and potentially preventing any of the peripheral systems from reaching maximal capacity.

As discussed in Chapter 1 of the present thesis, we are unaware of any previous studies that have investigated the effects of sequential single-limb exercise to exhaustion on perceived exertion, muscle activation levels and performance in the previously rested second limb within a single exercise session.

Therefore, the aim of this study was to determine whether antecedent fatiguing exercise during sequential single-leg cycling (Leg 1) alters perceived exertion and muscle activation levels and as a result influences performance in the previously rested leg (Leg 2) during an open-loop exercise protocol to exhaustion. We hypothesized that the antecedent fatiguing exercise of the first limb would alter the metabolic milieu of the entire system and therefore, increase perceived exertion and muscle activation levels in the previously rested second limb resulting in reduced exercise capacity. The single-limb exercise protocol used in the present study was similar to that of Bosch et al. (unpublished observations). However, in the present study, data regarding muscle activation levels was collected and the non-exercising limb was rested and not passively rotated during the alternate limb's exercise bout.

2.2 METHODS

2.2.1 Subject Selection

Seven healthy males were recruited to participate in the study. The study was approved by the Ethics and Research Committee of the Faculty of Health Sciences of the University of Cape Town. All subjects provided informed consent prior to participation. The data collected for this study formed part of a larger study, and therefore, the muscle glycogen content, plasma glucose and lactate concentrations, and FFA's are reported elsewhere (PhD thesis, Ms Amanda Claassen, 2005), and will not be reported in this thesis. The EMG data and its relationship with perceived exertion and exercise performance, however, remains unique to this thesis.

All subjects were between 18 and 30 years of age and were physically active, exercising at least three times a week. Exclusion criteria included any history or current signs of knee pathology that would influence exercise performance or be negatively affected by the exercise trial.

2.2.2 Anthropometry

The subjects' mass and height were recorded and the sum of seven skinfolds (biceps, triceps, subscapular, supra-iliac, abdominal, thigh and calf) was measured. Using these skinfold measurements, percentage fat was estimated using the equations of Durnin and Wormersley (1974).

2.2.3 VO_{2peak} and Peak Power Output Test

Maximal oxygen uptake ($VO_{2\ peak}$) and peak power output (PPO) of each subject were determined during an incremental test to exhaustion on a recumbent bicycle. Exercise was started at a workload of 100 W and increased by 25 W every 150 s

until the subject was exhausted. Exhaustion was defined as a >10% reduction in pedaling frequency, or an RER of >1.10, or both. PPO was defined as the highest workload the subject completed in W, plus the fraction of time spent in the final workload multiplied by 25 W. During the incremental exercise test, oxygen uptake (VO_2), CO_2 production (VCO_2) and ventilation volume (V_E) were measured over 30 s intervals by use of a breath-by-breath Oxycon Alpha Analyzer (Jaeger-Mijnhart, Wuerzburg, Netherlands). Each subject's PPO was used to determine the workrate utilised in the experimental trial, since each subject exercised at an intensity corresponding to 30% of their PPO.

2.2.4 Experimental Protocol

The day prior to each experimental trial, the subjects refrained from any strenuous exercise. On the day of the trial, subjects arrived at the laboratory after an overnight fast (10-12 hr).

2.2.4.1 Single Leg Protocol

The subject was seated on a recumbent stationary bicycle and the saddle adjusted to a position chosen by each subject as the most comfortable for his cycling performance. The subjects' right or left leg was randomly chosen to start the exercise trial. The foot of the active leg was secured to the pedal using inelastic adhesive tape to reduce any shifting from the pedal. The inactive (rested) leg was placed on a step adjacent to the recumbent bicycle. Subjects started single-limb cycling at a workload corresponding to 30% of their PPO until they reached exhaustion (Leg 1). Exhaustion was defined as the inability of the subject to maintain the required workload. After a rest period, during which a muscle biopsy was obtained, the protocol from Leg 1 was repeated on the previously inactive limb (Leg 2). At 30 min intervals during both Leg 1 and 2, subjects ingested a 10% glucose polymer solution (500ml) to maintain euglycaemic levels.

2.2.4.2 Blood Sampling and Analysis

Venous blood samples (~ 8 ml) were drawn at rest, every 30 min during the exercise trial and at fatigue, by inserting a flexible 20-gauge cannula into a forearm antecubital vein and attaching it to a three-way stopcock. The cannula was kept patent by flushing with 1 ml sterile saline after each blood sample. Two aliquots (2 x 3 ml) were placed into vacutainers containing lithium heparin for analysis of plasma epinephrine and norepinephrine concentrations. All samples were kept on ice and then centrifuged at 3000 rpm at 4°C for 10 minutes at the end of the trial. The supernatants were stored at -80°C for later analysis. Plasma catecholamines were measured using HPLC with electrochemical detection using the method described by Forster and Macdonald (1999) (Appendix 9.1).

2.2.4.3 VO_2 Measurements and Heart Rate

VO_2 was measured for 5 min every 15 min using a breath by breath computerised system (Oxycon Alpha Analyzer, Jaeger-Mijnhart, Wuerzburg, Netherlands). The flow meter was calibrated prior to each test using a Hans Rudolph 3 liter syringe (Vacumed, Ventura, CA), and the gas analyser was calibrated using a two-point calibration of fresh air and a 4% CO_2, 96% N_2 gas mixture. Heart rate was recorded at 5 sec intervals throughout the exercise trial using a Polar ^{TM} heart rate monitor.

2.2.4.4 Perceived Exertion

Prior to the start of the exercise trial, subjects were educated on the use of Borg's "Rating of Perceived Exertion" (RPE) scales (Borg, 1973). Two scales were used to quantify the subjects' level of exertion, the Borg 15-point RPE scale and the Borg category-ratio 10-point scale (Appendix 9.2 and 9.3). Printed scale instructions were given to subjects, as well as an explanation of each scale and a description of how the scales should be used (Appendix 9.2.1 and 9.3.1). Both scales were used to obtain RPE scores at 5 min intervals during the exercise trials. The Borg 15-point RPE scale was used for the subjects overall perception of effort. Subjects were asked to focus on their subjective feelings and not the

workload or physiological cues, and subsequently score their level of exertion on the 15-point scale where 6 means "no exertion at all" and 20 means "maximal exertion". The Borg category-ratio 10-point scale was used to quantify perception of effort in more localised areas such as the legs. The lowest point of reference was 0 being "nothing at all" and the highest anchored at 10 being "extremely strong". A further anchor marked with a dot "•" (absolute maximum) was available if subjects perceived anything stronger than a 10, for example, 11 or 12 or higher. Subjects were encouraged to use half values or decimals where appropriate, and ratings above the scale limits (greater than 10) if necessary (Borg, 1982).

2.2.4.5 Electromyography (EMG)

Electromyographic (EMG) activity was measured in the vastus lateralis muscle of the active leg. The electrode positioning was standardized for each subject and the electrode was not moved throughout the trial. The hair at the placement site was shaved and the skin abraded using industrial sandpaper. An alcohol swab removed any oil and dirt from the skin. This facilitated electrode adherence and conduction of the EMG signal. After preparation of the skin, a surface EMG triode electrode (1 cm radius, 1 cm inter-electrode distance, incorporated into a self-adhesive pad) (Thought Technology TriodeTM MIEPO100, Montreal, Canada) was placed on the skin over the belly of the vastus lateralis muscle, midway between the greater trochanter and femoral epicondyles along the line of the femur.

Prior to the trial, subjects' peak isometric force was assessed on the right and left legs using an isokinetic dynamometer (Kin-Com, Chattanooga Group, Inc., Chattanooga, USA) which was calibrated before each day of testing. Subject's hips and upper bodies were firmly strapped to the seat. The arm position for each test was standardized with each subject crossing his arms over his chest. All isometric tests were conducted at a knee angle of 60°, with 0° being the knee angle at full extension. The angle of 60° flexion has been shown to be the angle of maximal isometric force generation (Tihanyi, 1982). Each subject performed four warm-up isometric contractions of the knee extensors, at a voluntary force output

below their maximum, for 5 s each separated by a 10 s interval. The isometric test consisted of two maximum voluntary contractions (MVC) of 5 s each separated by 10 s intervals. Subjects were verbally encouraged to achieve their maximum potential. EMG amplitude of the vastus lateralis muscle was recorded during the isometric MVC tests and the MVC with the highest mean force measured over 5 s was used for subsequent analyses.

The purpose of the EMG measurement during the MVC is to allow the EMG activity measured during the subsequent cycling trial to be expressed as a percentage of the MVC (Hunter, 2002). Normalizing each subject's EMG muscle activity relative to their own maximal activity also excludes confounding factors such as electrode positioning, skin impedance and differences in percentage body fat.

The triode electrode was attached to the muscle 'belly' as described above and connected to a pre-amplifier. The amplifier was linked via fibre-optic cable to the Flexcomp/DSP EMG apparatus (Thought Technology, Montreal, Canada) and host computer. EMG activity was sampled at 1984 Hz, a high enough frequency for reliable data collection and quantitative data analyses (Hunter, 2003b). Prior to EMG sampling, EMG signals from the electrode were band-pass filtered (20-500 Hz) and amplified using standard differential amplifiers (Thought Technology, Montreal, Canada, common mode rejection ratio > 103 dB at one kHz, input impedance = one million MegOhms). The sampled EMG was passed through a 50 Hz line filter to remove interference from electrical sources to yield raw data. The 50 Hz filter was designed with a narrow stop-band to remove as little of the physiological signal as possible. Its use was necessary due to the intermittent simultaneous utilisation of motorised equipment in the laboratory, such as treadmills, during data capture. Mullany et al (2002) demonstrated that the presence of the 50 Hz filter did not affect the statistical power of their conclusions with similar frequency analyses and fewer subjects in their protocol. Movement artifact was removed from the raw signals with a high-pass second order Butterworth filter with a cut off frequency of 15 Hz, to produce filtered EMG data.

The filtering procedures were performed using MATLAB™ software (The MathWorks Inc.).

Filtered EMG data from the right and left limbs were only processed from the MVC that yielded the greatest force output. The full 5 seconds of filtered EMG data during the 5 s MVC were processed during the contraction. In a similar manner, 5 s of filtered EMG data were processed from 20 s of EMG data collected during the cycling trial every 5 min. The filtered EMG data were analysed as follows: the EMG data from each trial was zero-meaned, the zero-meaned filtered EMG amplitude of each cycling trial was then determined by taking the RMS of the filtered EMG signal, normalised to the RMS of the EMG taken during the MVC contraction (Mullany, 2000). Complete EMG data was available from only five of the seven subjects due to equipment malfunction during the EMG recording.

Constant workload trials to exhaustion often vary in duration and therefore subjects during this trial did not cycle for equal duration before exhaustion. The VO_2 data, heart rate, ratings of perceived exertion and EMG amplitude were time-normalized by expressing the time domain as a percentage of the total completed duration during the trial. This allowed comparisons at similar relative time points between constant workload trials in Leg 1 and Leg 2.

2.2.5 Statistical Analyses

All data are presented as means ± standard deviations. A paired T-test was used to examine differences in performance between trials (Leg 1 and 2). Repeated measures analyses of variance (ANOVA) were used to examine the differences between trials over time for epinephrine and norepinephrine concentrations, VO_2, heart rate, RPE and EMG amplitude. A Tukey's HSD post-hoc test was performed to identify differences between legs over time. An alpha level of ≤ 0.05 was considered statistically significant.

2.3 RESULTS

2.3.1 Subject Characteristics

The general subject characteristics are shown in Table 1. Subjects were moderately trained males as shown by the peak power output (PPO) and VO_2 max values.

Table 1: Subject characteristics of the experimental sample (n=7).

Age (yrs)	19 ± 1
Weight (kg)	84 ± 10
Height (m)	1.8 ± 0.1
Body fat (%)	14.8 ± 1.7
Peak power output (PPO)	278 ± 26
VO_2 max ($ml.kg^{-1}.min^{-1}$)	43 ± 5

All values are expressed as mean ± SD.

2.3.2 Performance Time (Exercise Duration)

The time to fatigue was not significantly different between Leg 1 and Leg 2 (60 ± 6 vs 56 ± 8 min, Leg 1 vs Leg 2 respectively) (p=0.07) (Figure 1a). All subjects, except for subject A (similar time) and subject D, cycled longer with Leg 1 than Leg 2, and all cycled for at least 40 minutes with both legs (Figure 1b).

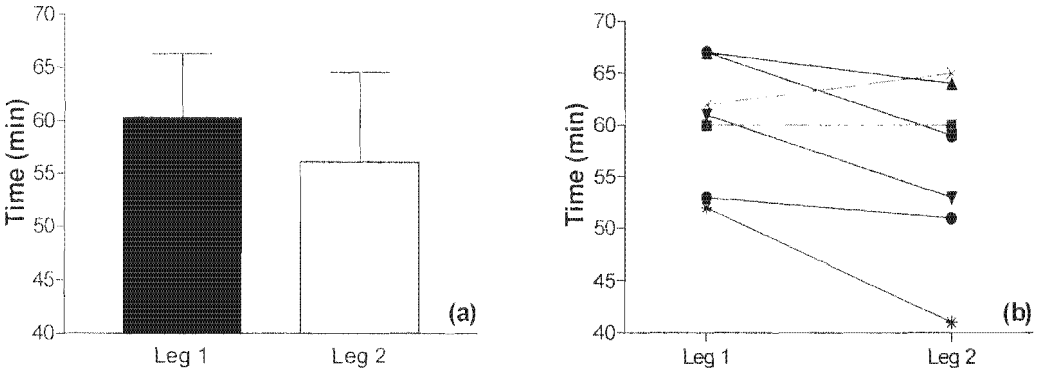


Figure 1: a) Overall performance time (min) of Leg 1 and Leg 2 and b) individual subject's performance times (min) during single-leg exercise to exhaustion of the experimental sample (n=7). Values are expressed as mean ± SD.

2.3.3 Plasma Epinephrine and Norepinephrine Concentrations

Plasma epinephrine and norepinephrine concentrations were both higher in Leg 2 compared to Leg 1 at the start of exercise (Figure 2a and b). Epinephrine concentrations increased from the onset of exercise in Leg 1 and Leg 2 ($p<0.001$), but remained significantly higher in Leg 2 than Leg 1 throughout the exercise bout ($p<0.01$). There was a significant trial x time effect for norepinephrine concentrations ($p=0.008$). Norepinephrine concentrations were significantly different between legs at rest, but increased comparably during exercise.

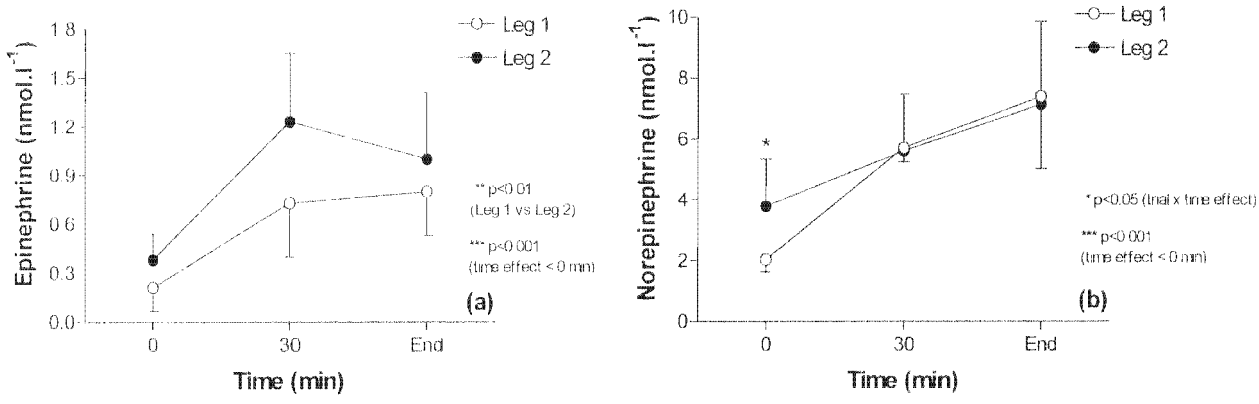


Figure 2: a) Serum epinephrine and b) norepinephrine concentrations during single-leg exercise to exhaustion of the experimental sample (n=7). Values are expressed as mean ± SD.

2.3.4 Oxygen Consumption and Heart Rate

The oxygen consumption was not significantly different between Leg 1 and Leg 2, but increased significantly over time in both trials ($p<0.01$) (Figure 3a). The mean heart rate response was significantly lower in Leg 2 compared to Leg 1 at 10% of the elapsed exercise time (131 ± 28 vs 121 ± 18 bpm, Leg 1vs 2) ($p<0.05$), but rose similarly throughout the exercise trials thereafter ($p<0.001$) (Figure 3b).

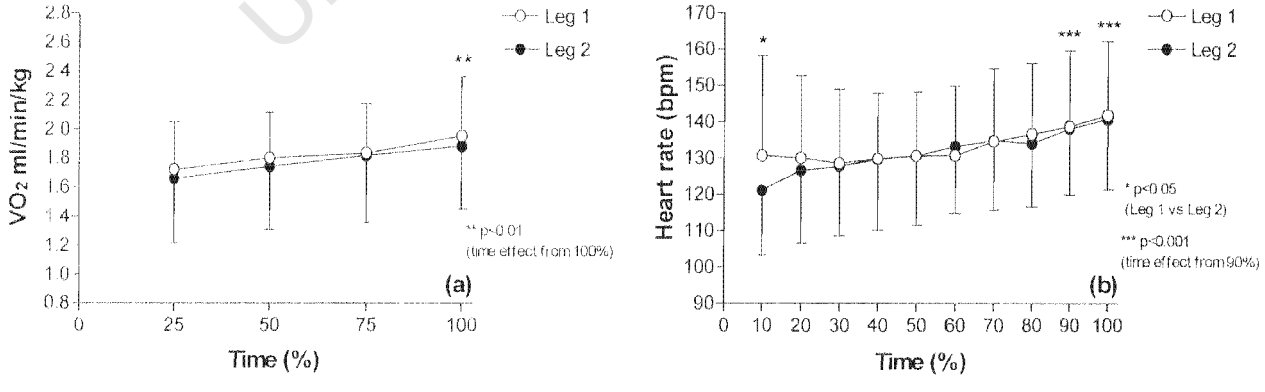


Figure 3: a) VO_2 data (ml/min/kg) and b) heart rate (bpm) during single-leg exercise to exhaustion of the experimental sample (n=7). Values are expressed as mean ± SD.

2.3.5 Perceived Exertion

There was a significant trial x time effect ($p<0.05$) with general RPE being higher in Leg 1 compared to Leg 2 at 10% of the elapsed exercise time (11± 2 vs 8 ± 2, Leg 1 vs 2). Thereafter, RPE was comparable between trials, increasing significantly over time in both Leg 1 and Leg 2 ($p<0.001$) (Figure 4a). The end exercise RPE was 17 ± 2 and 17 ± 2 for Leg 1and Leg 2, respectively.

The RPE characterising localised fatigue increased significantly over time in both legs ($p<0.001$). There was no difference in RPE between legs (Figure 4b). The rating of perceived exertion at the time of fatigue was 10.1 ± 0.6 and 9.2 ± 1.7 for Leg 1 and Leg 2, respectively.

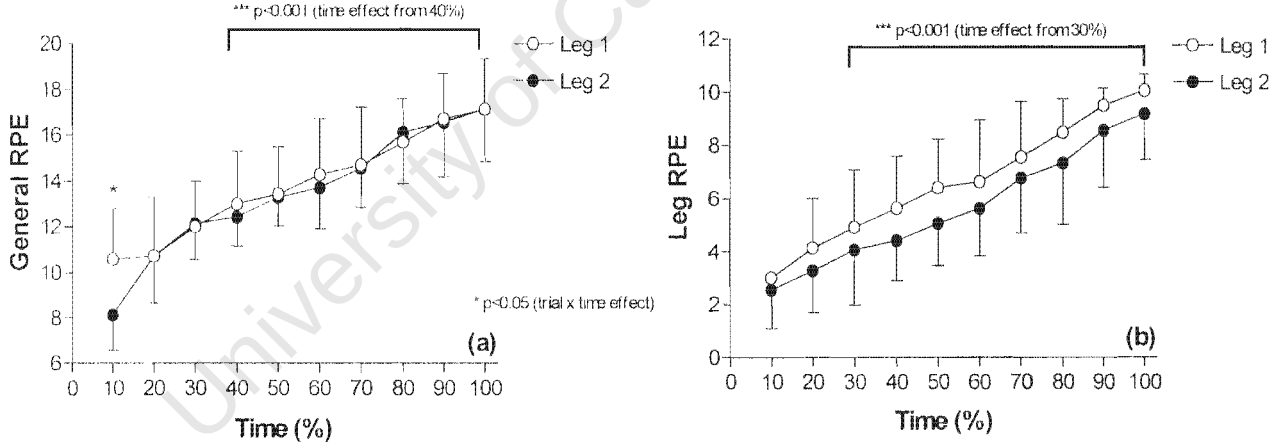


Figure 4: a) General RPE and b) leg RPE during single-leg exercise to exhaustion of the experimental sample (n=7). Values are expressed as mean ± SD.

There was a linear increase in both general and leg RPE with exercise duration for Leg 1 and Leg 2 (Figure 5a and b).

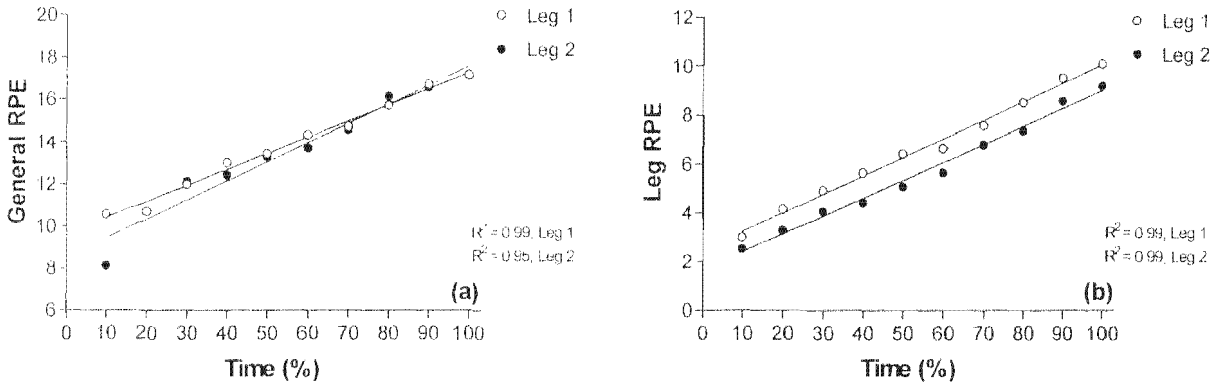


Figure 5: The rate of linear increase in a) general RPE and b) leg RPE during single-leg exercise to exhaustion of the experimental sample (n=7).

2.3.6 Electromyography (EMG) Amplitude

The EMG amplitude was significantly different between Legs 1 and 2 from the outset of exercise ($p<0.01$) (Figure 6). EMG amplitude in Leg 2 was higher than Leg 1 (32 ± 10 vs 27 ± 12 %, Leg 2 vs 1) at 10% of the elapsed exercise time and remained higher throughout the exercise trial.

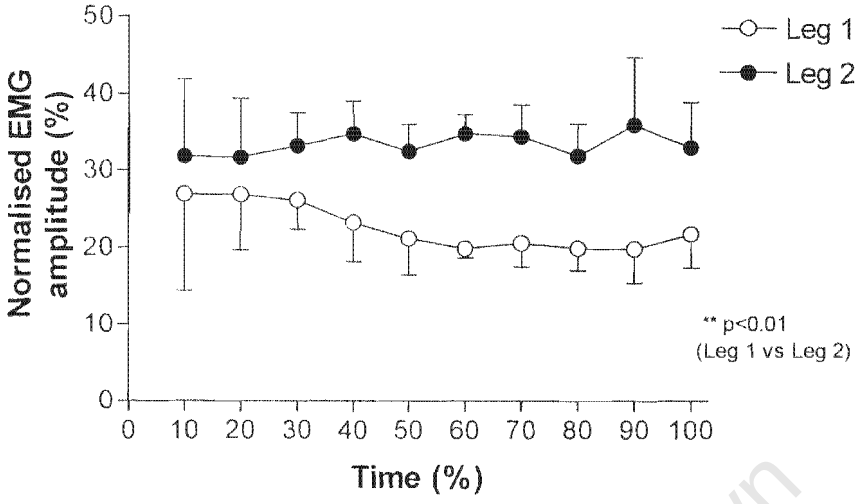


Figure 6: Normalised EMG amplitude during single-leg exercise to exhaustion of the experimental sample (n=5). Values are expressed as mean ± SD.

2.4 DISCUSSION

The purpose of the present study was to examine the effect of antecedent exertion during sequential single-leg cycling to exhaustion (Leg 1) on perceived exertion, muscle activation levels and performance in the previously rested leg (Leg 2). The major findings of this study were that perceived exertion and overall exercise performance were not altered by a prior bout of exhaustive exercise. However, heart rate and RPE were reduced at the onset of exercise in the previously rested leg, while catecholamine concentrations and muscle activation levels were raised in Leg 2.

The heart rate and RPE differences measured at the onset of exercise in Leg 2, however, did not persist throughout exercise and were comparable with Leg 1 following 10% of the elapsed exercise time in Leg 2. In contrast, the significantly elevated muscle activation levels in Leg 2 persisted throughout exercise, and

suggest an altered EMG/work relationship and may be the result of numerous contributing factors.

To our knowledge, only one *in vivo* study has shown indirect evidence that muscle activation levels and sympathetic nervous system activity are associated (Hunter et al, 2002b). Hunter et al (2002b) demonstrated that the administration of a β-blocker, which causes elevated epinephrine concentrations (Alway et al, 1987), caused a simultaneous increase in perceived exertion and muscle activation. It was suggested that the perceived exertion and neural output were increased to compensate for the impaired exercise performance associated with β-blockade. We have presently shown an association between elevated catecholamine concentrations at the onset of exercise in Leg 2 and muscle activation levels. However, whether this association is causal or not is not clear, nor is any potential mechanism for such an association. It is also noteworthy that the norepinpehrine levels in Leg 2 were different only at the onset of the trial, whereas, the epinephrine levels remained elevated throughout the exercise trial, similar to the EMG activity.

Previous research by Seals and Enoka (1989) demonstrated that increased muscle sympathetic nerve activity of non-active muscles during an isometric exercise protocol was directly related to the development of muscle fatigue, and was associated with elevated EMG activity. The mechanism controlling sympathetic activation during static exercise may, however, be different from dynamic exercise.

It is possible that the increased muscle activation levels in Leg 2 were the result of circulating metabolites elicited during the first exercise bout. This is however unlikely, since the data of Claassen (data reported elsewhere, Claassen, PhD thesis) suggest that the metabolic milieu was relatively similar between Leg 1 and Leg 2.

It must also be acknowledged that there are limitations to the measurement of EMG activity in only one muscle group during exercise. It is possible that the

increased activation of the vastus lateralis muscle group in Leg 2 was associated with altered muscle activation in other, non-measured muscle groups. The explanation for these potential differences in spatial patterns of muscle activation patterns by increased central command has previously been proposed (Thickbroom et al, 1998; Dettmers, 1995; Conwit et al, 1999; Kukulka and Clamann, 1981). Direct fatigue of a muscle has been shown to result in changes in voluntary activation patterns during subsequent voluntary contractions. For example, Akima et al (2002) examined four individual quadriceps muscles during knee extension exercise before and after fatiguing a single member of the quadriceps muscle group (vastus lateralis). They found that subjects were able to perform the submaximal knee extension exercise after the fatiguing bout by means of increased use of the other muscles. It was suggested that there was increased central command to the muscle so that the exercise task could be achieved despite acute fatigue of one its synergists (vastus lateralis muscle). In the present study, we have not directly fatigued the vastus lateralis muscle of Leg 2, but cannot exclude the possibility that the antecedent bout of fatiguing exercise resulted in changes in muscle activation in non-measured muscle groups allowing for performance of Leg 2, which would account for the absence of differences in VO_2 and heart rate in Leg 2, despite the elevated muscle activation levels in the measured muscle group.

Although overall perceived exertion was lower at the start of exercise in Leg 2 compared to Leg 1 (11± 2 vs 8 ± 2, Leg 1 vs Leg 2) (Figure 4a), it increased similarly thereafter despite the altered muscle activation / work relationship in Leg 2. It is possible, although speculative, that subjects perceived the exercise as unfamiliar and strenuous at the start of Leg 1, while in Leg 2, prior experience of this mode of exercise by the exercise bout in Leg 1, combined with a short rest period, subjects may have perceived the start of the subsequent exercise bout as less strenuous. It is interesting to note that the general RPE at termination of exercise in both Leg 1 and Leg 2 was submaximal (~ 17 units), even though the subjects were instructed to exercise in both exercise trials to "exhaustion", defined as the inability to maintain the required workload. This suggests that perceived exertion may be subconsciously calculated and that an anticipatory regulatory

process exists so that exercise may be "safely" completed maintaining a physical reserve capacity.

Previous research has shown that peripheral RPE (muscle and / or joints) tends to be higher than overall RPE during cycling exercise (Robertson et al, 1979a; Pandolf et al, 1975), specifically at lower cadences (Robertson et al, 1979b; Marsh and Martin, 1998; Jameson and Ring, 2000), which suggests that peripheral feedback may play an important role in influencing RPE and cadence selection during exercise. The leg RPE values were between "very strong and extremely strong" at exhaustion (10.1 ± 0.6 and 9.2 ± 1.7 for Leg 1 and 2, respectively) (Figure 4b), whereas general RPE values at exhaustion were not maximal (17 ± 2 for Legs 1 and 2) This indicates the presence of localised fatigue, and therefore, the local leg RPE seems to be the dominant effort sensation for this dynamic type of exercise. The subjects who performed this trial were unaccustomed to single-leg cycling, and in addition, found it difficult to maintain a smooth rhythmic cadence at exhaustion.

When expressed relative to elapsed time, both general and leg RPE increased at the same linear rate in both legs (Figure 5a and b). This finding indicates that perceived exertion may have scalar time based properties which supports earlier findings of Noakes (2004), and more recently Eston et al (unpublished data, personal communication with St Clair Gibson). Noakes (2004) reported a similar linear relationship between RPE and exercise duration during cycling to exhaustion with low or high preexercise intramuscular glycogen content (Baldwin et al, 2003). Similarly, Eston et al (unpublished data, personal communication with St Clair Gibson) also found a linear relationship between RPE and relative exercise duration during exercise to exhaustion in either a pre-fatigued or non-fatigued state.

Despite the altered physiological milieu and RPE at the onset of exercise, and the increased muscle activation levels that persisted throughout exercise in Leg 2, the performance times were not significantly different between Leg 1 and Leg 2. Any changes in physiological or perceptual variables at the start of exercise in Leg 2

had diminished by the time subjects reached volitional fatigue, and therefore, fatigue occurred at the same RPE and physiological state in both trials, despite different muscle activation levels.

It has previously been suggested that a teleoanticipatory strategy regulates exercise performance in response to changes in physiological variables (Ulmer, 1996; St Clair Gibson and Noakes, 2004). In the present study, work rate was fixed and changes in exercise intensity not possible. It is possible, however, that a teleoanticipatory system could still regulate the duration of exercise based on physiological changes, as well as previous experience of exercise. In the present study, the only measured physiological differences were elevated epinephrine levels and muscle activation levels in Leg 2. Ulmer's proposed relationship between efferent motor command and control of metabolic rate suggests that muscle activation levels would be altered by a feedback control system (Ulmer, 1996), possibly including sympathetic activity. It is therefore possible that the increased EMG activity was associated with elevated epinephrine levels and resulted in similar times to fatigue in Leg 1 and Leg 2, although the mechanism is at present unknown.

The similar exercise performance capacity found in this study is in contrast to previous research. Bosch et al (unpublished observations) showed a significant performance time difference of ~ 117 min during sequential single-limb cycling to exhaustion. However, the decreased performance time in the previously rested leg may be due to its passive rotation during exercise of the first leg, whereas in the present study, Leg 2 remained completely rested. Also, in the study of Bosch et al (unpublished observations), exercise with Leg 1 continued for substantially longer than in the present study, and this increased duration of exercise may have had implications for energy availability and motivation of subjects. A study examining the performance capacity of previously inactive muscle reported decreased performance due to increased blood lactate concentrations following activity of other muscles (Klausen et al, 1972). Furthermore, Karlsson et al (1975) investigated the effect of prior exhaustive exercise on the ability of previously rested muscles to perform, using alternate arm and leg exercise, and showed

decreased performance time in the second exercise bout. This decreased performance capacity of the previously inactive muscle was associated with metabolic changes in the muscle (increased blood lactate and/or H^+ concentrations) elicited during exhaustive exercise of other muscle groups.

In summary, the present study found that antecedent exhaustive single-limb exercise did not influence performance during a subsequent bout of single-limb exercise with the previously rested leg, despite differences in catecholamine concentrations, heart rate, RPE and EMG activity at the start of exercise in Leg 2. During exercise in Leg 2, these variables became similar to those measured in Leg 1, with the exception of epinephrine concentrations and EMG activity, which remained elevated until termination of exercise. The mechanism underlying this altered neuromuscular strategy remains unclear, but these data suggest that the increased muscle activation levels may be associated with increased circulating catecholamine concentrations.

Perceived exertion was, however, unrelated to muscle activation and catecholamine concentrations, and increased similarly for most of the trial until volitional fatigue where RPE values were comparable in Leg 1 and Leg 2. Therefore, the rate of change in perceived exertion associated with prior local muscle fatiguing exercise appeared to be dependent on the total exercise duration, irrespective of antecedent exercise, an altered physiological milieu or increased muscle activation levels. However, we have used a single-limb protocol in an open-loop exercise design and so the potential influence of circulating catecholamine concentrations on performance, RPE and muscle activation levels are not conclusively established, and further research is required to examine this association.

CHAPTER 3

THE EFFECT OF ELEVATED PLASMA EPINEPHRINE LEVELS ON MUSCLE ACTIVATION, PERCEIVED EXERTION AND SUBSTRATE METABOLISM DURING LOW-TO-MODERATE EXERCISE

Published in part in: Pflugers Arch – Eur J Physiol, 451: 727-737, 2006. **West SJ**, Goedecke JH, van Niekerk L, Collins M, St Clair Gibson A, MacDonald IA, Noakes TD, Lambert EV (2006) Effects of elevated plasma adrenaline levels on substrate metabolism, effort perception and muscle activation during low-to-moderate intensity exercise

3.1 INTRODUCTION

Chapter 2 of the present thesis evaluated the physiological and perceptual responses to an antecedent exposure to fatiguing exercise and found that prior exhaustive single-limb exercise resulted in increased muscle activation levels, but did not alter perceived exertion or overall exercise performance, despite an altered physiological milieu at the onset exercise in the previously rested limb. A potential interaction between the sympathetic nervous system and muscle activation was discussed. Previously, in a study by Seals and Enoka (1999), increased sympathetic activation has been associated with muscle fatigue, as demonstrated by an increase in EMG activity. Perceived exertion in Chapter 2, however, was unrelated to muscle activation and catecholamine concentrations. This is in contrast to previous research by Saito et al (1989), which showed that the perception of fatigue was related to increased sympathetic activation. However, both Seals and Enoka (1999) and Saito et al (1989) used an isometric exercise protocol, and the potential association between sympathetic activation and neural activation may be different during dynamic exercise.

During dynamic exercise, an increase in exercise intensity is associated with increased activation of the sympathetic nervous system (SNS), as well as the sympathoadrenal response, resulting in an increase in endogenous epinephrine (Epi) and norepinephrine (NorEpi) secretion (Kjaer, 1989). There is indirect evidence that the increased circulating catecholamine concentrations are responsible for the major sympathoadrenergic effects on energy metabolism during such exercise (Kjaer and Lange, 2000; Stallknecht et al, 2001).

Previous studies (Chesley et al, 1995; Febbraio et al, 1998; Howlett et al, 1999a; Kjaer et al, 2000; Watt et al, 2001; Wendling et al, 1996) have attempted to differentiate the role of circulating [Epi] from that of general sympathoadrenal response in regulating the metabolic sequelae during exercise of different intensities. These studies have typically infused Epi to levels at least twice the normal range measured during exercise. Collectively, these studies have

demonstrated that Epi infusion increases the mobilization of fuels, resulting in elevated circulating glucose and lactate concentrations (Chesley et al, 1995; Febbraio et al, 1998; Howlett et al, 1999a; Kjaer et al, 2000; Watt et al, 2001; Wendling et al, 1996). The effects of elevated circulating epinephrine concentrations are thus relatively well established. Less well known, however, is the effect of increasing epinephrine concentrations on exercise performance, muscle activation levels and ratings of perceived exertion.

In Chapter 2 it was found that an antecedent bout of fatiguing exercise resulted in elevated circulating epinephrine concentrations and muscle activation levels in the previously rested leg. An important consideration may be that this study utilized a single-limb exercise protocol that resulted in volitional fatigue after approximately 55 - 60 min of cycling. The effect of whole body exercise at a range of exercise intensities on this relationship is not known. Accordingly, the aim of the present study was to evaluate the role of raised circulating Epi concentrations, similar to those concentrations found during moderate-intensity exercise, on muscle activation levels, perceived exertion, circulating substrate availability and intramuscular substrate utilization during prolonged, low-intensity exercise.

3.2 METHODS

3.2.1 Subject Selection

Thirteen endurance trained male cyclists between the ages of 18 and 40 years were recruited to participate in the study. The study was approved by the Ethics and Research Committee of the Faculty of Health Sciences of the University of Cape Town. To participate in the study, subjects had to i) be between the ages of 21 and 45 yrs; ii) have completed a local 109 km cycle race in less than 4.5 hrs and iii) have no known metabolic conditions that may adversely affect intermediary metabolism. Subjects were excluded if they were taking any medications for chronic conditions such as high-blood pressure or asthma (e.g. β-adrenergic receptor agonists or antagonists). The risks and benefits of the trial

were carefully explained to the subjects and their informed consent was obtained in writing before participation in the trial.

Subjects were randomly assigned to one of two groups, MOD (moderate, n=6) and LOW (low, n=7). Subjects in the MOD group were required to cycle on two occasions. On one occasion, they cycled at 68% VO_{2max} and received a saline infusion (MOD 68% Sal). On the other occasion, they cycled at 34% VO_{2max} with an Epi infusion (MOD 34% Epi) which was designed to achieve similar Epi levels as those measured during exercise at 68% VO_{2max}. In the LOW group, two trials were also performed by each subject. Both trials were at 34% VO_{2max} but in one trial, subjects received a saline infusion (LOW 34% Sal), while in the other trial, they received an Epi infusion (LOW 34% Epi), which was again designed to achieve the Epi levels of exercise at 68% VO_{2max}. The characteristics of the subjects are presented in Table 1. There were no significant differences in anthropometric measurements or VO_{2max} and peak power output, as described below, between groups.

Table 1: Subject characteristics of the MOD and LOW experimental groups.

	MOD group (n=6)	**LOW group (n=7)**
Age (yrs)	28 ± 10	27 ± 10
Weight (kg)	75.6 ± 9.8	76.4 ± 9.8
Sum 7 skinfolds (mm)	31 ± 6	42 ±17
Body fat (%)	15.1 ± 1.6	15.1 ± 4.7
Peak power output (W)	302 ± 48	329 ± 48
VO_{2max} (ml/kg/min)	56.2 ± 4.1	58.6 ± 4.1

All values are expressed as mean ± SD.

3.2.2 Anthropometry

The subjects' mass, height and sum of seven skinfolds (triceps, biceps, subscapular, supra-iliac, abdominal, thigh and calf) were measured. Percentage body fat was estimated using the equations of Durnin and Womersley (1974).

3.2.3 VO_2 Peak and Peak Power Output Test

All subjects performed an incremental workload test to exhaustion on a cycle ergometer (Lode, Groningen, Holland) to determine their maximal oxygen consumption (VO_{2max}) and their peak power output (PPO). Work rates started at 3.33 W/kg body mass, and after 150 s the workload was increased by 50W and then by 25W every 150 s until the subject was exhausted. Exhaustion was defined as a >10% reduction in pedaling frequency, or an RER of >1.10, or both. PPO was defined as the highest exercise intensity the subject completed for 150 s in W, plus the fraction of time spent in the final workload multiplied by 25W.

During the incremental exercise test, oxygen uptake (VO_2), CO_2 production (VCO_2) and ventilation volume (V_E) were measured over 30 s intervals by use of a breath-by-breath Oxycon Alpha Analyzer (Jaeger, Wuerzburg, Netherlands). The reliability of the Oxycon Alpha Analyzer was tested on a weekly basis by burning absolute ethanol [99% analytical reagent, Associated Chemical Enterprises (Pty), Glenvista, South Africa] as a reference. The pneumotach was calibrated before each test with a Hans Rudolph 3-litre syringe (Vacumed, Vertura, CA) and the analyzers were calibrated with room air and a 4% CO_2-96% N_2 gas mixture. This information was used to adjust the work rate in the experimental trials to correspond to either 34% or 68% of VO_{2max}.

3.2.4 Experimental Protocol

As described previously, subjects were assigned to one of two groups – MOD and LOW. In MOD, subjects cycled once at 68% VO_{2max} while receiving a saline infusion (MOD 68% Sal), and once at 34% while receiving Epi infusion (MOD 34% Epi). In LOW, subjects cycled at 34% VO_{2max} receiving either saline infusion (LOW 34% Sal) or Epi infusion (LOW 34% Epi). The exercise intensities chosen for the present study correspond to 25% and 50% of PPO respectively, and are typically

used for low- (low enough not to stimulate SNS activation) and moderate-intensity exercise (high enough to stimulate SNS activation) (Mora-Rodriguez and Coyle, 2000; Wendling et al, 1996). The infusion rate (0.015 μg/kg/min) was designed to elevate plasma [Epi] to levels matching those observed during exercise between 65 and 70% VO_{2max} (Mora-Rodriguez and Coyle, 2000). Muscle biopsies were performed prior to and at the end of the 90-min exercise bout for the determination of muscle glycogen and triglyceride (TG) content. The following measurements were taken at 15 min intervals during the steady-state 90-min cycle: effort perception, electromyographic (EMG) activity and metabolic parameters which included gas exchange, heart rate and blood samples for the determination of plasma catecholamines, glucose, lactate and free fatty acid (FFA) concentrations.

3.2.4.1 Resting Measurements

The day prior to each experimental trial, the subjects were requested to abstain from any strenuous exercise and to follow the same diet. In addition, the subjects were required to avoid caffeine 12 hours before the trial. On the subsequent day, the subjects arrived at the laboratory between 7:00 and 7:30 AM after an overnight fast (10-12 hr). A muscle sample was obtained from the belly of the vastus lateralis muscle of the left leg, midway between the greater trochanter and femoral epicondyles along the line of the femur, before the start and immediately on completion of the exercise trials, by means of the percutaneous needle biopsy technique. Due to the invasiveness of the muscle biopsy procedure, the subjects did not serve as their own controls but were divided into two groups each receiving a total of four biopsies. The muscle sample was rapidly frozen in liquid nitrogen (N_2) and stored at -80^0C for subsequent analysis of glycogen and triglyceride content.

After the muscle biopsy was taken, a 20-gauge Teflon cannula (Jelco; Johnson and Johnson, Halfway House, South Africa) was placed in each arm and connected to a three-way stopcock (Uniflex; Mallinckrodt Medical, Hennef-Sieg, Germany). One arm was used for blood sampling during the trial, while the other

arm was used to infuse either saline or Epi during the trial. Thereafter, the subjects' peak isometric force was assessed on the lower right limb and EMG activity was measured as described below.

Subjects were then required to rest for at least 40 minutes to minimize the effects of the muscle biopsy and venipuncture. During this time period, the subjects were instructed on the use of the 15-point Borg-scale for the rating of perceived exertion (RPE), as described below (Borg, 1973).

Five minutes before the start of exercise subjects sat on the bike while resting oxygen consumption (VO_2) and carbon dioxide production (VCO_2) was measured for 5 minutes using the Oxycon Alpha Analyzer (Jaeger, Wuerzburg, Netherlands), and respiratory exchange ratio (RER) calculated. Immediately thereafter, a 10-ml resting blood sample was drawn and placed in the appropriate tubes for subsequent analysis of plasma Epi, NorEpi, glucose, lactate and serum FFA concentrations. Heart Rate was recorded continuously by means of a Polar™ Heart Rate Monitor (Polar Electro, Kempele, Finland).

3.2.4.2 Exercise Trials

After the resting measurements were performed, subjects then started cycling on the stationary cycle ergometer (Lode, Groningen, Holland) at a workload corresponding to either 34% or 68% VO_{2max} for 90 minutes, maintaining a pedaling rate of 90 rpm throughout both trials. Fifteen minutes into the exercise period, Epi or saline was infused at a constant rate (0.015 µg/kg/min) until the end of the trial (Mora-Rodriguez and Coyle, 2000). All infusions were controlled using a calibrated automatic syringe pump (Travenol Laboratories, Hooksett, NJ). Subjects were blinded to the type of infusion they were receiving. Immediately after the infusion started and at 15-min intervals throughout each cycle trial, VO_2 and VCO_2 were recorded for 5 minutes, and blood samples were drawn, as previously described. In addition, RPE and EMG activity (as described below) were recorded at 15 min intervals throughout the exercise trial.

3.2.4.3 Electromyography (EMG) Amplitude and Analysis

Electromyography (EMG) activity was measured from each subject's right vastus lateralis muscle and the peak isometric force assessed on the lower right limb on a Kin-Com isokinetic dynamometer (Chattanooga Group, Inc., Chattanooga, USA). The preparation of the skin, electrode positioning, the isometric MVC test, the purpose of the MVC, and the EMG sampling and analyses are described in Chapter 2.

3.2.4.4 Ratings of Perceived Exertion and Analysis

The use of Borg's RPE scale has been previously described in Chapter 2.

3.2.4.5 Blood Sampling and Analysis

Blood samples (10 ml) were placed in tubes containing potassium oxalate and sodium fluoride (2 ml) for the subsequent determination of plasma glucose and lactate concentrations, and lithium heparin tubes (5 ml) for the subsequent determination of plasma Epi and NorEpi concentrations. The remaining aliquot (3 ml) was placed into a tube containing gel and clot activator for the determination of serum FFA concentrations. The tubes were immediately placed on ice and, on completion of the trial, centrifuged at 3000 rpm and 4°C for 10 minutes. The supernatants were then transferred to microfuge tubes and stored at –20°C or –80°C for subsequent analysis of metabolite and hormone concentrations, respectively.

Plasma glucose concentrations were determined by the glucose oxidase method using a glucose analyzer (Glucose analyzer 2; Beckman Instruments, Fullerton, CA). Lactate concentrations were measured on the same plasma samples by enzymatic colorimetric assays (Lactate PAP, bioMerieux, Lyon, France). Serum FFA concentrations were measured using an enzymatic colorimetric assay (Halfmicro test; Roche, Mannheim). Plasma catecholamines were measured using HPLC with electrochemical detection using the method described by Forster and Macdonald (1999).

3.2.4.6 Intramuscular triglyceride and glycogen concentrations

A portion of the frozen muscle biopsy sample (~50 mg) was freeze-dried and dissected free of any visible fat or connective tissue. Muscle glycogen content was determined as glucose residues (glucose oxidase method; Glucose Analyzer 2, Beckman Instruments, Fullerton, CA) after hydrolysis of approximately half of the freeze dried muscle sample in 2 M HCl at 95°C for 3 h as previously described (Passonneau and Lauderdale, 1999). Glycerol concentrations were measured in the remaining portion of the freeze dried muscle using a commercial glycerol kit (Boehringer Mannheim, Mannheim, Germany) after the TG was lipolyzed to glycerol and FFAs, as described by Kiens and Richter (1996).

3.2.5 Statistical Analyses

All data are presented as means ± standard deviations. T-tests for independent samples were used to examine differences in subject characteristics between groups. Repeated-measures analyses of variance (ANOVA) were used to examine differences between trials over time for plasma metabolites and hormone concentrations, muscle substrate levels, RPE, gas exchange, heart rate and EMG activity. A Tukey's HSD post-hoc test was performed to locate differences over time. An alpha level of <0.05 was considered statistically significant.

3.3 RESULTS

3.3.1 Plasma Epinephrine (Epi) and Norepinephrine (Norepi) Concentrations

Plasma Epi concentrations were significantly higher in both MOD (34% Epi) and LOW (34% Epi) trials, when compared to the saline infusion in MOD (68% Sal) and LOW (34% Sal) trials (Figure 1a, $p<0.001$). Plasma NorEpi concentrations

were higher during exercise in the MOD (68% Sal) trial (Figure 1b, $p<0.001$), compared to 34% VO_{2max}trials, whether or not Epi was infused.

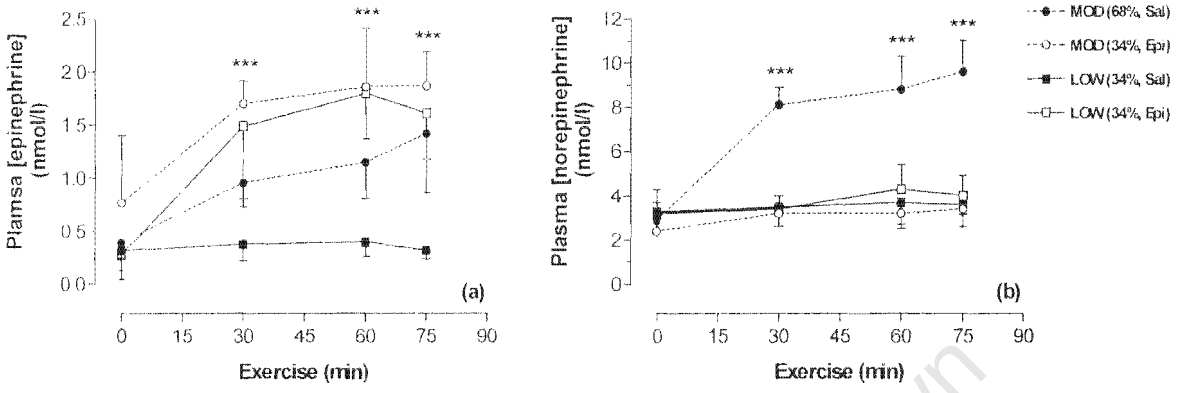


Figure 1: Plasma a) Epi and b) NorEpi concentrations during exercise at 34% VO_{2max}, with and without Epi infusion, and during exercise at 68% VO_{2max}. Values are expressed as mean ± SD.

*MOD trial (68% Sal and 34% Epi) plasma epinephrine levels: *** p<0.001 for trial and time (≥30 min)*
*LOW trial (34% Sal and 34% Epi) plasma epinephrine levels: *** p<0.001 for trial, time (≥30 min) and interaction effect*
*MOD trial (68% Sal and 34% Epi) plasma norepinephrine levels: *** p<0.001 for trial, time (≥30 min) and interaction effect*
LOW trial (34% Sal and 34% Epi) plasma norepinephrine levels: not significant

3.3.2 Intramuscular Triacylglycerol (IMTG) and Muscle Glycogen Content

The IMTG concentrations did not change significantly in either the MOD (68% Sal and 34% Epi) or LOW (34% Sal and 34% Epi) groups throughout the trials (Table 2).

Table 2: Intramuscular triacyclglycerol concentrations (μmol/g dry wt) pre- and post-exercise at 34% VO_{2max}, with and without Epi infusion, and at 68% VO_{2max}.

	PRE	POST
MOD group (n=6)		
68% saline	56.76 ± 46.58	30.87 ± 14.23
34% Epi	40.25 ± 18.13	42.98 ± 22.79
LOW group (n=7)		
34% saline	49.82 ± 30.91	34.81 ± 15.18
34% Epi	29.51 ± 11.87	36.40 ± 13.36

Values are means ± SD. MOD (68% Sal and 34% Epi): NS; LOW (34% Sal and 34% Epi): p=0.07 for trial x time.

The muscle glycogen concentrations decreased significantly from pre to post exercise in the MOD (68% Sal) trial (P<0.01) and decreased from pre to post exercise in the LOW (34% Sal) trial (p = 0.06) (Table 3). Rates of muscle glycogen utilization were not significantly different during exercise at 34 % VO_{2max} with Epi infusion.

Table 3: Muscle glycogen content (μmol/g dry wt) pre- and post-exercise at 34% VO_{2max}, with and without Epi infusion, and at 68% VO_{2max}.

	PRE	POST
MOD group (n=6)		
68% saline	406 ± 134	194 ± 36 **
34% Epi	369 ± 106	287 ± 79
LOW group (n=7)		
34% saline	503 ± 117	371± 119
34% Epi	449 ± 111	413 ± 84

Values are means ± SD. MOD (68% Sal): ** P< 0.01 for time; LOW (34% Sal): p = 0.06 for time, and LOW (34%Sal and 34% Epi): p=0.07 for trial x time.

3.3.3 Plasma Metabolite Concentrations

Plasma glucose and lactate concentrations in response to exercise at 34% VO_{2max}, with and without Epi infusion, and during exercise at 68% VO_{2max} are presented in Figures 2a and b. Epi infusion was associated with increased circulating plasma glucose and lactate concentrations during exercise at 34% VO_{2max} in both MOD (34% Epi) and LOW (34% Epi) trials ($p<0.001$). Plasma glucose concentrations declined to ~ 4 mmol/l during the MOD (68% Sal) trial, but increased to ~ 5.3 mmol/l during the MOD (34% Epi) trial ($p<0.05$). There was a significant time x trial interaction effect for plasma glucose concentrations during the LOW (34% Sal and 34% Epi) trials.

The moderate-intensity exercise with saline infusion, MOD (68% Sal) trial, resulted in a significantly greater increase in plasma lactate concentrations compared to the low-intensity exercise trial with Epi infusion, MOD (34% Epi) ($p<0.01$). Plasma lactate concentrations were, however, significantly higher during exercise at 34% VO_{2max} with Epi infusion, LOW (34% Epi) trial, compared to saline infusion, LOW (34% Sal) trial, ($p<0.01$). There was a significant time x trial interaction effect for serum FFA concentrations during the MOD (68% Sal and 34% Epi) trials. Serum FFA concentrations progressively increased during exercise at 68% VO_{2max} with saline infusion, however, during exercise at 34% VO_{2max} with Epi infusion, there was no further increase in serum FFA concentrations after 60 min of exercise. In contrast, there were no differences in serum FFA concentrations during the LOW (34% Sal and 34% Epi) trials.

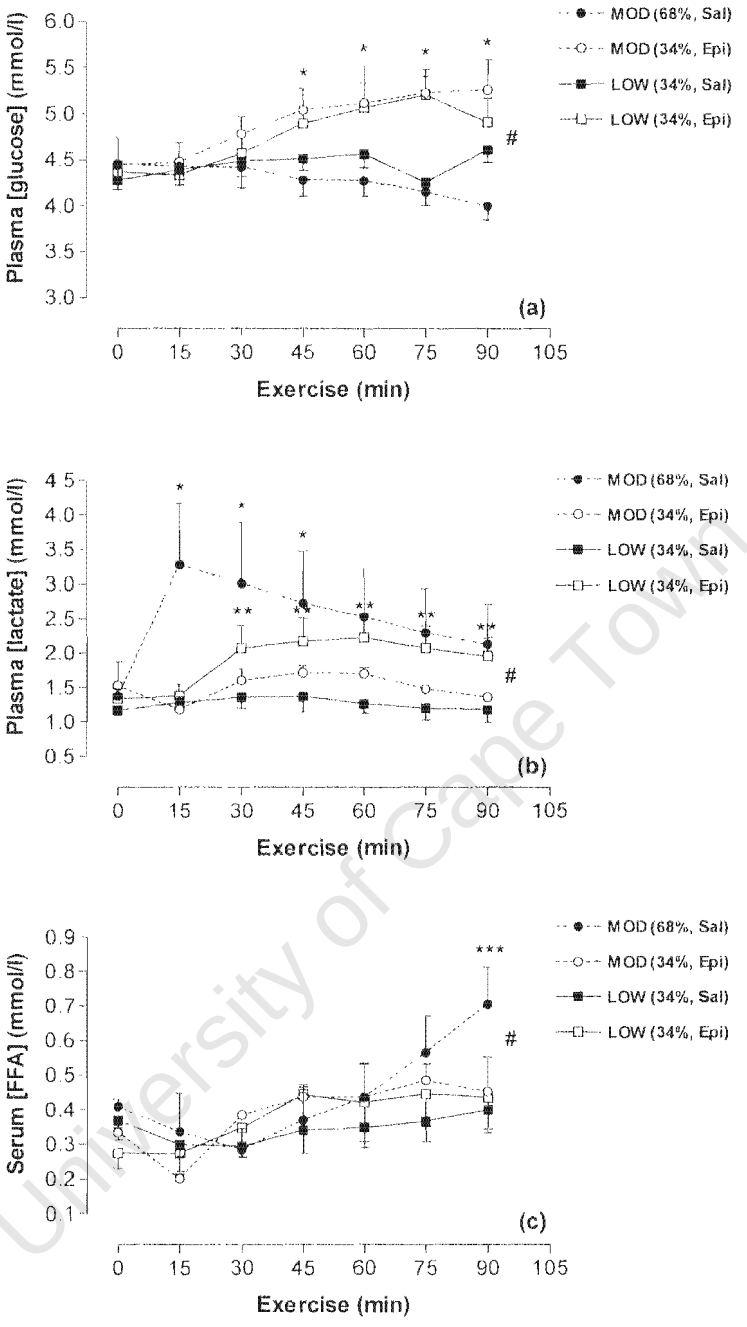


Figure 2: a) Plasma glucose, b) lactate and c) serum free fatty acid (FFA) concentrations during exercise at 34% VO_{2max}, with and without Epi infusion, and during exercise at 68% VO_{2max}. Values are expressed as mean ± SD.

*MOD trial (68% Sal and 34% Epi) plasma glucose levels: * p<0.05 for trial, # p<0.001 for trial x time effect (≥45 min)*
LOW trial (34% Sal and 34% Epi) plasma glucose levels: # p<0.001 for trial x time effect (≥45 min)
*MOD trial (68% Sal and 34% Epi) plasma lactate: * p<0.05 for time (15, 30 and 45 min), # p<0.01 for trial x time effect*
*LOW trial (34% Sal and 34% Epi) plasma lactate: ** p<0.01 for trial and time (≥30 min, # p<0.01for trial x time effect*
*MOD trial (68% Sal and 34% Epi) serum FFA levels: *** p<0.001 for time, # p<0.05 for trial x time effect (90 min)*
LOW trial (34% Sal and 34% Epi) serum FFA levels: not significant

3.3.4 Heart Rate and Gas Exchange

Heart rate was significantly higher during the MOD (68% Sal) trial compared to the MOD (34% Epi) trial ($p<0.001$), but was not altered by Epi infusion during the LOW (34% Sal and 34% Epi) trials (Figure 3a). There were also no effects of Epi infusion on VO_2, V_E or RER during exercise at 34% VO_{2max} in the LOW (34% Sal and 34% Epi) trials (Figures 3b and 3c). However, VO_2, V_E and RER were significantly higher during exercise at 68% VO_{2max} in the MOD (68% Sal) trial compared to the MOD (34% Epi) trial ($p<0.05$).

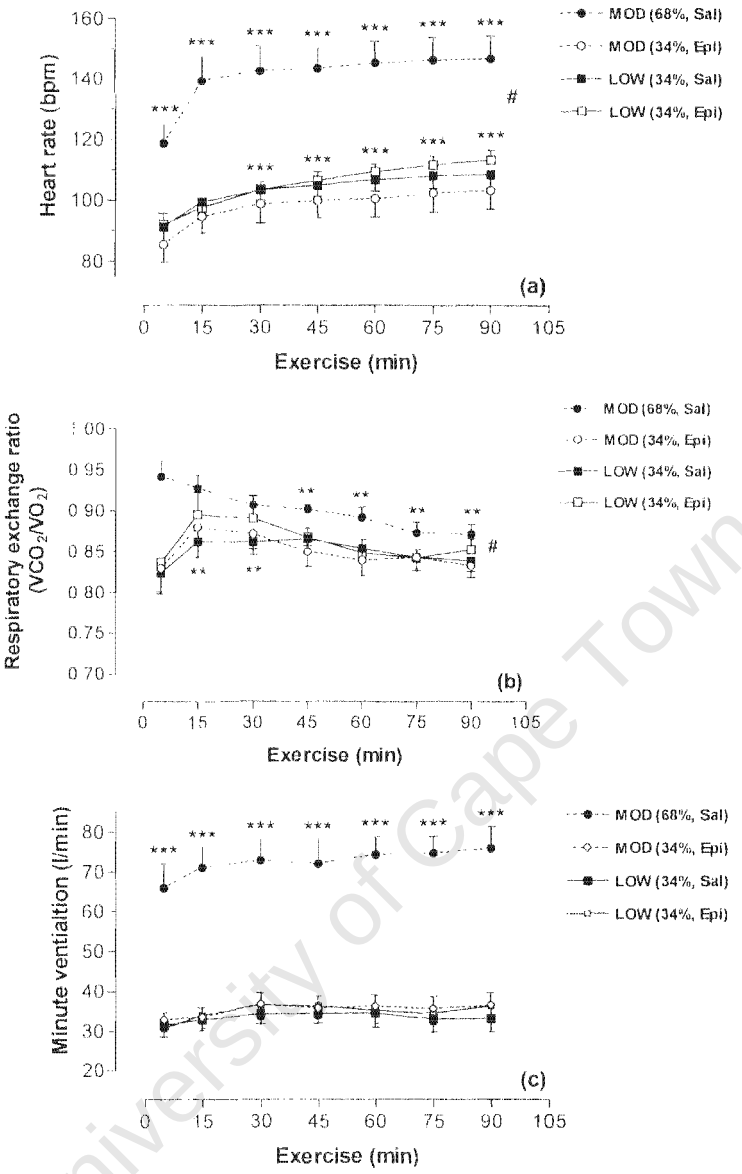


Figure 3: a) Heart rate, b) respiratory exchange ratio and c) minute ventilation during exercise at 34% VO_{2max} with and without Epi infusion, and during exercise at 68% VO_{2max}. Values are expressed as mean ± SD.

*MOD trial (68% Sal and 34% Epi) heart rate: *** p<0.001 for trial and time, # p<0.05 for trial x time effect (≥5 min)*

*LOW trial (34% Sal and 34% Epi) heart rate: *** p<0.001 for time (≥30 min)*

*MOD trial (68% Sal and 34% Epi) RER: ** P<0.01 for trial and time (≥45 min), # p<0.001 for interaction effect*

*LOW trial (34% Sal and 34% Epi) RER: ** p<0.01 for time (15, 30 min)*

*MOD trial (68% Sal and 34% Epi) minute ventilation: *** p<0.001 for trial and time (≥5 min)*
LOW trial (34% Sal and 34% Epi) minute ventilation: not significant

3.3.5 Perceived Exertion

The Epi infusion had no effect on ratings of perceived exertion in either trial. RPE increased significantly higher during exercise at 68% VO_{2max} in the MOD (68% Sal) trial ($p<0.05$) compared to exercise at 34% VO_{2max}, with or without Epi infusion, which was similar in the MOD (34% Epi) and LOW (34% Sal and 34% Epi) trials (Figure 4).

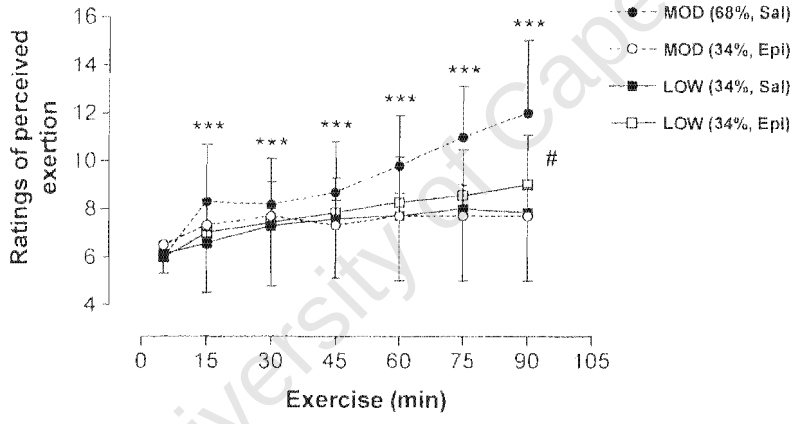


Figure 4: Ratings of perceived exertion during exercise at 34% VO_{2max} with and without Epi infusion, and during exercise at 68% VO_{2max}. Values are expressed as mean ± SD.

*MOD trial (68% Sal and 34% Epi) RPE: * p<0.05 for trial, *** p<0.001 for time (≥15 min), # p<0.001for trial x time effect*
*LOW trial (34% Sal and 34% Epi) RPE: *** p<0.001 for time (≥30 min)*

3.3.6 Electromyography (EMG) Amplitude

Normalised EMG amplitude was significantly higher during MOD (68% Sal) compared to MOD (34% Epi) trial ($p<0.05$) (~ 20 ± 9 vs. 12 ± 5 %, respectively), but was not altered by Epi infusion during LOW (34% Sal and 34% Epi) trials (Figure 5).

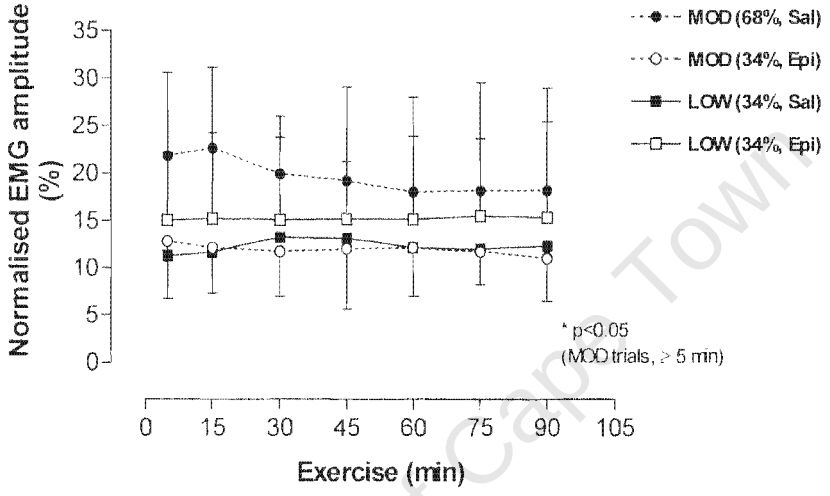


Figure 5: Normalised EMG amplitude during exercise at 34% VO_{2max} with and without Epi infusion, and during exercise at 68% VO_{2max}. Values are expressed as mean ± SD.

MOD trial (68% Sal and 34% Epi) EMG amplitude: $p<0.05$ for trial (≥5 min)
LOW trial (34% Sal and 34% Epi) EMG amplitude: not significant

3.4 DISCUSSION

The aim of the present study was to investigate the role of raised circulating Epi concentrations, similar to those found during moderate-intensity exercise, on muscle activation levels and perceived exertion during prolonged steady-state low-intensity exercise, as well as endogenous substrate utilization and circulating substrate availability. The design of this study was such that it allowed differentiation between the effects of physiological levels of humoral Epi, from the

exercise response itself. Previous studies have examined the role of Epi during high-intensity exercise, and have infused Epi to levels 2-6 times that of normal exercising levels while keeping the exercise intensity constant. In contrast, in the present study, the trained subjects cycled at a low intensity (34% VO_{2max}) and Epi was infused to levels to match those measured during moderate-intensity exercise (68% VO_{2max}).

The first important finding of this study was that there were no changes in muscle activation levels as a consequence of raised circulating Epi. EMG amplitude was only significantly greater during exercise at 68% VO_{2max} (MOD 68% Sal) compared to trials at 34% VO_{2max} with or without Epi infusion (Figure 5). In Chapter 2 of the present thesis, we demonstrated that an antecedent bout of single-limb exercise to fatigue resulted in elevated plasma epinephrine concentrations during a subsequent bout with the previously rested leg. The elevated epinephrine concentrations were associated with increased muscle activation levels, but no differences in heart rate, oxygen consumption or RPE over the course of the exercise bout. A possible relationship between the increase in epinephrine concentrations and the elevated muscle activation levels was discussed (Chapter 2), and therefore, the present study aimed to explore this possibility further. The present results suggest that such a relationship is not due to circulating catecholamines alone, since the Epi infusion did not influence EMG activity during cycling. We therefore speculated that in the absence of direct SNS activation, the humoral effects of increased Epi concentrations were not associated with changes in muscle activation levels.

The second important finding was that Epi infusion was not associated with altered RPE or physiological parameters, including heart rate and oxygen consumption during exercise at 34% VO_{2max} (Figures 4 and 3a and b, respectively). There was, however, an increase in fuel substrate mobilization (Figure 2a and b). We suggest that although the Epi infusion increased plasma Epi concentrations to those similar to moderate-intensity exercise, it was not sufficient to stimulate a cardiopulmonary response during the low-intensity exercise bout, despite being sufficient enough to alter fuel substrate mobilization.

The finding that RPE was not influenced by Epi infusion is in agreement with the findings of Womack et al (1998), who found that RPE was similar during a constant-power exercise test after a six week training programme, despite Epi infusion sufficient to raise plasma Epi concentrations to supraphysiological levels (~13.2 mmol/l). There was a linear increase in RPE, similar to the previous chapter, during exercise at 68% VO_{2max} (MOD 68% Sal). Therefore, it appears that the rise in RPE is a consequence of the increase in exercise intensity only, and is not directly related to elevated plasma Epi concentrations.

In contrast to the finding in the present study, previous studies have reported significant increases in heart rate with Epi infusion (Febbraio et al, 1998; Kreisman et al, 2000; Watt and Hargreaves, 2002). However, these studies utilized much higher exercise intensities ranging from 50 to 70% of VO_{2max}. Therefore, the effects of epinephrine infusion on the cardiopulmonary response may only manifest when there is a concomitant increase in SNS activation with higher exercise intensities. Furthermore, the Epi infusion in the present study resulted in less than a 2-fold increase in plasma Epi concentrations (Fig 1a). In contrast, previous studies that have shown an effect on cardiopulmonary responses have produced increases in plasma epinephrine concentrations that ranged from approximately 3-fold (Febbraio et al, 1998) to 10-fold (Kreisman et al, 2000; Watt and Hargreaves, 2002).

The current finding that elevated circulating Epi concentrations does not alter perceived exertion or muscle activation may also be attributable to methodological differences between the present study and the previous study. In Chapter 2, single-limb, open loop exercise at a relatively high intensity was used, compared to whole body, closed loop exercise at an intensity that failed to produce volitional exhaustion in any subject in the present study. Indeed, the RPE measured after 90 minutes during the low-intensity exercise at 34% VO_{2max} was only ~ 8. Therefore, it is possible that a higher exercise intensity would have produced differences in perception of effort and physiological variables, as described previously in other studies.

Similar to the findings of previous studies (Chesley et al, 1995; Febbraio et al, 1998; Howlett et al, 1999a; Kjaer et al, 2000; Kreisman et al, 2000; Watt et al, 2001; Wendling et al, 1996), we found that Epi infusion resulted in an increase in the mobilization of fuels, such that plasma glucose and lactate concentrations were elevated (Figure 2a and b). These alterations did not, however, exert any effect on performance ability, as all subjects completed the required 90 minutes of exercise at 34% VO_{2max} at the same RPE values (Figure 4). The RPE measured during exercise at 34% VO_{2max} was thus dissociated from the changes in plasma glucose and lactate concentrations. Previous studies have shown that increases in circulating levels of blood glucose and rates of CHO oxidation following CHO supplementation have caused an attenuation in RPE during prolonged exercise at a given workload (Coggan and Coyle, 1987; Robertson et al, 1990; Burgess et al, 1991; Kang et al, 1996; Utter et al, 1997). In the present study, however, the plasma glucose concentrations were within the euglycaemic range, and therefore, the similar RPE values may be an indication that the RPE is sensitive to blood glucose levels only outside a normal physiological range. Studies have shown that there is a relationship between lactate concentrations and RPE (Gamberale, 1972; Hetzler et al, 1991). In the present study, no such association was found, though this may be attributed to the low exercise intensity and consequent low lactate concentrations produced, since these previous studies have found this relationship at higher intensities only (Kosta and Cafarelli, 1982; Robertson et al, 1986; Swank and Robertson, 1989).

In conclusion, Epi infusion, at levels similar and somewhat higher to those seen with prolonged, moderate intensity exercise (68% VO_{2max}) was associated with increased circulating plasma glucose and lactate concentrations. Increased circulating Epi alone, however, did not change perceived exertion, muscle activation levels or substrate oxidation, or alter the cardiovascular and respiratory responses to low-intensity exercise. It appears that increases in circulating Epi, and the metabolic changes associated with it during low-intensity exercise, are not sufficient afferent feedback for cardiopulmonary and peripheral responses or changes in perceived exertion and muscle activation levels which we found at

moderate- and higher-intensity exercise. Furthermore, these data suggest that at low exercise intensities, muscle activation levels and perceived exertion are not directly associated with elevated plasma glucose and lactate concentrations (an altered metabolic milieu).

CHAPTER 4

THE EFFECT OF ANTECEDENT SHORT TERM HIGH-FAT FEEDING ON PERFORMANCE, PERCEIVED EXERTION AND MUSCLE ACTIVATION LEVELS DURING ENDURANCE EXERCISE

Published in part in: J Appl Physiol Jan:100(1):194-202, 2006. Havemann L, **West SJ**, Goedecke JH, MacDonald IA, St Clair Gibson A, Noakes TD, Lambert EV (2006) Fat adaptation followed by carbohydrate loading compromises high-intensity sprint performance

4.1 INTRODUCTION

The results of Chapter 3 demonstrated that in the absence of direct sympathetic activation, increased circulating epinephrine alone did not alter muscle activation levels, perceived exertion, cardiovascular and respiratory responses, and substrate oxidation. Previous research has shown however, that increased sympathetic activation with a concomitant increase in exercise intensity, is associated with increased 'sensations of fatigue' (Saito et al, 1989), muscle fatigue (Seals and Enoka, 1989), as well as increased heart rate and RER (Kreisman et al, 2000), and increased glucose production and uptake (glycogenolysis) (Kreisman et al, 2000; Febbraio et al, 1998). High-fat feeding has also been associated with increased sympathetic activation (increased epinephrine and norepinephrine concentrations) and perceived exertion, suggesting a possible association between high-fat feeding and integrated brain control mechanisms. Therefore, the present study sought to further explore the relationship between an antecedent metabolic perturbation, in this case a high-fat dietary intervention, and sympathetic activation, perceived exertion and muscle activation levels, and how these perturbations may impact on overall exercise performance.

Numerous studies have demonstrated that short-term high-fat adaptation induces increased sympathetic activation during exercise (Jansson et al, 1982; Sasaki et al, 1991). Furthermore, ingestion of a prolonged high-fat diet (HFD) (7-wk period) has also been associated with increased sympathetic activation (Helge et al, 1996), along with increased perception of effort and reduced exercise capacity (unpublished data, data shown in Helge, 2002), possibly as a consequence of the increased sympathetic activation. While the ingestion of a HFD may result in a greater training capacity (training at a relatively high intensity), studies have shown the cost of a HFD to be an increase in subjective ratings of perceived exertion. Increased perceived exertion has been reported during submaximal exercise (30% - 90% VO_{2peak}) (14.3 ± 0.7 vs 12.6 ± 0.6) (Prusaczyk at al, 1992), as well as during high intensity sprint bouts (~85% VO2peak) (16.0 ± 1.3 vs 13.8 ±

1.8) following 3 days of a HFD compared to 3 days of a high CHO diet (HCD) (Stepto et al, 2002). Although the mechanism remains unclear, it is possible that altered autonomic nervous system activity may contribute to the increased perceived exertion, and therefore the relationship between the increase in fat intake in the diet, sympathetic activation and perception of effort during exercise requires further investigation.

There have been conflicting results with regard to high-fat feeding benefiting exercise performance, where previous studies have shown fat adaptation either enhances performance (Lambert et al, 1994), fails to alter (Goedecke et al, 1999) or impairs exercise capacity (Simonsen et al, 1991; Starling et al, 1997). The inconsistent effects of a high-fat diet on exercise performance may be a consequence of the various methodological designs utilized and the impracticality of the diet duration (2- to 7-wk periods) for subjects to maintain. A recent dietary strategy involving a period of fat-loading (5-6 days), followed by 1 day of carbohydrate (CHO) loading prior to an exercise event, has shown to increase fat oxidation, increase muscle glycogen stores and reduce muscle glycogen utilization during exercise (Burke et al, 2000; Burke et al, 2002; Carey et al, 2001). This strategy however, has only been tested under time-trial conditions (~25 min – 1hr) following prolonged submaximal steady-state exercise and have resulted in no overall improvements in performance. Since this particular nutritional strategy optimizes muscle glycogen stores (predominant fuel during high-intensity exercise) and promotes glycogen sparing, it would be an ideal method to investigate performance during endurance exercise that simulates actual race conditions, which includes high intensity sprints (>85% VO_{2peak}).

Therefore, the aim of this study was to examine the effect of an antecedent high-fat diet followed by 1 day of CHO-loading on heart rate variability as a proxy measure of sympathetic activation, perceived exertion, muscle activation levels and performance during a self-paced 100-km cycling time-trial including high-intensity sprints that simulates actual race conditions. A self-paced exercise model was selected since this allows subjects to alter work rate in response to factors including RPE, energy availability and sympathetic activation. In Chapter 2

and Chapter 3, we utilized fixed work rate protocols, and found no differences in muscle activation as a result of antecedent fatiguing exercise or epinephrine infusion. By allowing subjects to self-select their power output, we aimed to examine whether a high fat intake and resultant sympathetic activation exerted effects on performance that were mediated by a neural control strategy. It has previously been shown that differences in muscle activation, measured using EMG techniques, are associated with changes in performance (Tucker et al, 2004; Morrison et al, 2004).

4.2 METHODOLOGY

4.2.1 Subject Selection

Eight endurance-trained male cyclists were recruited to participate in the study. The study was approved by the Ethics and Research Committee of the Faculty of Health Sciences of the University of Cape Town. The data collected for this study formed part of a larger study, and therefore, the plasma lactate and FFA's, and the VO2, VCO2 and RER data are reported elsewhere (PhD thesis, Ms Lise Havemann), and will not be reported in this thesis. The EMG data and its relationship with perceived exertion and exercise performance, however, remains unique to this thesis.

To participate in the study, subjects had to i) have completed a local 109 km cycle race in less than 4.5hrs and ii) have no known metabolic conditions that may adversely affect intermediary metabolism. Subjects were excluded if they were taking any medications for chronic conditions such as high-blood pressure or asthma (e.g. β-adrenergic receptor antagonists). The risks and benefits of the trial were carefully explained to the subjects and their informed consent was obtained in writing before participation in the trial. The subject characteristics are presented in Table 1.

Table 1: Subject characteristics of the experimental sample (n=8).

	Experimental Sample
Age (yrs)	26.0 ± 3.3
Weight (kg)	81.3 ± 9.6
Height (m)	1.8 ± 0.1
Body fat (%)	14.0 ± 2.8
VO_{2peak} (ml/kg/min)	57.8 ± 5.5
W_{peak} (W)	361.0 ± 35.5

Values are expressed as mean ± SD.

4.2.2 Preliminary Testing

All preliminary testing methods performed in the present study, which included an anthropometrical assessment and the determination of peak power output (W peak) and peak oxygen consumption (VO_{2peak}), was similar to the methodology described previously in Chapter 3.

4.2.3 Experimental Design

Each subject performed two trials in a randomised, single blind crossover design separated by a two-week washout period. Both trials required subjects to complete an 8-day diet, training sessions and performance testing. Subjects visited the laboratory on days 1, 3, 5, 7 and 8 to perform the training and performance testing.

4.2.3.1 Dietary Control

Each subject previously completed a 3-day dietary record to determine the subjects' energy intake and macronutrient consumption. Subjects were required to ingest either a high-fat diet (~68% energy from fat) for 6 days followed by 1 day of

carbohydrate (CHO) loading (~90% energy from CHO), or a CHO diet (~68% energy from CHO) for 6 days followed by 1 day of CHO loading (~90% energy from CHO) (Table 2). The 1 day of CHO intake was to restore muscle and liver glycogen stores. The purpose of the dietary strategy chosen was to enhance the contribution from fat to oxidative metabolism during exercise thereby sparing muscle glycogen without compromising pre-exercise endogenous CHO stores (Hawley et al, 1998). A registered dietician prepared individualised meal plans. Pre-packed meals were provided to the subjects to control dietary intake. Subjects were asked to honestly record any deviations from the diet. The macronutrient compositions of the diets were covertly manipulated in an effort to blind the subjects regarding the contents of the diets.

Table 2: Mean dietary intake during HFD and HCO treatments (n=8).

	Energy (cal)	CHO			FAT			PROT		
		g	g/kg	%E	g	g/kg	%E	g	g/kg	%E
HFD	3560	150*	1.85*	16.8*	270*	3.33*	68.2*	134	1.65	15.0
	±246	±14	±0.10	±0.6	±18	±0.16	±0.6	±8	±0.09	±0.5
HCD	3550	602*	7.48*	67.8*	68*	0.83*	17.1*	134	1.66	15.1
	±206	±32	±0.46	±0.7	±6	±0.03	±0.6	±10	±0.10	±0.4

Values are expressed as mean ± SD. *Significant difference between HFD and HCD ($p<0.001$). CHO, Carbohydrate; E, Energy; HFD, High-Fat Diet; HCD, High Carbohydrate Diet.

4.2.3.2 Exercise Training

Subjects visited the laboratory on days 1, 3 and 5 after a 10-12 hr fast to perform an exercise training session. On day 1, subjects completed a 100-km familiarization time trial (TT) on the subjects own bicycles, mounted on a Kingcycle Trainer (EDS Portaprompt, Ltd, UK). The calibration and reliability of the Kingcycle has been previously described (Palmer et al, 1996). The TT included a series of high-intensity bouts, five 1-km sprints at 10, 32, 52 72 and 99 km and four 4-km sprints at 20, 40, 60 and 80 km. The subjects were instructed to

complete the sprints as well as the total distance in the fastest time possible. No feedback was given to the subject during the TT except for their elapsed distance. The familiarization TT functioned as a screening trial to ensure the subjects were adequately trained to be able to complete the trial, and to ensure a relatively homogenous group, since training status is known to influence the metabolic response to dietary manipulations (Goedecke et al, 2000).

On days 3 and 5, subjects performed a 60 minute steady-state cycle (SS) at 70% VO_{2peak} on an electronically braked cycle ergometer (Lode, Groningen, Holland). The SS training sessions were performed to collect data regarding heart rate variability (described later), to monitor their physiological and metabolic responses to the dietary interventions, and to ensure the subjects' training was consistent during the trial. This was considered important since differences in training during the adaptation phase of the diet may have resulted in a variety of individual responses.

4.2.3.3 Performance Testing

On day 7, subjects visited the laboratory after a 10-12 hour overnight fast to perform a 60 minute SS cycle at 70% VO_{2peak}. Prior to the exercise test, HRV was recorded as described below. During the steady-state exercise, electromyography (EMG) and ratings of perceived exertion (RPE) (described below) were measured every 15 minutes. Blood samples were also drawn every 15 minutes for the subsequent analysis of plasma glucose (described below). On day 8, subjects (CHO loaded) again reported to laboratory after an overnight fast to complete a 100-km performance TT. HRV was again measured prior to the 100-km TT. A blood sample was taken immediately prior to the 1-km sprints at 32, 52, 72 and 99 km for the subsequent analysis of plasma glucose and catecholamine concentrations. RPE was recorded before and after every 1-km and 4-km sprint. EMG activity was measured during the midpoint of the 1-km sprints (10.5, 32.5, 52.5, 72.5 and 99.5 km), the midpoint of the 4-km sprints (22, 42, 62 and 82 km) and at three non-sprint distances (5, 55 and 95 km) during the 100-km TT. Power output and heart rate were measured continuously throughout the trial. Subjects

ingested a 10% glucose polymer solution (200ml every 20 minutes) during both trials (100-km TT) to maintain euglycaemic levels.

4.2.3.4 Measurements

Heart rate variability (HRV) was recorded prior to the 100-km TT's and the steady-state exercise trials using a heart rate monitor (Body IQ, South Africa). HRV has been implicated as an indirect measure of autonomic nervous system activation (Ori et al, 1992). Heart rate measurements were recorded during the HRV test, while subjects breathed rhythmically (12 breaths/min) for 5 minutes of supine lying, followed by 5 minutes of standing. Power spectrum analysis for low frequency [LF] (indicative of sympathetic activation) and high frequency [HF] (indicative of parasympathetic activation) was performed based on the HRV interval, using MATLAB™ software (The MathWorks Inc.). The natural logarithms of LF and HF power, as well as the ratio of LF power to HF power were calculated from the power spectrum values. All the other measurements, including blood sampling, heart rate, perceived exertion and EMG activity, were performed as previously described in Chapter 3.

4.2.4 Statistical Analysis

All data are presented as means ± standard deviations. A two-way analysis of variance (ANOVA) with repeated measures was used to compare variables during the performance testing between the two experimental diets, and to assess significant main effects and interactions. Where significant differences occurred, a Tukey's HSD post hoc analysis was used to examine the differences. Statistical significance was accepted when $p<0.05$.

4.3 RESULTS

4.3.1 HRV during Exercise Training and Performance Testing

The mean normalized heart rate variability values for low frequency (LF), reflecting sympathetic modulation for supine and standing, during exercise training and performance testing are presented in Table 3. There were no significant differences between responses to the HFD and HCD for high frequency (HF) or LF:HF ratio (data not shown). Although not significant, there was a tendency towards a significant diet effect (p=0.056) for the LF supine values (Table 3).

Table 3: Mean resting heart rate variability (expressed as the natural log [ln]) in response to the HFD and HCD of the experimental sample (n=8).

	Day 1	Day 3	Day 5	Day 7	Day 8	P value
LFsup						
HFD	6.65±0.73	6.31±0.75	6.67±1.10	6.12±1.13	6.21±0.73	NS (0.056)
HCD	5.78±0.59	6.26±0.92	5.87±0.70	5.27±0.92	6.07±1.15	
LFstand						
HFD	6.90±1.55	7.07±0.90	7.68±0.84	7.19±0.89	7.14±0.63	NS
HCD	7.04±1.11	6.92±1.03	7.18±1.06	6.98±0.97	7.03±0.93	

Values are expressed as mean ± SD. LFsup, low frequency supine; LFstand, low frequency standing; HFD, high-fat diet; HCD, high carbohydrate diet; NS, not significant.

4.3.2 Steady-state (SS) Cycle – Day 7

The mean exercising data of the SS cycle is presented in Table 4. Plasma glucose concentrations ranged from ~ 3.9 to 4.5 mmol/L and euglycaemia was similarly maintained throughout exercise in both HFD and HCD trials. Heart rate rose progressively ($p<0.001$) during exercise, but was significantly higher during the HFD trial ($p<0.05$). The RPE was similar between dietary treatments and

increased significantly ($p<0.001$) during the steady-state exercise. The EMG amplitude of the HFD trial decreased significantly over time ($p<0.05$) but was not different between the trials.

Table 4: Mean exercising values during the steady-state cycle of the experimental sample (n=8).

	15 min	30 min	45 min	60 min	P value
Heart rate (bpm)					
HFD	149 ± 9	152 ± 7	156 ± 7	158 ± 7	<0.05 trial
HCD	143 ± 11	148 ± 8	151 ± 6	154 ± 8	<0.001 time
RPE					
HFD	12 ± 2	13 ± 1	14 ± 1	14 ± 2	<0.001 time
HCD	11 ± 2	12 ± 1	13 ± 1	13 ± 1	
Normalised EMG amplitude (%)					
HFD	36 ± 13	29 ± 7	27 ± 9	25 ± 9	<0.05 time
HCD	37 ± 8	32 ± 11	35 ± 13	32 ± 8	

Values are expressed as mean ± SD. RPE, ratings of perceived exertion; HFD, high-fat diet; HCD, high carbohydrate diet; NS, not significant.

4.3.3 Performance 100-km TT – Day 8

The performance time of the 100-km TT was not significantly different between trials ($p=0.234$) (156 min 54 sec vs 153 min 10 sec, HFD-CHO vs HCO-CHO) (Figure 1a). Three out of the eight subjects improved their performance on the HFD-CHO, with no order effect observed ($p=0.28$) (Figure 1b).

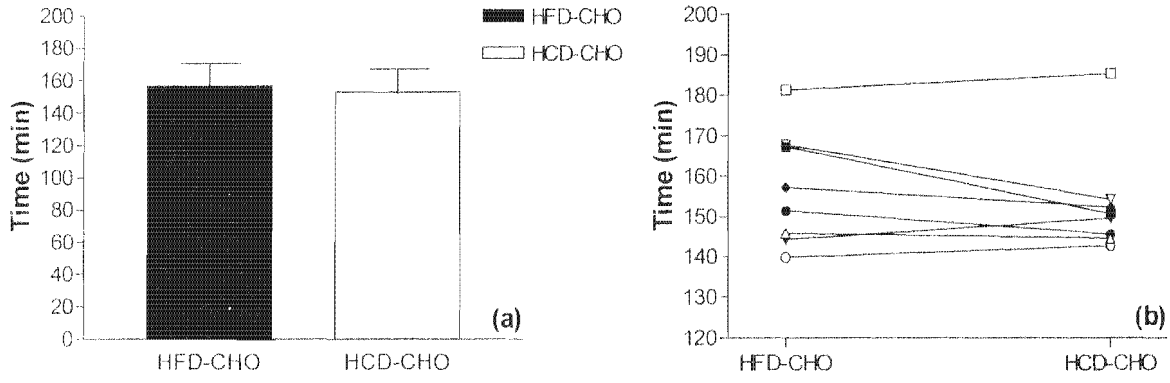


Figure 1: a) Overall performance time and b) individual performance times of the 100-km TT on Day 8 of the experimental sample n=8). Values are expressed as mean ± SD.

Plasma glucose concentrations were not significantly different between HFD-CHO and HCD-CHO and subjects remained euglycemic throughout the 100-km time-trial. Plasma catecholamine concentrations increased significantly during both trials ($p<0.001$), but were also not different between the two dietary treatments.

There were no significant differences during the 4-km sprints between the HFD-CHO and HCD-HCO as presented in Table 5. The mean power output and sprint times of the 4-km sprints decreased significantly over time during both HFD-CHO and HCD-HCO trials ($p<0.01$ and $p<0.05$, respectively). Conversely, RPE measured immediately after 4-km sprints increased similarly over time ($p<0.001$) in both HFD-CHO and HCD-HCO trials.

Table 5: Variables measured during the 4-km sprints of the experimental sample (n=8).

	20 km	40 km	60 km	80 km	P value
Power (watts)					
HFD-CHO	289 ± 50	291 ± 50	279 ± 50	268 ± 48	<0.01 time
HCD-CHO	308 ± 56	308 ± 61	305 ± 62	295 ± 55	
Sprint times (sec)					
HFD-CHO	336 ± 26	338 ± 26	340 ± 24	347 ± 29	<0.05 time
HCD-CHO	327 ± 27	330 ± 31	328 ± 28	335 ± 31	
Heart rate (bpm)					
HFD-CHO	166 ± 6	166 ± 8	167 ± 6	168 ± 7	NS
HCD-CHO	166 ± 7	166 ± 9	164 ± 10	166 ± 9	
RPE					
HFD-CHO	17 ± 2	18 ± 1	18 ± 2	19 ± 1	<0.001 time
HCD-CHO	16 ± 3	17 ± 2	18 ± 2	18 ± 2	
Normalised EMG amplitude (%)					
HFD-CHO	34 ± 13	31 ± 11	31 ± 13	32 ± 12	NS
HCD-CHO	31 ± 10	28 ± 6	31 ± 5	29 ± 8	

Values are expressed as means ± SD. RPE, ratings of perceived exertion; HFD-CHO, 6d high-fat diet + 1d carbohydrate loading; HCD-CHO, 6d high carbohydrate diet + 1d carbohydrate loading; NS, not significant.

In contrast to the 4-km sprint performance, the mean power output during the 1-km sprints was significantly lower after HFD-CHO compared to HCD-CHO ($p<0.05$, time x trial interaction) (Figure 2a). Consequently, the 1-km sprint times tended to be slower following the HFD-CHO compared to the HCD-CHO treatment ($p=0.07$). The heart rate was comparable for both trials (Figure 2b). RPE measured immediately after the 1-km sprints rose progressively over time ($p<0.01$) but was not different between dietary treatments (Figure 2c). Normalised EMG amplitude measured during the 1-km sprints was also similar for both trials (Figure 2d).

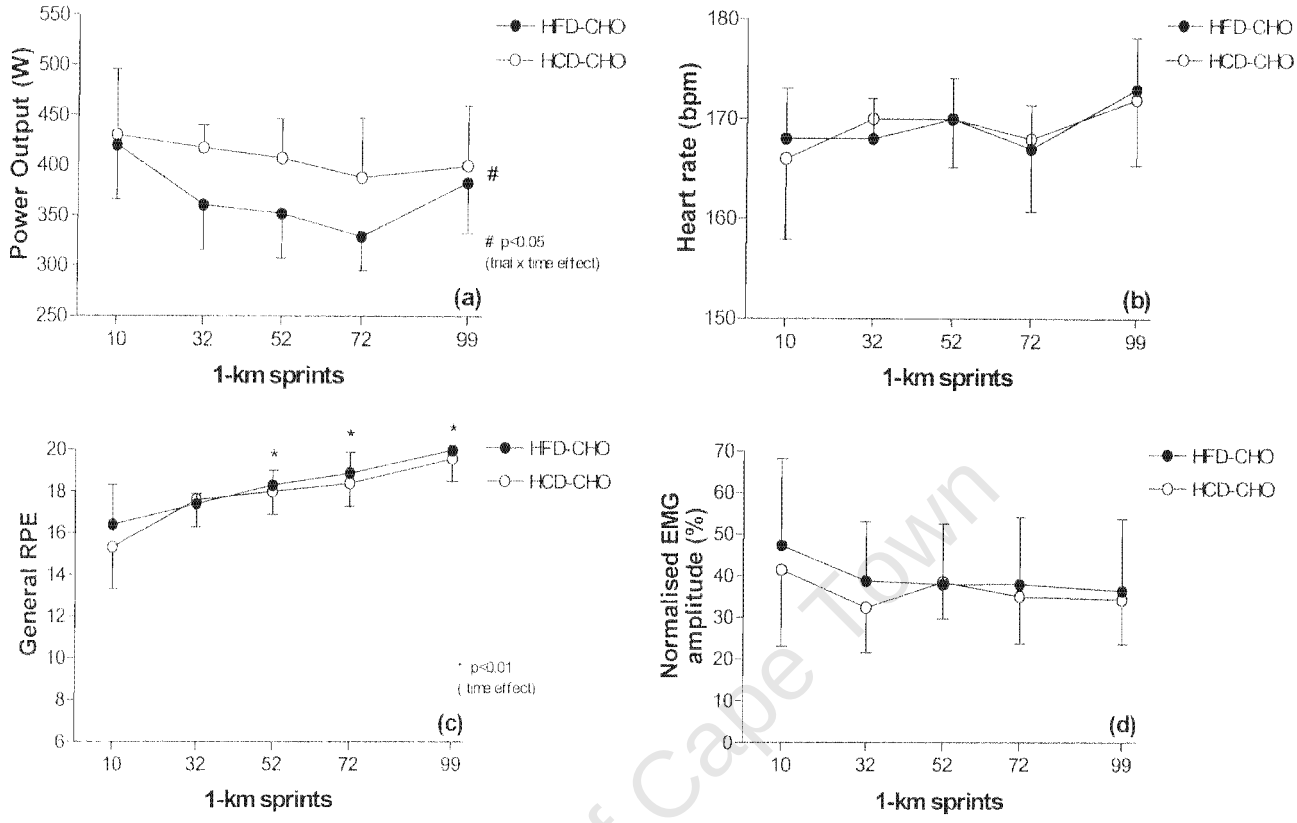


Figure 2: a) Power output (W), b) heart rate (bpm), c) RPE and d) normalised EMG amplitude during the 1-km sprints of the 100-km TT on Day 8 of the experimental sample n=8). Values are expressed as mean ± SD.

4.4 DISCUSSION

In the present study, we examined the effect of an antecedent high-fat diet, followed by 1 day of CHO-loading, on perceived exertion, muscle activation levels and performance during endurance exercise that simulated a race situation. To our knowledge, this is the first study to investigate the effect of high-fat feeding, followed by CHO-loading, using an exercise protocol that included high intensity sprint bouts, as well as exploring the effect of antecedent dietary manipulation (high-fat diet) on muscle activation levels during exercise. The main finding of this study was that high-intensity sprint performance was compromised after exposure to the HFD-CHO dietary treatment, possibly by increased sympathetic activation

or altered contractile function, or altered neural commands. The overall exercise performance, however, was not affected by antecedent high-fat feeding, despite impaired high-intensity sprint performance during the 1-km sprints.

Overall performance of the 100-km TT, as well as the 4-km sprint performance times, were not significantly different between dietary treatments which is consistent with other studies investigating the effects of high-fat feeding on exercise performance using a similar dietary intervention protocol to the present study (CHO restoration diet before exercise). Burke et al (2000) (2002) and Carey et al (2001) both showed no benefit of fat adaptation during a 7kJ/kg TT performance (~ 30 min) after a 2 hour submaximal steady-state cycle and a 1 hour TT performance following a 4 hour submaximal steady-state cycle, respectively. They did, however, show individual response differences following the high-fat diet with five of the seven subjects improving performance (Burke et al, 2000; Carey et al, 2001).

No previous studies have used high intensity exercise bouts within performance trials simulating actual race conditions. The mean power output in both studies by Burke et al (2000) (2002) during the 7kJ/kg TT after fat adaptation was 281 W (76% of PPO) and 302 W (76% of PPO) respectively, and 312 ± 15 W (77.4% of PPO) during the 1 hour TT of Carey et al (2001). The present study included 1-km sprints in the endurance TT which were performed at a power output > 90% PPO. In contrast to our original hypothesis, the high intensity 1-km sprint power output was actually compromised by the high-fat dietary treatment. The power output during the high intensity 1-km sprints of the 3 subjects whose overall 100-km TT performance was improved by the high-fat feeding was also compromised.

The impaired 1-km sprint performance in HFD-CHO may be related to changes in sympathetic activation associated with the high-fat feeding. This is reflected in the increase in low frequency power spectrum for heart rate variability following the high-fat intake that persisted after 1 day of CHO-loading ($p=0.056$) (Table 3). Heart rate variability has been shown to be a practical, non-invasive and reliable measure of sympathetic modulation (Goldberger, 1999). Furthermore, heart rate

was significantly higher during the steady-state cycle on Day 7 following the HFD (Table 4), and similar during the 1-km sprints of the 100-km TT on Day 8 (Figure 2b) despite reduced power output, suggestive of increased sympathetic activation during the HFD-CHO trial. Studies have previously reported an increase in sympathetic activation, as measured by plasma norepinephrine, during exercise following high-fat feeding (Jansson et al, 1982; Sasaki et al, 1991; Helge et al, 1996), as well as an association with increased perceived exertion (Prusaczyk et al, 1992; Helge et al, 1996; Stepto et al, 2002). In the present study, the RPE values were similar immediately following the 1-km sprints for both dietary treatments, despite the reduced power output in the HFD-CHO trial, which suggests increased perception of effort for less power produced (Figure 2c).

In Chapter 3 of the present thesis, it was found that neither performance nor RPE were influenced by epinephrine infusion. However, in that chapter, the exercise intensity was very low (34% VO_{2max}) and the work rate was not free to vary. It was suggested that the low exercise intensity contributed to the absence of an effect of elevated epinephrine concentrations. In the present study, subjects were able to subjectively alter power output, and maintained average work rates of approximately 70% of PPO, increasing up to almost 100% of PPO during the 1 km sprints. Consequently, alterations in sympathetic activation due to high-fat feeding may have exerted more of a measurable effect on power output than in Chapter 3. Also, the epinephrine infusion in Chapter 3 resulted in increased humoral levels only, whereas in the present study, sympathetic activation may have been altered by the HFD, as suggested by the heart rate variability.

The EMG amplitude was similar between dietary treatments, and failed to track the change in power output during the 1-km sprints. This suggests that the impaired power output in HFD-CHO trial occurred despite similar levels of muscle activation in the measured muscle group (Figure 2d). In contrast to our findings, St Clair Gibson et al (2001c) reported no effect of dietary manipulation on neuromuscular activity during a self-paced 100-km TT and showed similar reductions in power output and EMG activity during the 1-km and 4-km sprints between the placebo and CHO-loading exercise trials. More recently, however,

Nikolopolous et al (2004) showed that CHO ingestion immediately before and during cycling to fatigue at a fixed work rate, significantly attenuated the increase in surface EMG activity, but attributed this effect to changes in afferent sensory input. It is not clear how high-fat feeding altered power output without a change in muscle activation during the present study, but it may be related to the increased sympathetic activation associated with high-fat intake. It has previously been shown that an increase in sympathetic activation is associated with increases in EMG activity, however this measure was during static and not dynamic exercise (Seals and Enoka, 1989). It is also possible that the unchanged muscle activation, despite reduced power output, was the result of the development of peripheral fatigue due to altered contractile function and/or metabolic substrate perturbations (Hakkinen and Komi, 1983).

There was an increase in power output during the final 1-km sprint in both the HFD-CHO and HCD-CHO trials, even though the EMG amplitude did not track the increase in power output. In this trial, muscle activation was only measured from the vastus lateralis muscle and therefore, it is possible that the muscle activation of the entire quadriceps femoris was altered as subjects fatigued, and the maintenance or increase in power output during the last high intensity sprint was the result of motor unit rotation/substitution (Westgaard and De Luca, 1999) or additional activation of non measured synergistic muscles (Akima et al, 2002; Dimitrijevic et al, 1992). The increased power output during the final 1-km sprints suggests the presence of a motor unit reserve during the earlier part of the trial, when power outputs were different between dietary treatments. This pacing strategy, even though subjects were verbally encouraged to exercise as hard as possible during each sprint, may be indicative of a teleoanticipatory central control system (Ulmer, 1996).

According to this theory, the subjects would control the metabolic rate through the altering of muscle performance. In the present study, the impairment of sprint performance may be due to metabolic changes induced by the antecedent high-fat diet. Because this study employed a closed loop self-paced exercise protocol, subjects were able to alter their power output during the 1-km sprints. This is an

important difference between the present study and Chapter 2 and 3 of the thesis where power ouput was not free to vary.

Finally it is notable that the RPE values measured during the sprints were not different between dietary treatments, despite differences in power output. The RPE therefore is not simply a measure of exercise workrate but rather incorporates afferent feedback including factors such as metabolic and sympathetic activity. Had the power output been increased in HFD-CHO to reach levels similar to those measured in HCD-CHO, the RPE would have presumably been higher, possibly resulting in a limiting maximal RPE being achieved before the known end point of exercise. This would have impaired overall exercise performance. The reduced power output in HFD-CHO is therefore responsible for the subject's ability to complete the bout without attaining a limiting RPE. Therefore, we propose that a central "programmer" regulates RPE, muscle performance and hence physiological systems to ensure that the subjects complete the exercise bout without premature fatigue or bodily harm (Noakes and St Clair Gibson, 2004; Noakes et al, 2004; St Clair Gibson and Noakes, 2004).

In conclusion, ingestion of an antecedent HFD for 6 days, followed by 1-d of CHO-loading, did not effect overall exercise performance but impaired high-intensity sprint performance, despite similar levels of muscle activation, effort perception and heart rate. The relationship between self-paced exercise performance and these variables was thus altered during the 1-km sprints. The mechanism associated with the decrement in sprint performance is not clear, but could possibly be related to increased sympathetic activation, or altered contractile function. The ability to increase power output at the end of the trial suggests the presence of a motor unit reserve during the earlier part of the trial, when the power outputs were different between dietary treatments. Furthermore, reduced power output during the 1-km sprints following antecedent high-fat feeding did not affect overall exercise performance which suggests a compensatory increase in performance during the non-sprint cycling sections. Further research is required to investigate mechanisms associated with high-fat feeding and compromised high-intensity sprint performance.

CHAPTER 5

THE EFFECT OF ANTECEDENT HYPOGLYCAEMIA ON PERCEIVED EXERTION, MUSCLE ACTIVATION LEVELS AND PERFORMANCE DURING STEADY-STATE AND SELF-PACED EXERCISE

5.1 INTRODUCTION

In the previous chapter, we demonstrated that antecedent short-term exposure to a high-fat diet, even with 1 day of CHO repletion, resulted in reduced performance during high-intensity 1-km sprints. Furthermore, this occurred, despite no change in perceived exertion, muscle activation levels, heart rate and overall exercise performance. Indirect evidence suggests that the high-fat feeding resulted in increased sympathetic activation, which may have contributed to a change in "pacing" strategy. The present study sought to investigate the effects of antecedent hypoglycaemia, which is not uncommon for athletes to experience in a fasted state or during prolonged endurance exercise, on subsequent exercise performance, muscle activation levels and perceived exertion.

Recurrent hypoglycaemia has been shown to reduce an individual's ability to recognise symptoms of hypoglycaemia (Bolli, 1999). This phenomenon is known as "hypoglycaemia unawareness" and has most often been described in type I diabetic patients (Davis et al, 1992), as well as being described in normal healthy individuals (Heller and Cryer, 1991). Antecedent periods of hypoglycaemia have also been shown to reduce or blunt subsequent counter-regulatory responses to hypoglycaemia (Heller and Cryer, 1991; Davis et al, 2000b). The metabolic, neuroendocrine and autonomic nervous system counter-regulatory responses to hypoglycaemia are similar to that of an acute, high-intensity bout of exercise, and may therefore also blunt counter-regulatory responses to subsequent physiologic stresses. Recent studies demonstrated that antecedent hypoglycaemia (Davis et al, 2000a) and antecedent prolonged exercise (Galasseti et al, 2001a; 2001b) blunt neuroendocrine and metabolic responses to a subsequent bout of exercise and hypoglycaemia, respectively, in normal healthy individuals.

Substantial research has been done examining the ability of fuel substrates, for example carbohydrate, to enhance exercise performance and attenuate the fatigue process (Coyle et al, 1986; Brewer et al, 1988; el Sayed et al, 1997). However, the mechanisms by which performance is improved by fuel substrate

manipulation are unclear. In view of this, there is still much debate on the effect of alterations in blood glucose concentrations on exercise performance. Many studies have found that preserving blood glucose concentrations enhances performance or prolongs exercise time to fatigue (Coggan and Coyle, 1987; Bosch et al, 1993; Febbraio et al, 2000), where others have showed that the prevention, or reversal of hypoglycaemia (plasma glucose <2.5 mmol/L) during exercise failed to improve endurance capacity (Felig et al, 1982; Jentjiens et al, 2003).

More recently, research from our laboratory showed that CHO supplementation during prolonged exercise had an ergogenic effect in subjects with decreased glycogen content, however, this effect was variable between individuals (Claassen et al, 2005). Furthermore, this study observed an inverse relationship between exercise duration and blood glucose concentrations at exhaustion during a placebo trial where subjects, who became the most hypoglycaemic, exercised for the longest duration. Although the individual variability was large, it suggests that the effects of hypoglycaemia are disparate.

While previous studies have focused on the role of fuel substrate availability in the development of fatigue during prolonged exercise, recent research has suggested a possible contribution from neural control mechanisms. Nybo (2003) examined the effect of glucose supplementation on prolonged cycling exercise followed by maximal voluntary isometric contractions. It was found that the placebo trial resulted in the development of hypoglycaemia which impaired cycling performance. Average force production during the isometric MVC was also reduced compared to the glucose trial, and this was associated with reduced CNS activation. In contrast, force output and central activation remained unaffected when euglycaemia was maintained by CHO ingestion. This study provides evidence of the existence of a neural regulatory strategy that alters muscle fiber activation in response to reduced fuel availability. More recently, Nikolopolous et al (2004) demonstrated that carbohydrate ingestion significantly attenuated the rise in surface (EMG) activity in the exercising limb during sustained cycling exercise to fatigue at a fixed power output. This was, however, attributed to

changes in afferent sensory input. Furthermore, carbohydrate ingestion has been shown to lower overall perception of effort during prolonged running (Utter et al, 2004), as well as general overall and leg ratings of perceived exertion during prolonged cycling (Kang et al, 1996). Kang et al (1996) suggested that carbohydrate availability during endurance exercise may be a sensory cue, but that other physiological factors may also influence perceptual intensity at exhaustion.

To our knowledge, no previous studies have investigated the effect of an antecedent exposure to a physiological stress (eg. hypoglycaemia or exercise (Davis et al, 2000a; Galassetti et al, 2001a)) on subsequent exercise performance and neuromuscular activity such as muscle activation levels, as well as perceived exertion in well-trained healthy individuals. It is unclear how the potentially altered counter-regulatory responses may affect exercise performance, muscle activation and perceived exertion. It is possible that antecedent exposure to hypoglycaemia may result in downregulation of the central command, reduced conscious perception of the symptoms of hypoglycaemia (hypoglycaemia unawareness), and therefore, improved overall exercise performance. The interaction, however, between the conscious perception of fatigue, antecedent hypoglycaemia and performance is unknown, and to what it extent it varies between individuals, perhaps based on antecedent exposures.

Therefore, the aim of this thesis chapter specifically was to determine the effect of antecedent hypoglycaemia on perceived exertion, muscle activation and exercise performance in healthy well-trained individuals during a subsequent (next day) bout of steady-state and self-paced exercise.

5.2 METHODOLOGY

5.2.1 Subject Selection

Ten healthy trained male cyclists engaged in regular endurance training were recruited to participate in this study. The study was approved by the Ethics and Research Committee of the Faculty of Health Sciences of the University of Cape Town. The data collected for this study formed part of a larger study, and therefore, the substrate and metabolic data measured during the antecedent hypoglyceamia/euglycaemia clamp and during subsequent exercise trials are reported elsewhere (PhD thesis, Ms Amanda Claassen, 2005), and will not be reported in this thesis. The EMG data and its relationship with perceived exertion and exercise performance, however, remains unique to this thesis.

Subjects were excluded if they had any chronic medical conditions such as hypertension or diabetes, which required medication and may have altered metabolic responses to exercise, or if they were currently taking chronic steroidal medications for conditions such as asthma or allergies. The risks associated with the trial were carefully explained to the subjects and their informed consent was obtained in writing before participation in the trial. The subject characteristics are presented in Table 1.

Table 1: Subject characteristics of the experimental sample (n=10).

	Mean ± SD
Age (yrs)	25 ± 4
Weight (kg)	73 ± 7
Height (cm)	177 ± 7
Body fat (%)	9 ± 3
VO_{2peak} (ml/kg/min)	64 ± 3
W_{peak} (W)	367 ± 26

Values are expressed as mean ± SD.

5.2.2 Preliminary Testing

Subjects were required to fill out a 3-day dietary record, and a questionnaire on training history to determine regular dietary and training habits (data reported elsewhere, Claassen, PhD thesis). The preliminary testing methods, which included an anthropometrical assessment, and the determination of peak power output (W_{peak}) and peak oxygen consumption (VO_{2peak}), were measured as described previously in Chapter 3.

5.2.3 Experimental Design

Each subject performed two trials consisting of two consecutive days of testing in a randomised, single blind crossover design separated by a one-month washout period (Figure 1).

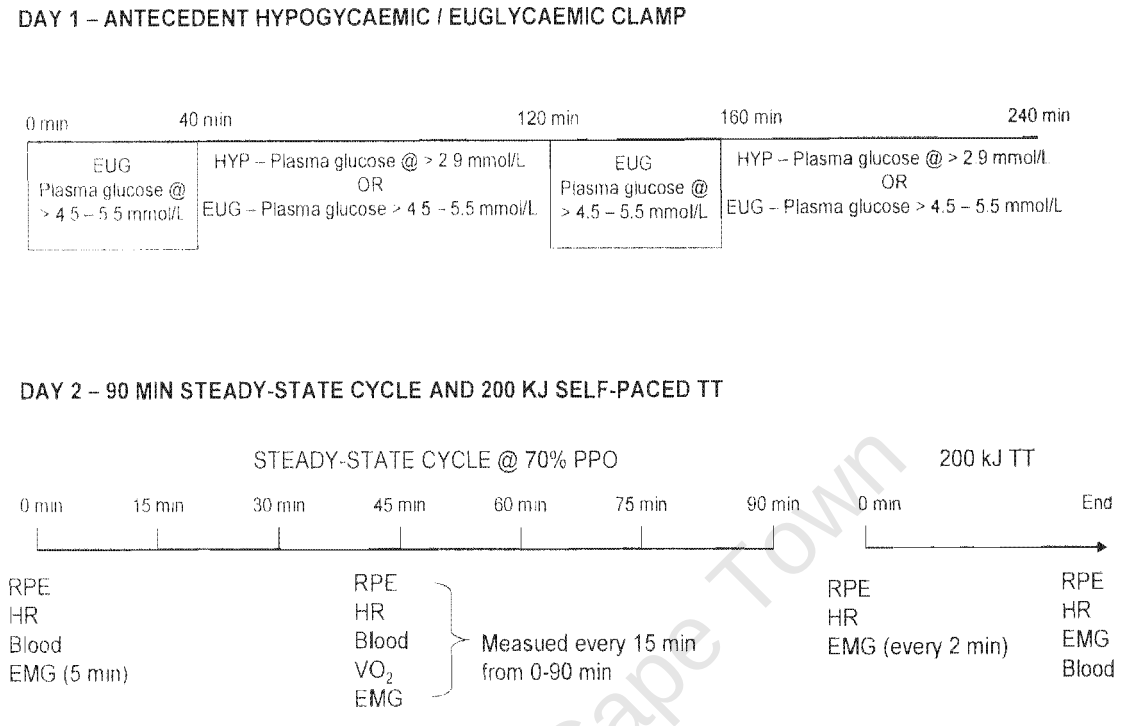


Figure 1: Schematic diagram of the experimental design. EUG, euglycaemia; HYP, hypoglycaemia; TT, time-trial; RPE, ratings of perceived exertion; HR, heart rate; EMG, electromyography.

5.2.3.1 Trial A - Day 1: Antecedent hypoglycaemic exposure

The subjects reported to the laboratory the day before the exercise trial following a 10-12 hr overnight fast. Thereafter, they underwent an initial 40 min bout of "clamped" euglycaemia before the first hypoglycaemic bout. Subjects were then exposed to 2 x 80 min bouts of "clamped" hypoglycaemia, separated by a further 40 min of "clamped" euglycaemia (Davis et al, 2000a; Galassetti et al, 2001a).

The clamp consisted of a prime, constant infusion of insulin with a variable rate of 20% dextrose infusion in order to clamp the blood glucose concentration at the required level. The dose of Actrapid (Nova Nordisk) was calculated according to the subjects weight and mixed into 50 ml sterile saline, the subjects received 1.5 mU/kg/min throughout the clamping procedure. Euglycaemia or hypoglycaemia was attained by frequent blood sampling and adjusting the glucose infusion rate

according to the small changes in plasma glucose concentrations. Every 5 min, less than 1 ml of blood was sampled and then rapidly centrifuged, the plasma sample was then introduced into a Beckman glucose analyzer (Glucose analyzer 2; Beckman Instruments, Fullerton, CA).

Euglycaemia was maintained (plasma glucose at 5.5 mmol/L, range >4.5 mmol/L - ~7 mmol/L) by varying the 20% dextrose infusion rate. The glucose infusion rate was steadily reduced at 39 min to decrease blood glucose to 2.9 mmol/L (± 0.5 mmol/L) to induce the first 80 min bout of controlled hypoglycaemia. After the first 80 min, the glucose infusion rate was increased accordingly to achieve euglycaemia for a period of 40 min, and then decreased again for the second 80 min bout of hypoglycaemia (identical to the first bout).

The subject was medically monitored at all times during the clamp. Intravenous glucose (20% dextrose) was available and connected to the subject for the duration of the clamp, as well as a 50 ml bolus of 50% dextrose (delivered intravenously) and glucagon (1 mg, administered intramuscularly) if the subjects showed severe symptoms of hypoglycemia such as faintness, weakness, sweating, headache, tremor, palpitations and/or blood glucose levels below 2.2 mmol/L. The 1 mg Glucagon (dissolved in 10 ml of 0.9% saline (dilluent)) acts by mobilising hepatic glycogen reserves, and works independently of glucose ingestion. At the end of the trial, glycogen reserves would be replenished with oral glucose (and food intake) once the patient had recovered.

The insulin infusion was terminated at the end of the second bout of hypoglycaemia, and the glucose infusion rate increased to stabilise blood glucose concentrations at euglycaemic levels. After this, subjects received a drink and a CHO-rich meal containing low and high glycaemic index foods. Blood glucose was monitored and subjects were allowed to leave the laboratory once stable blood glucose levels were reached.

5.2.3.2 Trial A – Day 2: Exercise Trial (HYP)

The morning after the glucose clamp procedure, subjects reported to the laboratory again after a 10-12 hr overnight fast to perform a 90 min cycle at 60% of their pre-determined maximum workload capacity (W_{peak}; equivalent to ~ 70% VO_{2peak}), followed by a self-paced 200 kJ time-trial (TT) on a Lode cycle ergometer. The TT was a measure of the subjects exercise performance.

Every 15 min during the 90 min steady-state cycle, the subjects performed a 10 kJ sprint. The sprints and self-paced TT were performed to determine a fatiguing profile and to characterise changes in muscle activation patterns (measured by EMG described below). Ratings of perceived exertion (RPE), heart rate (HR) and VO_2 (described below) were measured every 15 min before the sprints during the steady-state cycle. RPE and HR were measured again before and after the 200 kJ TT. EMG amplitude was measured at 5, 40 and 80 min during the steady-state cycle and during each 10 kJ sprint. During the TT, EMG amplitude was measured every 2 min.

An artificially flavoured drink was provided, *ad libitum,* during the steady-state cycle. No carbohydrate was given to the subjects during the exercise trial, however their blood glucose was measured every 15 min, and the exercise terminated if the subjects blood glucose concentrations decreased below 2.5 mmol/L. Carbohydrate beverages and intravenous glucose were available to restore blood glucose concentrations to euglycaemic levels (~5.5 mmol/L) if necessary.

5.2.3.3 Trial B – Day 1: Antecedent placebo exposure

A month after Trial A, performed in a randomised order, subjects reported to the lab following an overnight fast. Thereafter, they underwent a 240 min, hyperinsulinaemic, euglycaemic clamp (plasma glucose ~5.5 mmol/L) using a primed, constant insulin infusion (at a rate of 9 pmol/kg/min) and a variable 20% dextrose (glucose) infusion rate to maintain plasma glucose at 5.5 mmol/L (same procedure as described above, Trial A, Day 1).

Following the two hyperinsulinaemic, euglycaemic clamps, subjects had to consume a standardised evening meal and snack before returning to the laboratory the following morning for the exercise trial.

5.2.3.4 Trial 2 – Day 2: Exercise Trial (EUG)

Followed the same procedure as Trial A, Day 2.

5.2.4 Measurements

5.2.4.1 Blood Sampling, Heart Rate and VO_2

The blood samples, heart rate and VO_2.were collected as previously described in Chapter 3.

5.2.4.2 Perceived Exertion

The use of Borg's RPE scale has been previously described in Chapter 3.

5.2.4.3 Electromyography (EMG)

The EMG collection and analysis was performed as previously described in Chapter 3.

5.2.5 Statistical Analyses

All data are presented as means ± standard deviations. A two-way analysis of variance (ANOVA) with repeated measures was used to compare variables during the steady-state cycle and TT between the two experimental conditions, and to assess significant main effects and interactions. Where significant differences occurred, a Tukey's HSD post hoc analysis was used to examine the differences.

A dependent t-test was used to evaluate differences before and after the self-paced TT. Statistical significance was accepted when $p<0.05$.

5.3 RESULTS

5.3.1 Sprint Performance during the 90 min Cycle

The 10 kJ sprint times were not significantly different between HYP and EUG trials during the 90 min cycle (Figure 2a) (HYP: 20.65 ± 2.35, EUG: 20.29 ± 2.24 sec, average sprint time). The individuals average sprint times are shown in Figure 2b. Seven out of the ten subjects average sprint performance was better after the HYP exposure (~ 1.02 sec), although this difference was not significant.

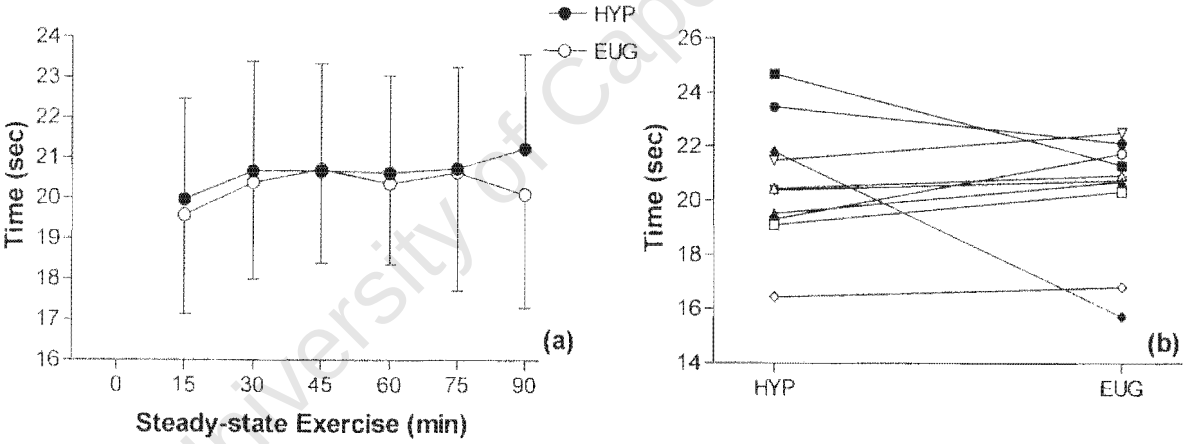


Figure 2: The a) 10 kJ sprint times (sec) and the b) average individual sprint times (sec) during the 90 min cycle of the experimental sample (n=10). Values are expressed as mean ± SD.

5.3.2 Plasma Glucose, Epinephrine and Norepinephrine Concentrations during the 90 min Cycle

The plasma glucose concentrations were similar during both HYP and EUG trials and within the euglycaemic range. Plasma epinephrine concentrations increased

similarly during both trials ($p<0.01$) (Figure 3a), however norepinephrine concentrations were significantly lower during exercise after HYP exposure compared to EUG ($p<0.05$) (Figure 3b).

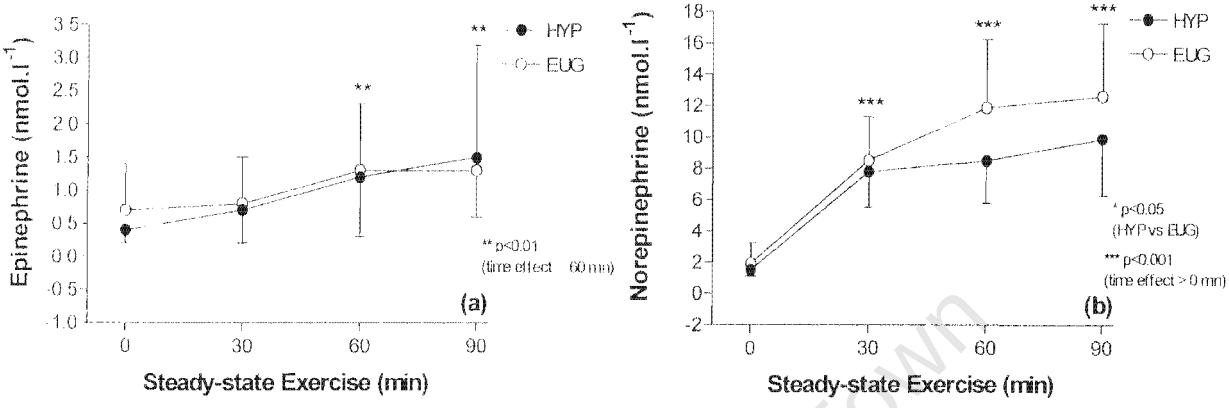


Figure 3: a) Epinephrine and b) norepinephrine concentrations (nmol.l-1) during the 90 min cycle of the experimental sample (n=10). Values are expressed as mean ± SD.

5.3.3 Oxygen Consumption, RER and HR during the 90 min Cycle

Submaximal oxygen consumption was significantly higher after the antecedent hypoglycaemic exposure ($p<0.05$) (Figure 4a). The RER decreased progressively over time during both trials ($p<0.001$) and there was a tendency for a significant interaction effect ($p=0.05$) (Figure 4b). The pre sprint HR rose similarly from 15 min during exercise in both trials ($p<0.001$).

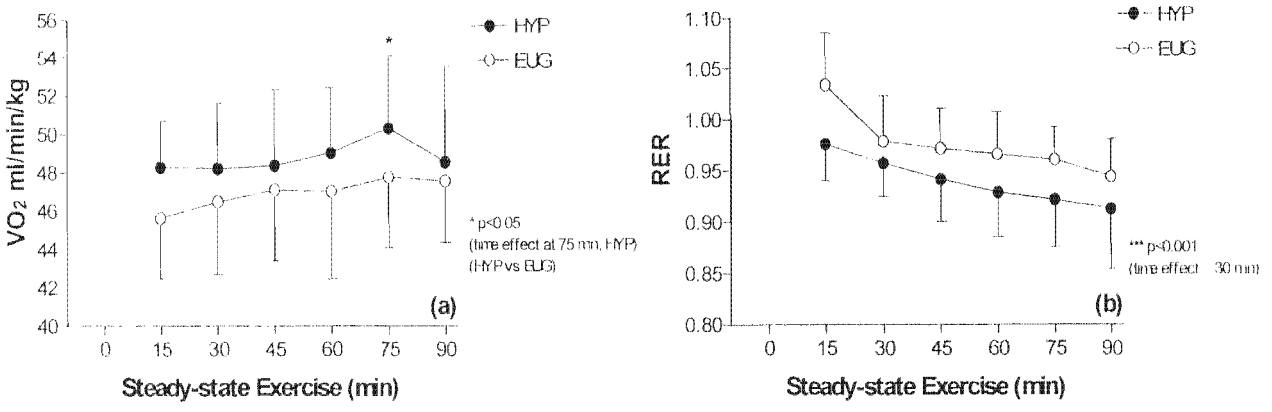


Figure 4: a) VO_2 data (ml/min/kg) and b) RER during the 90 min cycle of the experimental sample (n=10). Values are expressed as mean ± SD.

5.3.4 Perceived Exertion during the 90 min Cycle

There were no significant differences in RPE between HYP and EUG trials, despite differences in oxygen consumption. General RPE increased progressively during the cycle from 6.70 ± 2.21 (HYP) and 6.00 ± 0.00 (EUG) after 15 min of exercise to 14.50 ± 1.58 and 14.50 ± 1.17 at 90 min, respectively ($p<0.001$) (Figure 5a). Head and leg RPE were similar during both trials, and rose progressively over time ($p<0.001$) (Figure 5b and c, respectively).

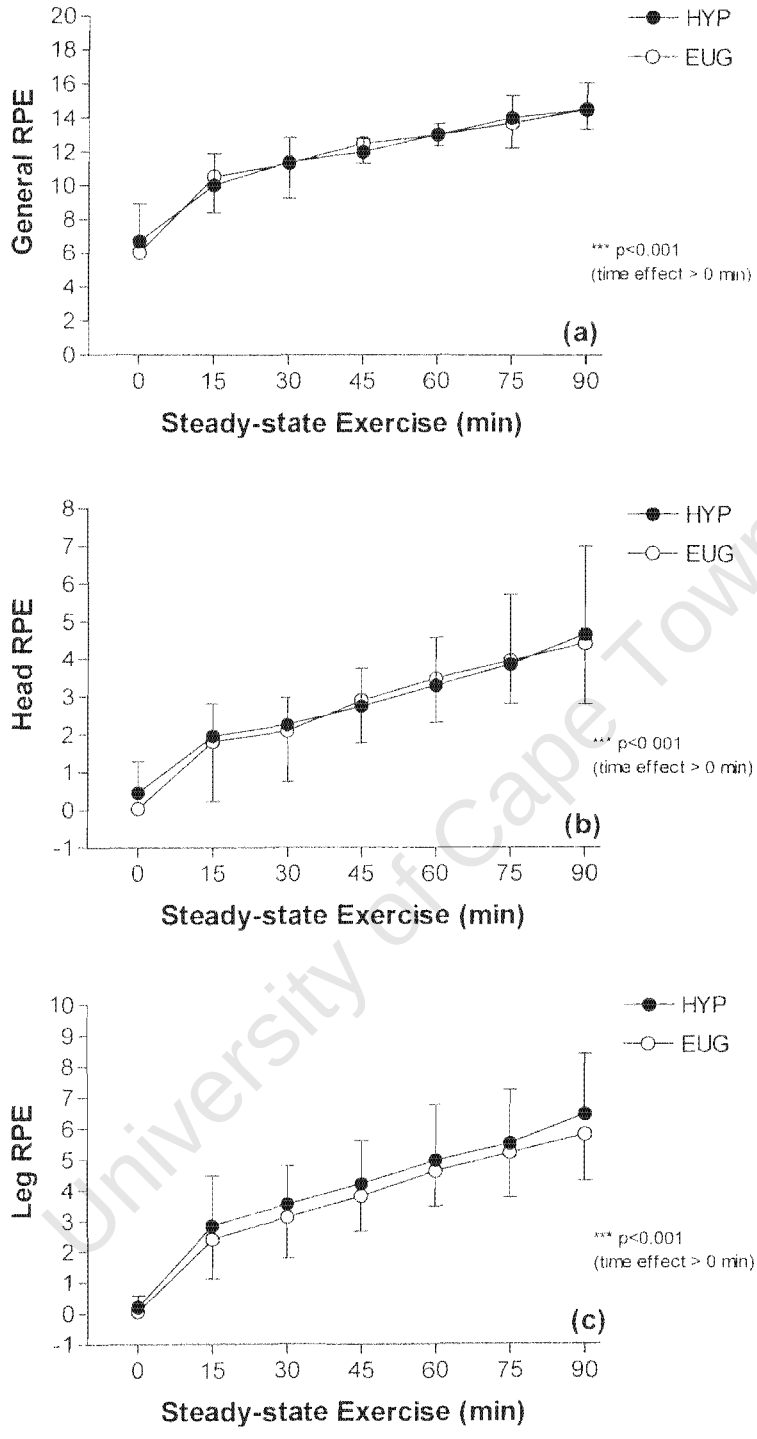


Figure 5: a) General RPE, b) head RPE, and c) leg RPE during the 90 min cycle of the experimental sample (n=10). Values are expressed as mean ± SD.

5.3.5 EMG Amplitude and Power Output during the 90 min Cycle

The non-sprint (steady-state) EMG amplitude measured at 5, 40 and 80 min is shown in Figure 6. EMG amplitude decreased significantly ($p<0.005$) during the EUG trial from 5 min to 40 and 80 min although there were no significant differences in muscle activation levels between trials.

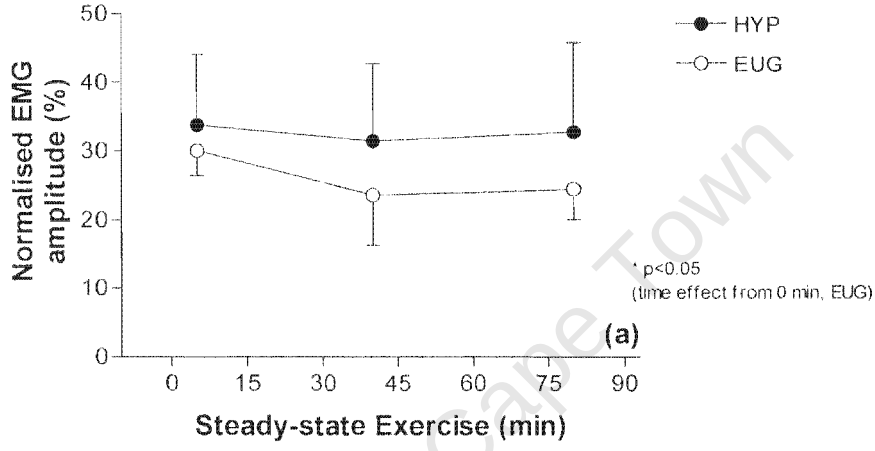


Figure 6: Normalised EMG amplitude (%) during the non-sprints of the 90 min steady-state cycle of the experimental sample (n=10). Values are expressed as mean ± SD.

Similarly, there was no significant difference in EMG amplitude during the 10 kJ sprints. The EMG amplitude decreased by ~ 13.04 % (HYP) and ~ 24.1 % (EUG) ($p<0.005$) from the first sprint to the second sprint, but did not change significantly over the remaining four sprints (Figure 7a). The power output of the 10 kJ sprints was not significantly different between trials (Figure 7b).

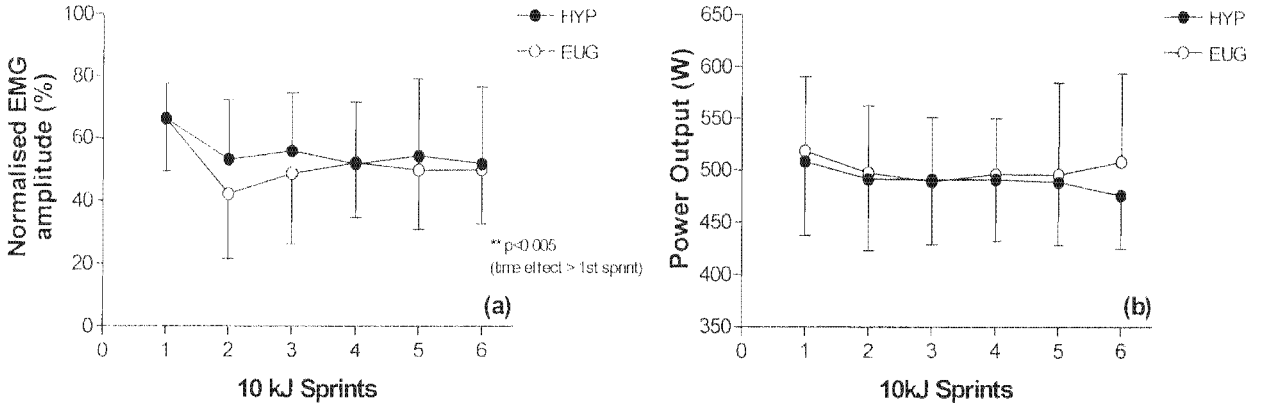


Figure 7: a) Normalised EMG amplitude (%) and b) power ouput (W) during the 10 kJ sprints of the 90 min cycle of the experimental sample (n=10). Values are expressed as mean ± SD.

5.3.6 Performance Time of the Self-paced TT

The performance time of the 200 kJ self-paced TT was similar between HYP and EUG conditions (695.8 ± 64.5 sec vs 704.4 ± 80.3 sec, respectively) (Figure 8a). The subjects individual TT performance is displayed in Figure 8b. Six out of 10 subjects performed better after the HYP exposure (~ 17 sec, n=5; ~ 105 sec, n=1) suggesting the response was variable between the two trials.

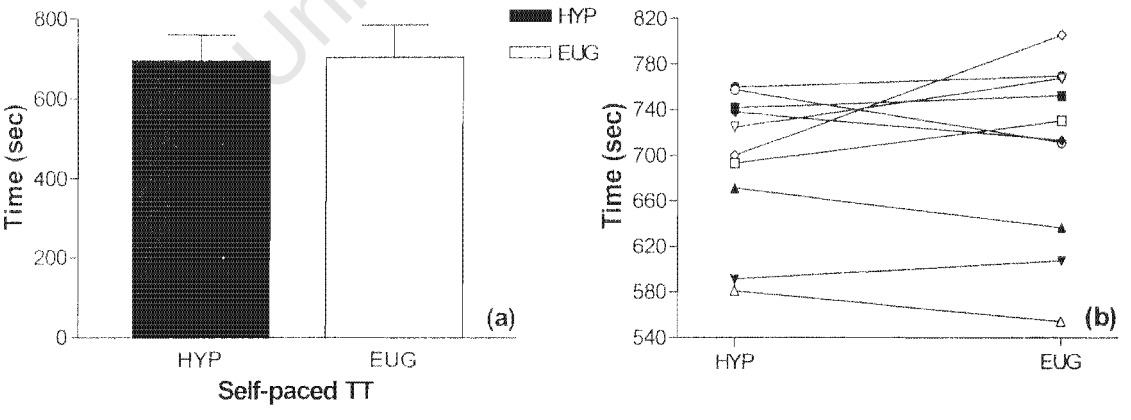


Figure 8: a) Overall performance time (sec) and b) subjects individual performance times (sec) of the 200 kJ self-paced TT of the experimental sample (n=10). Values are expressed as mean ± SD.

5.3.7 Plasma Glucose, Catecholamine Concentrations and Heart Rate during the Self-paced TT

Plasma glucose, catecholamine concentrations and HR at the end of the self-paced TT are presented in Table 2. Plasma glucose, epinephrine and norepinephrine concentrations measured at the end of the TT were not significantly different between trials. HR was similar pre and post TT for both trials.

Table 2: Plasma glucose (mmol/L), epinephrine and norepinephrine concentrations and HR (bpm) after the 200kJ self-paced TT of the experimental sample (n=10).

	HYP	EUG
Plasma Glucose (mmol/L)	6.4 ± 1.3	5.3 ± 1.3
Epinephrine (nmol.l-1)	2.22 ± 1.37	2.45 ± 1.34
Norepinephrine (nmol.l-1)	21.16 ± 4.17	23.42 ± 4.30
Heart Rate (bpm)	183.5 ± 8.4	182.3 ± 10.7

Values are expressed as mean ± SD.
Epinephrine and norepinephrine concentrations (n=8).

5.3.8 Perceived Exertion during the Self-paced TT

Rating of perceived exertion (RPE) measured pre and post the 200 kJ self-paced TT were similar for both HYP and EUG trials (Figure 9).

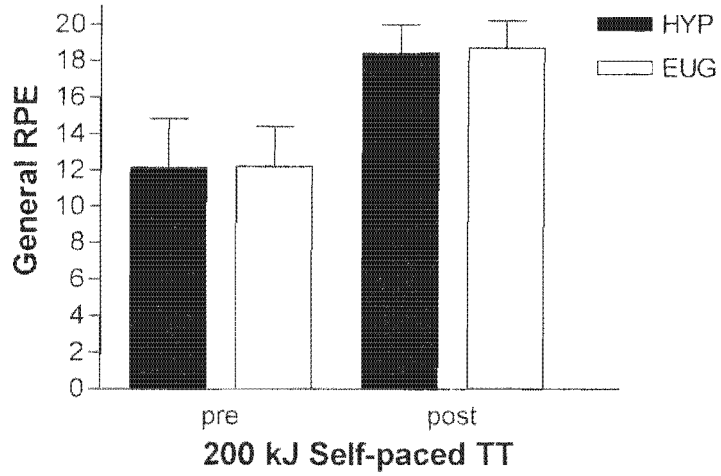


Figure 9: General RPE pre and post the self-paced TT of the experimental sample (n=10). Values are expressed as mean ± SD.

5.3.9 EMG Amplitude during the Self-paced TT

There was a significant trial x time interaction ($p<0.01$) for the normalised EMG amplitude (Figure 10), suggesting that the EMG amplitude was different between trials at the start of the self-paced TT (HYP: 42.81 ± 11.34, EUG: 33.00 ± 9.71 %) but finished the same at the end of the TT (HYP: 35.02 ± 11.39, EUG: 35.35 ± 10.53 %).

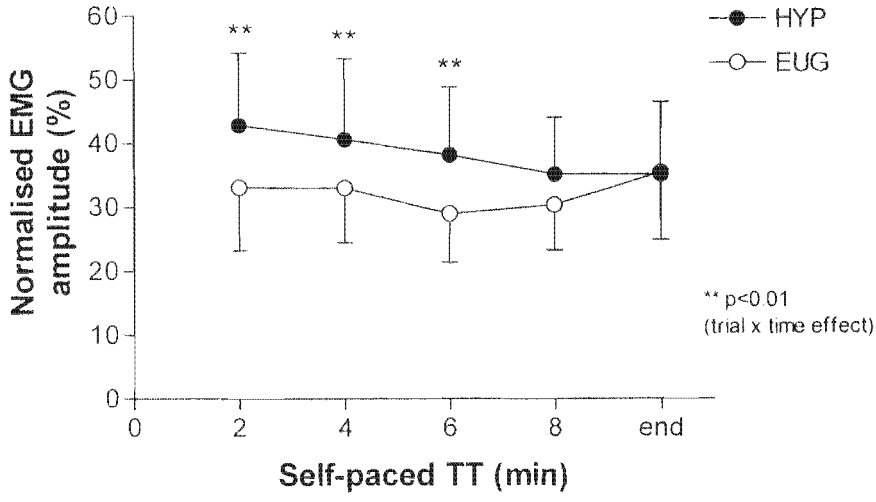


Figure 9: Normalised EMG amplitude (%) during the self-paced TT of the experimental sample (n=10). Values are expressed as mean ± SD.

5.4 DISCUSSION

The main aim of the present study was to determine the effect of antecedent hypoglycaemia on exercise performance, perceived exertion and muscle activation levels during a subsequent (next day) bout of steady-state exercise, interspersed with high-intensity sprints, and self-paced exercise in well-trained, healthy subjects. Overall exercise performance was not significantly altered by prior hypoglycaemia although there was a variable effect on individual exercise performance. Furthermore, the results demonstrate that antecedent hypoglycaemia (2 x 80 min bouts) had no effect on perceived exertion, muscle activation levels, the ability to maintain blood glucose homeostasis, and subsequent counter-regulatory responses during the prolonged exercise with high-intensity sprints. Antecedent hypoglycaemia did, however, increase submaximal oxygen consumption and blunt the sympathetic neural response. The measured variables were all similar during the self-paced TT, except for muscle activation which was altered at the onset of the TT in response to antecedent hypoglycaemia.

There was no significant difference between trials in the 10 kJ sprint times and in performance time of the self-paced TT due to the large inter-subject variability. The sprint times (n=7) and the TT performance (n=6) were generally better after the HYP exposure ranging from ~ 0.39 to 2.45 sec and ~ 9 to 105 sec respectively, but the magnitude of improvement by the few subjects after the EUG exposure was on average ~ 3.6 sec during the sprints (n=3) and ~ 33 sec in the TT (n=4). We are not aware of any previous studies examining the effect of antecedent hypoglycaemia on subsequent high intensity exercise bouts or performance during a self-paced exercise trial. This finding suggests that antecedent hypoglycaemia has a variable effect on the individual's ability to regulate work during high intensity exercise or a self-paced TT. It is possible that this individual variability and diverse effect on pacing strategy may be based on the subjects' training status and their recurrent antecedent exposure to hypoglycaemia during regular strenuous exercise training.

Furthermore, antecedent hypoglycaemia did not alter perceived exertion during either the steady state cycling bout or the self-paced TT. The RPE values measured during the steady state bout were submaximal, ranging from ~ 10.0 (HYP) and 10.5 (EUG) after the first sprint to ~ 14.5 (HYP and EUG) after the last sprint (Figure 5a), despite a conscious effort and encouraged to cycle "as fast as possible". During the subsequent self-paced TT, the RPE increased further, reaching peak values at the completion of the required work (Figure 9). Interestingly, the inter-individual differences in RPE between subjects were remarkably low. Thus, irrespective of large differences between the subject's performances in HYP and EUG (Figure 2b and 8b), the RPE increased similarly.

This suggests that the RPE is not simply a consequence of the exercise work rate, but is instead reflective of the entire hormonal, metabolic and physiological milieu of the exercising athlete. It has been suggested that the perceived exertion, or the *conscious sensation of fatigue*, may be related to a central process where it is interpreted and controlled at a subconscious level against an expected outcome (Hampson et al, 2001; Tucker et al, 2004; St Clair Gibson and Noakes, 2004).

This is possible in the present study, since the RPE increased similarly irrespective of differences in performance times between subjects, which are presumably the result of altered physiological processes in response to the antecedent hypoglycaemia. Since the power output was free to vary, it may be argued that the subjects simply reduced power output in response to physiological changes against an expected outcome, the end point of the exercise trial. This would result in a similar measurement for RPE as well as altered pacing strategies. This has been termed teleoanticipation (Ulmer, 1996), and accounts for variations in performance between subjects, even though RPE was not different between subjects.

Antecedent hypoglycaemia did not alter the muscle activation levels during the 10 kJ sprints of the 90 min cycle trial. EMG amplitude was reduced (~13%, HYP; ~24%, EUG) in both trials from the first to the second sprint and remained lower than the first sprint throughout the trial (~14%, HYP; ~17% EUG) (Figure 7a). This decline in EMG amplitude during prolonged exercise with high intensity sprints is consistent with those of previous studies (St Clair Gibson et al, 2001c; Kay et al, 2001).

These findings from the present study during the prolonged exercise with high-intensity sprints (submaximal effort perception and reduced muscle activation) suggests the presence of a central factor that subconsciously controls perceived exertion and regulates skeletal muscle activation by reducing neural drive and altering performance, despite the sprints being a maximal effort. Subjects appeared exhausted at the end of the 90 min cycle trial and indicated verbally that they were sprinting at maximal effort during the high-intensity sprints.

Conversely, muscle activation during the 200 kJ self-paced TT was significantly altered by antecedent hypoglycaemia, despite similar performance times. EMG amplitude was increased at the onset of the TT in the HYP compared to the EUG trial (~43%, HYP; ~33%, EUG), but progressively decreased resulting in similar muscle activation levels at the completion of the TT (~35%, HYP; ~35%, EUG) (Figure 10). It is possible that the increased muscle activation at the onset of the

HYP trial may be a result of reduced norepinephrine concentrations (described below) measured at the end of the preceding 90 min exercise bout. This blunted sympathetic neural response and its possible association with increased muscle activation levels is in contrast with the findings of previous studies in the present thesis. In Chapters 2 and 4, we found that altered muscle activation and power output were potentially related to increased sympathetic activation. It is unclear from these data in the present study how reduced SNS activity may be associated with increased muscle activation levels. Furthermore, it is unlikely that the increased muscle activation at the onset of exercise was due to metabolic changes in the peripheral organs as the circulating metabolites measured in the present study were not different.

A limitation of our study was that muscle activation was only measured from one muscle group, vastus lateralis, of the lower limb. It is possible that other lower limb muscles, not measured in the present study, may have received either inhibitory or excitatory commands different to those received in the vastus lateralis as cadence was free to vary. A previous study has shown that different muscles are used at different cadences during cycling indicating that muscle activation changes and fatigue are dependent on cadence (Takaishi et al, 1994; Takaishi et al, 1996). Alternatively, Akima et al (2002) showed that vastus lateralis fatigue increased the use of the other three quadricep muscles during knee extension exercise.

Antecedent exposure to two 80 min bouts of moderate hypoglycaemia (2.9 mmol/L) did not alter the cardiovascular response, which is in agreement with previous studies (Davis et al, 1997; Davis et al, 2000b), nor the metabolic (blood glucose, hepatic glucose production, lactate and FFA concentrations) (data reported elsewhere, Claassen PhD thesis) response during the prolonged exercise bout performed the next day. It did, however, increase submaximal oxygen consumption which corresponded with a decrease in RER suggesting that antecedent hypoglycaemia results in increased fat utilization during subsequent exercise. In addition, antecedent exposure to hypoglycaemia had a variable effect on the hormonal response. The insulin, cortisol (data reported elsewhere,

Claassen PhD thesis) and epinephrine concentrations were not reduced, though norepinephrine levels were significantly blunted by antcedent hypoglycaemia compared to euglycaemia. In a study by Kuipers et al (1999), subjects who developed rebound hypoglycaemia demonstrated a significantly lower norepinephrine response during an exercise bout (40 min at 60% PPO) compared to the non-hypoglycaemic subjects, indicating lower activation of the sympathetic-mediated counter-regulatory response in the hypoglycaemic group. Insulin and epinephrine concentrations, however, remained similar between groups (Kuipers et al, 1999).

It is difficult to distinguish between the effects of catecholamine concentrations as they function together in a co-ordinated manner. It would seem, therefore, that the reduced norepinephrine during exercise did not affect the other physiological levels due to the appropriate epinephrine response, suggesting a possible redundant control system. This explanation, however, is speculative since the present study did not measure the widespread effect on multiple neuroendocrine counter-regulatory responses (e.g. glucagon, growth hormone, pancreatic polypeptide). Plasma glucose concentrations were matched in the present study and were within a euglycaemic range throughout both trials. These results are in contrast to studies showing that prior hypoglycaemia results in blunted counter-regulatory responses in healthy individuals (Davis et al, 1997; Davis, et al, 2000a; 2000b).

In the study of Davis et al (2000a), similar to the present study, antecedent hypoglycaemia (2 x 2 hr bouts at 2.9 ± 0.1 mmol/L) resulted in significant blunting of neuroendocrine (glucagon, insulin, catecholamines) and metabolic (endogenous glucose production, lipolysis, ketogenesis) responses during subsequent (next day) exercise. Euglycaemia was maintained during the 90 min steady-state exercise (~ 50% $VO_{2\ max}$) via an exogenous glucose infusion and subjects required a 10-fold higher rate of glucose infusion to maintain euglycaemia during exercise after antecedent hypoglycaemia compared to euglycaemia. It is therefore difficult to deduce, whether the blunted neuroendocrine and metabolic responses were caused by, or resulted in the

higher rate of glucose infusion during the subsequent exercise (Davis et al, 2000a). Furthermore, a study by the same group (Galassetti et al, 2001a) demonstrated that two prior exercise bouts of 90 min at 50% $VO_{2\ max}$ with euglycaemic glucose infusion resulted in reduced neuroendocrine and metabolic responses during next day hypoglycaemia (2 hrs at 2.9 mmol/L) in normal healthy individuals, which appears to be similar to the counter-regulatory responses observed after antecedent hypoglycaemia.

The difference in results between the present study and those by the Davis group may be attributed to a number of factors. Firstly, in the present study we did not infuse glucose during the exercise trial in order to establish the physiological blood glucose response whereas Davis et al (2000a) infused glucose to maintain euglycaemia. It is therefore possible, that when exogenous glucose is infused during exercise, the functioning interplay of metabolic and neuroendocrine responses involved in preserving glucose counter-regulation is not necessary. Secondly, the intensity of exercise differed between studies (50% $VO_{2\ max}$ {72} compared to 70% $VO_{2\ max}$). The exercise intensity in the present study was higher, in addition to the 10 kJ maximal sprints performed every 15 min. While exercise intensity has been shown to activate the sympathetic system (Leal-Cerro et al, 2003), thus stimulating counter-regulatory responses and maintaining blood glucose regulation, it is likely that the higher exercise intensity in the present study was sufficient during the subsequent exercise bout in activating the counter-regulatory responses and maintaining blood glucose homeostasis.

Lastly, the training status of the individuals used in these studies was different. The present study used a group of well-trained endurance cyclists with an average $VO_{2\ max}$ of 64±3 ml/kg/min (range 60 to 70), whereas the subjects of Davis and co-workers were sedentary or actively participating in competitive sports with a group average $VO_{2\ max}$ of 43±3 ml/kg/min (range 21 to 54). Previous research has shown that physical training reduces the reliance on CHO as an energy source (Coggan, 1997), enhances insulin sensitivity (Borghouts and Keizer, 2000), and alters sympathetic and parasympathetic activation (Kjaer, 1998; Iellamo et al, 2002; Carter et al, 2003).

In conclusion, this study demonstrated that in overnight-fasted, well-trained healthy individuals, antecedent exposure to hypoglycaemia had no effect on overall exercise performance, perceived exertion, muscle activation levels and the metabolic counter-regulatory responses to subsequent prolonged exercise (70% $VO_{2\ max}$). There was, however, a blunted sympathetic neural response (norepinephrine concentrations), as well as an increase in oxygen consumption and a corresponding decrease in RER. Prior hypoglycaemia increased muscle activation levels at the onset of the self-paced TT despite no change in performance time, perceived exertion, plasma glucose, catecholamine concentrations and heart rate. The altered muscle activation at the onset of the self-paced TT may be related to the reduced sympathetic neural response measured during the preceding exercise bout. However, this association cannot be conclusively determined from these data. Furthermore, there was no direct relationship between the changes in norepinephrine concentrations and perceived exertion or performance.

CHAPTER 6

THE EFFECT OF ANTECEDENT MAXIMAL AND FATIGUING ISOMETRIC EXERCISE ON PERCEPTUALLY GUIDED ISOMETRIC FORCE PRODUCTION

Published in part in: Eur J Appl Physiol Dec:95(5-6):537-42, 2005. **West SJ**, Smith L, Lambert EV, Noakes TD, St Clair Gibson. Submaximal force production during perceptually guided isometric exercise

6.1 INTRODUCTION

A variety of psycho-physiological methods have been used to specifically investigate "effort perception". These methods have been discussed in detail in the review of the literature (Chapter 1). Briefly, magnitude estimation methods whereby individuals are required to subjectively judge their level of effort with a number or verbal rating have reported a high correlation between isometric force and perceived exertion (Pincivero et al. 2000a). This relationship, however, remains variable during magnitude production efforts where individuals are asked to exercise to intensities determined by their perceptual feelings of exertion. Previous research has shown that in the absence of external feedback, the ability of individuals to accurately match voluntary muscle force to target contraction intensities is inconsistent, especially at high perceived intensities where force output has been shown to be under-produced (Cooper et al. 1979;Jackson and Dishman 2000; Kumar and Simmonds 1994; Pincivero et al. 2003a). In addition, many experimental designs using the magnitude production method have enabled individuals to cognitively establish a perceptual range by allowing the individual to experience perceptual scale anchors, such as a maximal voluntary contraction (MVC), prior to the testing protocol (Kumar and Simmonds 1994; Pincivero et al. 2001; Pincivero et al. 2003a; Pincivero et al. 2004). Effort perception may be influenced by a maximal contraction being performed immediately prior to a submaximal contraction, resulting in perceptual underestimation (Hutton et al. 1984). However, it has also been shown that previous exercise experience to a small extent is necessary to accurately gauge RPE (Horstman et al, 1979c). Thus, prior experience of an MVC may, in some models, provide anchors for the perceptual range of effort, or alternatively may actually alter the perceptual response range.

Furthermore, the relationship between antecedent muscle fatigue and effort perception during exercise is still not well understood. Previous research has shown that when the maximum force generating capacity of the muscle is reduced as a result of fatigue, individuals' perceptual feelings of exertion during exercise

are increased (Cafarelli and Bigland-Ritchie 1979; Pincivero and Gear 2000b), which suggest that the relationship between effort perception and the force generating capacity of the muscle is altered. It is therefore likely, that the individuals' effort perception is altered by the previous performance of a fatiguing contraction, which may impair their ability to estimate force during subsequent contractions (Enoka and Stuart 1992).

The previous four chapters of this thesis have examined muscle activation levels and perceived exertion during either constant workload or self-paced **dynamic** exercise. The results demonstrate that perceived exertion is not directly associated with changes in power output and muscle activation, and increases at a linear rate relative to elapsed time. To examine perceived exertion more specifically, Chapter 6 of the present thesis examined perceived exertion (perceptual response range) during **isometric** exercise which is a more controlled method of assessing perceived exertion compared to dynamic activity. This study aimed to determine the effect of prior exposure to an MVC and fatiguing contractions, which would act as perceptual anchors, on the perceptual response during subsequent isometric contractions.

Therefore, the aims of this chapter, using a magnitude production model, were to: 1) investigate the perceptual response during submaximal isometric contractions prior to the performance of an MVC as a perceptual anchor; 2) examine the effect of antecedent fatiguing isometric exercise on the subsequent perceptual response during isometric contractions; and 3) compare the force achieved relative to the target force at varying relative intensities of an MVC.

6.2 METHODOLOGY

6.2.1 Subject Selection

Thirty healthy, active individuals (15 male and 15 female) were recruited to participate in the study. The subjects were young adults with a mean age of 24 ±

3 yr, mean weight of 66.7 ± 12.1 kg and height of 170.4 ± 7.5 cm. The study was approved by the Ethics and Research Committee of the Faculty of Health Sciences, University of Cape Town. All subjects provided informed consent prior to participation.

All subjects were physically active at least three times a week and performed a mean of 9 ± 6 hrs/week physical activity. Exclusion criteria included any history or current signs of knee pathology that would influence exercise performance or be negatively affected by the testing protocol. Subjects were required to complete a questionnaire to establish age, training history and current training status, and the presence of previous and current injuries.

6.2.2 Experimental Design

Subjects were required to visit the laboratory on five different occasions, each separated by a rest day. The first visit was standard for all subjects, while the following four visits were randomised. During all tests (described below), the subjects were blinded to the force output they were producing, as this knowledge may have influenced their performance. This was achieved by placing a cover over the Kin-Com isokinetic dynamometer monitor displaying the force data.

A standardized warm up consisting of three stretches was performed before each test. The stretches were held for 30 s and included a standing quadriceps, hamstring and gastrocnemius stretch. Subjects' isometric force was assessed on the lower right limb using a Kin-Com isokinetic dynamometer (Chattanooga Group, Inc., Chattanooga, USA). Their hips and upper bodies were firmly strapped to the seat. The arm position for each test was standardized with each subject crossing his or her arms over the chest. All isometric tests were conducted at 60° knee flexion, with 0° being the limb in full extension. The angle of 60° flexion has been shown to be the angle of maximal isometric force generation (Tihanyi et al. 1982). Each subject performed four warm up isometric contractions of the knee extensors, at a voluntary force output below their maximum, for 5 s

each separated by 10 s intervals. The same investigator performed all testing procedures for all the subjects, as well as the verbal encouragement.

The absolute peak isometric force in Newtons (N) for each required contraction intensity was measured on the Kin-Com isokinetic dynamometer, which was calibrated before each day of testing. Peak force was the highest isometric force measured over 5 s. The relative peak isometric force, expressed as a percentage, was the absolute values normalised to the MVC, at each perceived contraction intensity. Muscle activation (EMG amplitude) was not measured in the present study as we have previously shown that perceived exertion is not directly associated with changes in muscle activation and power output (Chapters 2 and 4).

6.2.3 Experimental Protocol:

6.2.3.1 Standard Naïve Test

All subjects performed the standard naïve test on their first visit to the laboratory. During the standard naïve test, subjects were not initially exposed to an MVC, but were required to perform three voluntary isometric contractions at intensities they felt represented 25%, 50% and 75% of what they could produce during a maximum effort (MVC). For example, subjects were asked to "contract their muscles at an intensity they felt was 25% of their maximum". The order of the three contraction intensities was randomised. The fourth voluntary isometric contraction was a maximal contraction (100% MVC). Therefore, the three submaximal contractions were "naïve" from the perspective that no maximal contraction had occurred, which could be used by the subjects as a perceptual anchor. The investigator used verbal encouragement to facilitate the 100% MVC during the standard naïve test, and during the test days that followed. Contractions lasted 5 s, and were separated by 10 s intervals or more, if additional rest time was requested.

6.2.3.2 Control Tests 1 and 2

The control tests were performed on two separate visits to the laboratory to examine a possible learning effect. Subjects were required to perform isometric contractions at the following intensities: 25%, 50%, 75% and 100% of their perceived maximum. For example, subjects were asked to "contract their muscles at an intensity they felt was 25% of their MVC". The order of contraction intensities was randomised. Contractions lasted 5 s, and were separated by 10 s intervals or more, if additional rest time was requested. This exercise protocol was also repeated after the 20% and 100% MVC fatiguing contractions, described below.

6.2.3.3 Post 20% MVC Test

Prior to this testing session, a required contraction intensity for each subject was calculated as 20% of their 100% MVC, performed during the standard naïve test. Subjects then performed a voluntary isometric contraction to fatigue at this intensity. During the contraction, subjects were instructed to watch a monitor, which displayed the required level. The subjects were blinded to the absolute force they were generating during the fatiguing contraction. Subjects rested for 10 min before completing the same protocol used in the control tests. A 10 min rest period was chosen to remove the physical component of the fatigue trial but still have an effect on the perceptual response during subsequent testing.

6.2.3.4 Post 100% MVC Test

Subjects performed a voluntary isometric contraction at 100% MVC until fatigue. For example, subjects were instructed to "contract their muscles maximally for as long as they could until volitional fatigue". Thereafter subjects rested for 10 min before completing the same protocol used in the control tests. A 10 min rest period was chosen to remove the physical component of the fatigue trial but still have an effect on the perceptual response during subsequent testing.

6.2.4 Statistical Analyses

The statistical software package Statistica 6.1 (StatSoft, Inc., Tulsa, OK, USA) was used to analyse the data. All data are presented as means ± standard deviations. A two-way analysis of variance (ANOVA) with repeated measures was used to compare absolute and relative force output, as well as absolute and relative force errors, at the different perceptual intensities between the various experimental conditions, and to assess significant main effects and interactions. Where significant differences occurred, a Tukey's HSD post hoc analysis was used to examine the differences. Statistical significance was accepted when $p<0.05$.

6.3 RESULTS

6.3.1 Absolute Force

The results demonstrate that absolute force produced by the subjects increased with increasing contraction intensities at 25%, 50%, 75% and 100% of subjective maximum ($p<0.001$) during the standard naïve test, both control tests and the post 20% and 100% MVC tests (Table 1). Absolute force at 25% and 50% of perceived maximum was lower during the standard naïve test compared to control test 2, post 20% MVC and 100% MVC tests. At 75% of perceived maximum, force was also significantly lower ($p<0.001$) during the standard naïve test compared to the post 100% MVC test. Maximum isometric force (100% MVC) was not different between experimental conditions.

Table 1: The absolute force produced during the standard naïve test, control tests 1 and 2, post 20% MVC test and post 100% MVC test of the experimental sample (n=30).

Contraction Intensity (%)	Standard Naïve Test (Force in N)	Control Test 1 (Force in N)	Control Test 2 (Force in N)	Post 20% MVC Test (Force in N)	Post 100% MVC Test (Force in N)
25	152 ± 93 [a]	197 ± 122	209 ± 100 [b]	230 ± 101 [c]	226 ± 87 [c]
50	236 ± 131 [a]	279 ± 133	294 ± 111 [b]	308 ± 129 [c]	315 ± 109 [c]
75	324 ± 141 [a]	351 ± 128	351 ± 133	366 ± 145	391 ± 129 [c]
100	587 ± 148	599 ± 172	608 ± 172	587 ± 191	604 ± 173

Values are expressed as mean ± SD, absolute peak force is reported in Newton's (N)

Main effects, intensity: p = 0.00001
Main effects, trial: p = 0.0005
Interaction effect: p = 0.009
[a vs b] *indicates a significant (p<0.01) difference between the standard naïve test and control test 2*
[a vs c] *indicates a significant (p<0.001) difference between the standard naïve test, the post 20% MVC test and the post 100% MVC test*

6.3.2 Relative Force

The relative force was significantly lower during the standard naïve test, compared to control tests 1 and 2, and both the post 20% MVC and 100% MVC tests, at all contraction intensities (p<0.001) (Table 2) (Figure 1).

Table 2: The relative isometric force at the target contraction intensities during the standard naïve test, control tests 1 and 2, post 20% MVC test and post 100% MVC test of the experimental sample (n=30).

Contraction Intensity (%)	Standard Naïve Test (%)	Control Test 1 (%)	Control Test 2 (%)	Post 20% MVC Test (%)	Post 100% MVC Test (%)
25	24.9 ± 12.1	31.6 ± 15.3	34.5 ± 14.5	39.1 ± 12.0	37.6 ± 12.2
50	39.1 ± 16.2	45.0 ± 13.6	48.1 ± 12.4	53.0 ± 15.4	52.7 ± 13.4
75	54.3 ± 16.0	62.0 ± 23.4	56.6 ± 20.6	62.0 ± 14.3	65.3 ± 14.2

Values are expressed as mean ± SD, relative force is reported as a percentage (%) of the absolute peak force at each perceived contraction intensity.

Main effects, intensity: p = 0.00001

Main effects, trial: p = 0.00001

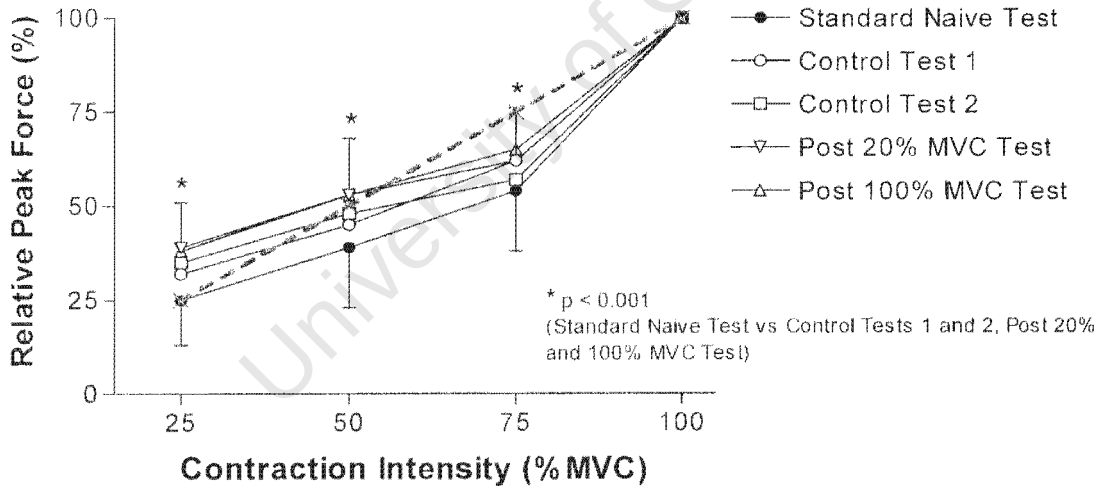
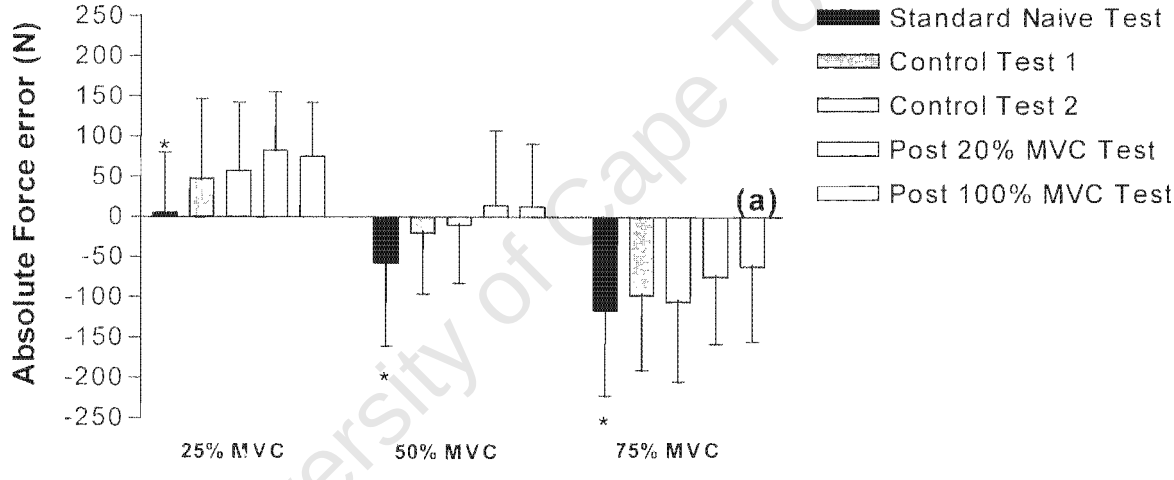


Figure 1: The relative peak isometric force at the target contraction intensities during the standard naïve test, control tests 1 and 2, post 20% MVC test and post 100% MVC test of the experimental sample (n=30). Values are expressed as mean ± SD. Dotted line represents the required contraction intensity relative to the maximum voluntary contraction (100% MVC).

6.3.3 Force Errors

Comparisons were made between the target contraction intensities and the subjects' absolute and relative isometric force, and the errors were then calculated (Figure 2a and b). The absolute force errors of the standard naïve test were significantly different ($p<0.001$) from control test 2, post 20% MVC and 100% MVC tests, at all intensities (Figure 2a). The relative force errors of the standard naïve test were significantly different ($p<0.001$) from control tests 1 and 2, post 20% MVC and 100% MVC tests at all intensities (Figure 2b).



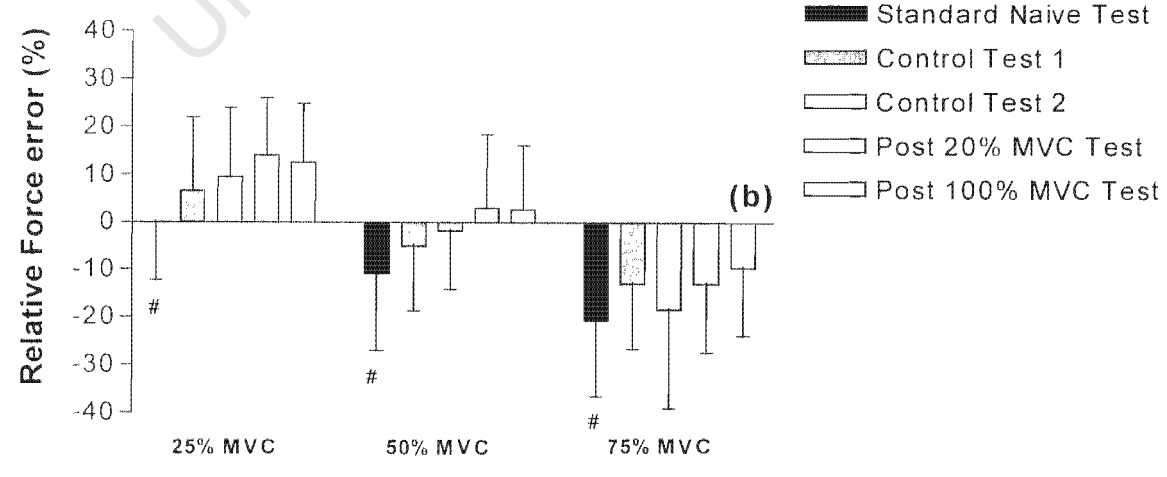


Figure 2: The errors of the a) absolute isometric force (N) and the b) relative isometric force (%) at the target contraction intensities of 25, 50 and 75% MVC during all experimental conditions of the experimental sample. Values are expressed as mean ± SD. (+ represents underestimation of force, - represents overestimation of force).

** p<0.001 standard naïve test vs control test 2, post 20% MVC and 100% MVC tests at all intensities (absolute isometric force)*
 # p<0.001 standard naïve test vs control tests 1 and 2, post 20% MVC and 100% MVC tests at all intensities (relative isometric force)

Subjects consistently over-produced isometric force (perceptually underestimated) at 25% MVC, except during the standard naïve test. Isometric force at 50% and 75% MVC intensities was lower on most occasions than equivalent percent values of the target contraction intensities (perceptual overestimation). Subjects tended to be less accurate at matching absolute force with the target contraction intensities at extreme ends of the force domain and were the most accurate at 50% MVC (Table 3).

Table 3: The absolute and relative force errors during all five experimental conditions at the target contraction intensities of the experimental sample (n=30).

Contraction Intensity (%)	Absolute Peak Force Error (Force in N)	Relative Peak Force Error (Force as a %)
25	53.68	8.54
50	-12.06	-2.42
75	-91.20	-14.98

(+ represents underestimation of force, - represents overestimation of force)

6.4 DISCUSSION

The most important finding of the present study was that isometric force, preceding a maximal voluntary contraction (used as a perceptual anchor), was

most accurate at the low perceptual intensity and significantly under-produced (perceptually overestimated) at higher perceptual intensities. Exposure to a 100% MVC resulted in a better judgement of force during subsequent voluntary contractions at 50% and 75% MVC guided by the subjects' perceptual feelings of exertion. Antecedent submaximal and maximal fatiguing isometric exercise did not affect the subjects' subsequent perceptual response range, however, it appears that the fatiguing isometric exercise may have resulted in an increased overestimation of force at the low perceptual intensity. In addition, matching isometric force with target contraction intensities tended to be less accurate at opposite ends of the force domain (25% and 75% MVC) during all five experimental conditions.

Absolute force produced during the standard naïve test, where the maximal voluntary contraction (100% MVC) was performed last, was the most accurate at 25% MVC, but was lower at all subjective intensities than the isometric force produced during the other four test days. It is unclear why subjects were able to accurately judge force at the low contraction intensity (25% MVC) during the standard naïve test. Exposure to a 100% MVC resulted in a more accurate judgement of force during subsequent tests at contraction intensities of 50% and 75% MVC which suggests that a perceptual range at higher contraction intensities was not established with no prior knowledge of one's maximal force generating capacity, and therefore, a dissociation exists between effort perception and force output at submaximal contraction intensities of 50% and 75% MVCin the naïve state. Horstman et al (1979c) have suggested that although pre-exercise warm up may not influence the results, previous experience of the exercise may be necessary to accurately judge effort perception. In contrast, Hutton et al. (1984) proposed that transient postcontractile potentiation of spinal reflex pathways may summate with previously set motor commands or act independently to produce errors in effort perception. Our results support the notion that antecedent experience of a maximal contraction did, at least in the 50% and 75% contraction intensities, improve the subject's ability to estimate force output based on perceived exertion.

In the present study we utilised two different antecedent fatiguing perturbations, a maximal 100% MVC and a submaximal 20% MVC fatigue test. Both central and peripheral fatigue has been shown to develop during maximal and submaximal sustained voluntary muscle contractions (Bigland-Ritchie et al. 1983b). Isometric exercise was used during the fatigue tests to be consistent with the test protocol that followed. It has previously been found that muscle fatigue impairs the ability of subjects to make judgements of force (Gandevia and McCloskey 1978; Jones and Hunter 1983a), as well as increases effort perception (Jones and Hunter 1983b). Our results suggest, however, that a previous bout of fatiguing isometric exercise did not impair the subjects' subsequent ability to match isometric force with target contraction intensities, but it appears that the fatiguing isometric exercise may have resulted in an increased overestimation of force at the low perceptual intensity of 25% MVC, similar to what occurred in ontrol tests 1 and 2.

A 10 min rest interval was given to subjects in the present study after the fatiguing isometric exercise before performing the test protocol. Rest intervals of 40 s compared to 160 s (Pincivero et al. 1999), as well as 1-3 min (Woods et al. 2004) between exercise bouts have previously shown no effect on perceived exertion, and therefore demonstrate that perceived exertion may not be affected by the rest interval length, or may not be a sensitive indicator of muscle recovery. It has been suggested that rest intervals of 2 – 4 min are adequate for minimising muscle fatigue (Pincivero et al. 1999). It is possible therefore, that the length of recovery time in the present study may have been a limitation and was too long for the physical effects of the fatigue tests to have an effect on the perceptual response range.

The ability to accurately judge force based on perceptual feelings of exertion had a tendency to be poorer at opposite ends of the force domain where force was over produced at 25% MVC and consistently under produced at 75% MVC after the performance of an MVC as a perceptual anchor (Table 3). The most accurate judgement of isometric force occurred at 50% MVC. The present finding is in agreement with previous research by Jones and Hunter (1982) who showed that

forces under 40% MVC were consistently over produced, forces above 60% MVC under produced and the most accurate estimation occurred around the middle of the force domain. Similar results were also found by Kumar and Simmonds (1994) who used a pinch grip, power grip and stoop lifting activity to measure accuracy and the reliability of effort perception during these activities. This study found a systematic bias at all submaximal force contractions except at 40% MVC. The force at 60% and 80% MVC was lower whereas at 20% MVC was higher than the values based on the MVC. Jackson and Dishman (2000) measured perceived submaximal force production using a chest press exercise in the order of 25%, 50%, 75% of maximum and a final MVC, and reported an over production of force at 25% MVC and lower forces produced at both 50% and 75% MVC when compared to the expected force production. It appears that the consistency of these results occurs whether the contraction intensities are presented in either a linear fashion (Jackson and Dishman 2000) or in a randomised manner (Jones and Hunter 1982; Kumar and Simmonds 1994; Pincivero et al. 2003a), as in the present study.

The reason for this perceptual over-and-underestimation is not immediately clear. It is possible that since the large musculature of the leg extension is more commonly involved in gross movements relating to power, it is relatively insensitive in identifying and performing the lower levels of force such as those required in fine movements, thus resulting in perceptual underestimation (Jones and Hunter 1982). The reason for perceptual overestimation may be a teleological one, in that subjects subconsciously under produce force at higher intensities as a protective mechanism, in order to maintain a reserve capacity, and therefore, prevent mechanical and metabolic damage which may occur from maximal force generation. Another explanation for the perceptual inaccuracies may be the absence of a memorised anchoring cue, which allows the subjects to cognitively establish a perceptual range by a low and a high reference point ((Pincivero et al. 2003a; Pincivero et al. 2003b). This would be particularly true of the higher end of the force-producing spectrum, since most individuals do not perform maximal leg exercise during activities of daily living.

In conclusion, subjects were most accurate at estimating force production at the low perceptual intensity of 25% MVC under naïve conditions, but under produced (perceptually overestimated) isometric force during the higher contraction intensities preceding a maximal voluntary contraction (100% MVC). Exposure to a maximal voluntary contraction enabled subjects to establish an improved perceptual response range during voluntary contractions at 50% and 75% MVC that resulted in a better judgement of force guided by perceptual feelings of exertion. Antecedent fatiguing isometric exercise did not alter the subjects' subsequent perceptual response range, irrespective of whether it was submaximal or maximal fatiguing exercise. It appears, however, that antecedent fatiguing exercise may have increased overestimation of force at the low perceptual intensity of 25% MVC. Furthermore, it was clearly observed that the ability of subjects to match absolute force with target contraction intensities during all five testing conditions was most accurate at 50% MVC and was poorer at extreme ends of the force domain, 25% and 75% MVC. Further work, therefore, is required to investigate the cause of perceptual overestimation at the higher contraction intensities and the acute effects of fatigue on perceived exertion using the magnitude production method.

CHAPTER 7

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The effort continua established by Borg represents the relationship between the physiological demands of the exercise performance and the perception of effort associated with that performance. According to Borg, the variables in all three continua, namely the perceptual, physiological and performance continuum, are not linearly related to each other but rather complement each other and therefore the contribution of each continuum is "weighted" differently during different exercise tasks. An integrative exercise model, based on Ulmer's theory of teleoanticipation, proposes that exercise performance is regulated by a feedforward/feedback control system where both afferent and efferent feedback are integrated. A "central programmer" thus incorporates this feedback, as well as taking into account antecedent experiences, training, metabolic reserves, actual metabolic rate and the time necessary to complete an exercise task, and as a result alters/modifies the metabolic milieu and muscle output accordingly. Therefore, it appears there is a complex interplay between afferent and efferent processes in regulating exercise performance where all physiological systems interact continuously to maintain homeostasis and prevent bodily damage/harm.

Accordingly, the aim of this thesis, using Borg's concept of the effort continua, was to assess the integrative regulation of exercise by examining the effect of various antecedent exposures and physiological perturbations on the relationship between perceived exertion, muscle activation levels and performance during both open and closed loop exercise at either a self-paced or constant workload.

The first study of this thesis examined the effect of antecedent fatiguing exercise on perceived exertion, muscle activation levels and overall exercise performance. We tested this by using a sequential single-limb exercise model during an open loop protocol to exhaustion. We aimed to determine whether the humoral effects and physiological responses elicited during exercise to exhaustion of the first limb (Leg 1) altered perceived exertion, muscle activation levels and exercise performance of the previously rested limb (Leg 2). We found that antecedent

exhaustive single-limb exercise **did not** significantly influence performance during a subsequent exercise bout with the previously rested leg, despite an altered physiological milieu and EMG activity during exercise in Leg 2. The increased epinephrine concentrations and EMG activity in Leg 2 remained elevated until fatigue. The mechanism underlying these changes remains unclear, but these data suggest that the increased muscle activation levels may be associated with increased circulating epinephrine concentrations. Perceived exertion was, however, **unrelated** to muscle activation and epinephrine concentrations, and increased similarly for most of the trial until volitional fatigue. Thus, the rate of change in perceived exertion associated with prior local muscle fatiguing exercise appeared to be dependent rather on the exercise duration, irrespective of either antecedent exercise, an altered physiological milieu or increased muscle activation levels.

The second study further evaluated the relationship between altered muscle activation levels and increased circulating catecholamine concentrations, in particular the interaction between the infusion of epinephrine (perturbation of epinephrine concentrations) and exercise intensity on muscle activation levels and perceived exertion during a steady-state closed loop exercise protocol. Epinephrine infusion resulted in epinephrine levels similar to those found during moderate-intensity exercise, but without the concomitant sympathetic activation related to increased exercise intensity. Plasma glucose and lactate concentrations were increased by epinephrine infusion, but muscle activations levels, perceived exertion or cardiovascular and respiratory responses during low-intensity steady-state exercise were **not** different. It appears that increases in circulating epinephrine, and the metabolic changes associated with epinephrine infusion during low-intensity exercise, may not be sufficient afferent feedback for cardiopulmonary and peripheral responses or changes in perceived exertion and muscle activation levels. Furthermore, these data suggest that there is **no direct relationship** between increased plasma glucose and lactate concentrations and muscle activation levels or perceived exertion during dynamic low-intensity exercise. Thus, circulating epinephrine levels do not appear to be directly

responsible for alterations in either RPE or efferent motor command. This may however be due to the low, fixed exercise intensity employed in this study.

In the third study, a relationship between metabolic activity and sympathetic activation levels was examined with particular reference to perceived exertion, muscle activation levels, fuel and hormonal responses, and performance. We found that ingestion of an antecedent high-fat diet for 6 days, followed by 1 day of CHO-loading, resulted in **no change** in overall exercise performance but high-intensity sprint power performance was impaired during prolonged self-paced exercise. Muscle activation levels, effort perception and heart rate were similar between dietary treatments. The relationship between self-paced exercise performance and these variables was thus altered during the 1-km sprints. However, the mechanism associated with the decrement in sprint performance is not clear, but could possibly be related to increased sympathetic activation, or altered contractile function. Furthermore, the reduced power output during the 1-km sprints following antecedent high-fat feeding did not affect overall exercise performance which suggests a compensatory increase in performance during the non-sprint cycling sections. We suggest that during self-paced exercise of known duration, the selected power output is altered based on afferent feedback and supports the notion of teleoanticipation.

Given the different findings for sprint and overall exercise performance in the previous trials, the fourth study used an antecedent hypoglycaemic exposure to determine its effect on performance, perceived exertion and muscle activation levels during a subsequent (next day) bout of steady-state, interspersed with high-intensity sprints, and self-paced exercise. The results suggest that antecedent exposure to hypoglycaemia had **no** effect on overall exercise performance perceived exertion, muscle activation levels and the metabolic counter-regulatory responses to subsequent prolonged exercise (70% $VO_{2\ max}$), however, the sympathetic neural response (norepinephrine) was blunted. Prior hypoglycaemia increased muscle activation levels at the onset of the self-paced TT despite **no** change in performance, effort perception, plasma glucose, catecholamine concentrations and heart rate. The altered muscle activation may be related to the

reduced sympathetic neural response measured during the steady-state exercise bout. However, this association cannot be directly determined from these data. As with the findings of the previous studies in this thesis, perceived exertion was **unrelated** to any measured physiological variables during both the steady-state and self-paced exercise bouts.

The relationship between force production, muscle activation levels and perceived exertion during constant workload or self-paced dynamic open and closed loop exercise protocols was examined in the previous four studies. The results showed that there was **no direct** relationship between perceived exertion and any measured variable except the end point of the exercise trials. The fifth study, therefore, aimed at exploring perceived exertion more specifically using an isometric exercise protocol, examining the effect of prior exposure to a perceptual anchor (maximal voluntary contraction) and antecedent fatiguing exercise on the perceptual response during subsequent isometric contractions. The estimated isometric force, preceding a maximal voluntary contraction was most accurate at the low perceptual intensity of 25% MVC in the naïve state, but under produced during the higher contraction intensities of 75% MVC. Exposure to a maximal voluntary contraction enabled subjects to establish an improved perceptual response range during voluntary contractions at 50% and 75% MVC. Antecedent fatiguing isometric exercise **did not** alter the subjects' subsequent perceptual response range, irrespective of whether it was submaximal or maximal fatiguing exercise. However, it seems antecedent fatiguing exercise may have increased overestimation of force at the low perceptual intensity of 25% MVC. Furthermore, it was clearly observed that the ability of subjects to match absolute force with target contraction intensities during all testing conditions was **most accurate** at 50% MVC and was poorer at extreme ends of the force domain, 25% and 75% MVC after performance of an MVC as a perceptual anchor. This suggests that at medium-range isometric intensities, the brain mechanism functions well but is prone to error at high and low intensities. It is possible that this altered relationship between perceived exertion and isometric exercise intensity, particularly at high and low intensities, may occur during dynamic exercise.

In conclusion, the present thesis examined the hypothesis that antecedent exposures and physiological perturbations would alter the perceptual and physiological milieu and, as a result, impact on perceived exertion, muscle activation levels and overall exercise performance. A further hypothesis tested was that the variables in the perceptual, the physiological and the performance continuum of Borg's effort continua would not be linearly related but rather "weighted" differently during each specific exercise protocol.

The main finding from the present thesis was that the antecedent exposures and physiologcial perturbations induced by the interventions did not alter **perceived exertion** or **overall exercise performance** during any study. We found a performance difference only in Chapter 4 where we examined antecedent high-fat feeding and showed sprint performance but not overall exercise performance was impaired by an antecedent high-fat diet. The RPE, however, was not different in that study or in any other study, with RPE increasing linearly in all exercise trials, irrespective of the mode of exercise, exercise intensity and duration.

The rate of increase in **perceived exertion** has been suggested to be a function of afferent feedback from numerous systems which include metabolic circulatory and nervous sytems. We have shown no effect of any measured physiological perturbation or difference, including antecedent fatiguing exercise, epinephrine infusion, high-fat dietary intervention, and antecedent hypoglycaemia. These perturbations resulted in changes in epinephrine and norepinephrine concentrations, plasma glucose and lactate concentrations, oxygen consumption, heart rate variability, self-selected power output, and muscle activation levels. Therefore, the generation and regulation of RPE appears to be more likely a function of exercise duration, with initial values determined, in part, by antecedent exposure or prior experience and knowledge of the end point of exercise. These findings therefore support Borg's theory of an effort continua that the perceptual, physiological and performance variables are not all linearly related to each other but rather their contribution complements each other and is "weighted" differently during each specific exercise task, and Ulmer's concept of teleoanticipation, where the endpoint of the exercise task sets the perceived exertion.

The interpretation may, however, be different during incremental exercise to volitional fatigue compared to self-paced exercise of a known duration. Using a magnitude production model and examining perceived exertion as a regulator of exercise intensity during isometric exercise, it appears antecedent exposure to perceptual anchors may be necessary to establish improved judgement of force production. In addition, the finding of poor force production at opposite ends of the force continuum suggests that while the brain mechanism functions well at medium-range intensities, it is prone to error at high and low isometric exercise intensities. It is possible that this altered relationship between perceived exertion and exercise intensity, particularly at high and low intensities, may occur during dynamic exercise.

Although overall exercise performance and perceived exertion were not altered in response to antecedent exposures and physiological perturbations used in the present thesis, there were changes in **force production** and **muscle activation levels** during both open and closed loop exercise at a self-paced and constant workload. In addition, a possible association exits between altered sympathetic activation, force production and muscle activation levels during dynamic exercise, although only with a concomitant increase in exercise intensity, as was demonstrated by the study involving the perturbation of epinephrine levels during low-intensity exercise. **Overall performance** during the different dynamic exercise trials, however, was not affected by these alterations in force production, muscle activation levels and sympathetic activation. This suggests a possible incorporation of a teleoanticipatory strategy during exercise whereby performance was regulated in response to changes in physiological variables against an expected outcome. Thus, in the context of an integrative regulatory system, the changes in the physiological systems elicited by the various stimuli (antecedent exposures and physiological perturbations), appear to have caused compensatory responses in the peripheral and central processes to ensure overall exercise performance was maintained.

Further research should examine in greater detail the relationship between sympathetic activation, force production and muscle activation levels during steady-state and self-paced open and closed loop exercise protocols. Furthermore, future research should explore the mechanisms associated with antecedent high-fat feeding and compromised high-intensity sprint power output, as well as the effect of antecedent hypoglycaemia on the regulation of skeletal muscle activation, during self-paced exercise. In addition, the magnitude production model of perceived exertion requires further investigation, specifically examining the acute effects of antecedent fatiguing exercise on the perceptual response during subsequent isometric exercise, and the cause of perceptual overestimation during high-intensity isometric exercise.

CHAPTER 8

LIST OF REFERENCES

Ahlborg, G., Hagenfeldt, L., & Wahren, J. (1975). Substrate utilization by the inactive leg during one-leg or arm exercise. *J Appl Physiol* **39**, 718-723.

Ahlborg, G. (1985). Mechanism for glycogenolysis in nonexercising human muscle during and after exercise. *Am J Physiol* **248**, E540-E545.

Akima, H., Foley, J. M., Prior, B. M., Dudley, G. A., & Meyer, R. A. (2002). Vastus lateralis fatigue alters recruitment of musculus quadriceps femoris in humans. *J Appl Physiol* **92**, 679-684.

Albertus, Y., Tucker, R., St Clair Gibson, A., Lambert, E. V., Hampson, D. B., & Noakes, T. D. (2005). Effect of distance feedback on pacing strategy and perceived exertion during cycling. *Med Sci Sports Exerc* **37**, 461-468.

Allen, P. D. & Pandolf, K. B. (1977). Perceived exertion associated with breathing hyperoxic mixtures during submaximal work. *Med Sci Sports* **9**, 122-127.

Alway, S. E., Hughson, R. L., Green, H. J., Patla, A. E., & Frank, J. S. (1987). Contractile properties of the human triceps surae following prolonged exercise and beta-blockade. *Clin Physiol* **7**, 151-163.

Armada-da-Silva, P. A., Woods, J., & Jones, D. A. (2004). The effect of passive heating and face cooling on perceived exertion during exercise in the heat. *Eur J Appl Physiol* **91**, 563-571.

Astrand, P. O. & Rodahl, K. (1977). *Textbook of Work Physiology*, pp. 141-570. McGraw Hill, New York.

Baldwin, J., Snow, R. J., Gibala, M. J., Garnham, A., Howarth, K., & Febbraio, M. A. (2003). Glycogen availability does not affect the TCA cycle or TAN pools during prolonged, fatiguing exercise. *J Appl Physiol* **94**, 2181-2187.

Bangsbo, J., Graham, T. E., Kiens, B., & Saltin, B. (1992). Elevated muscle glycogen and anaerobic energy production during exhaustive exercise in man. *J Physiol* **451**, 205-227.

Banister, E. W. (1979). The perception of effort: an inductive approach. *Eur J Appl Physiol Occup Physiol* **41**, 141-150.

Bar-Or, O., Skinner, J. S., Buskirk, E. R., & Borg, G. A. (1972). Physiological and perceptual indicators of physical stress in 41 to 60-year-old men who vary in conditioning level and body fat. *Med Sci Sports* **4**, 96-100.

Bar-Or, O. (1977). Age-related changes in exercise prescription. In *Physical Work and Effort* , ed. Borg, G. A., pp. 255-266. Pergamon Press, Solna, Sweden.

Bernardi, M., Felici, F., Marchetti, M., Montellanico, F., Piacentini, M. F., & Solomonow, M. (1999). Force generation performance and motor unit recruitment strategy in muscles of contralateral limbs. *J Electromyogr Kinesiol* **9**, 121-130.

Bigland-Ritchie, B. (1981a). EMG/force relations and fatigue of human voluntary contractions. *Exerc Sport Sci Rev* **9**, 75-117.

Bigland-Ritchie, B. (1981b). EMG and fatigue of human voluntary and stimulated contractions. *Ciba Found Symp* **82**, 130-156.

Bigland-Ritchie, B., Johansson, R., Lippold, O. C., & Woods, J. J. (1983a). Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. *J Neurophysiol* **50**, 313-324.

Bigland-Ritchie, B., Johansson, R., Lippold, O. C., Smith, S., & Woods, J. J. (1983b). Changes in motoneurone firing rates during sustained maximal voluntary contractions. *J Physiol* **340**, 335-346.

Bigland-Ritchie, B. (1984a). Muscle fatigue and the influence of changing neural drive. *Clin Chest Med* **5**, 21-34.

Bigland-Ritchie, B. & Woods, J. J. (1984b). Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle Nerve* **7**, 691-699.

Bilodeau, M., Arsenault, A. B. , Gravel, D., & Bourbonnais, D. (1990). The influence of an increase in the level of force on the EMG power spectrum of elbow extensors. *Eur J Appl Physiol Occup Physiol* **61**, 461-466.

Bilodeau, M., Cincera, M., Arsenault, B., & Gravel, D. (1997). Normality and Stationarity of EMG Signals of Elbow Flexor Muscles During Ramp and Step Isometric Contractions. *J Electromyogr Kinesiol* **7**, 87-96.

Bolli, G. B. (1999). How to ameliorate the problem of hypoglycemia in intensive as well as nonintensive treatment of type 1 diabetes. *Diabetes Care* **22 Suppl 2**, B43-B52.

Borg, G. A. & Linderholm, H. (1967). Perceived exertion and pulse rate during graded exercise in various age groups. *Acta Medica Scandinavica* **Suppl**, 194-206.

Borg, G. A. (1973). Perceived exertion: a note on "history" and methods. *Med Sci Sports* **5**, 90-93.

Borg, G. A. (1977). *Physical Work and Effort* Oxford: Pergamon Press.

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* **14**, 377-381.

Borghouts, L. B. & Keizer, H. A. (2000). Exercise and insulin sensitivity: a review. *Int J Sports Med* **21**, 1-12.

Bosch, A. N., Dennis, S. C., & Noakes, T. D. (1993). Influence of carbohydrate loading on fuel substrate turnover and oxidation during prolonged exercise. *J Appl Physiol* **74**, 1921-1927.

Boutcher, S. H., Seip, R. L., Hetzler, R. K., Pierce, E. F., Snead, D., & Weltman, A. (1989). The effects of specificity of training on rating of perceived exertion at the lactate threshold. *Eur J Appl Physiol Occup Physiol* **59**, 365-369.

Brewer, J., Williams, C., & Patton, A. (1988). The influence of high carbohydrate diets on endurance running performance. *Eur J Appl Physiol Occup Physiol* **57**, 698-706.

Brooks, G. A., Fahey, T. D., White, T. P., & Baldwin, K. M. (2000). *Exercise physiology: human bioenergetics and its applications*, 3rd ed. Mayfield Publishing Company, California.

Burden, A. & Bartlett, R. (1999). Normalisation of EMG amplitude: an evaluation and comparison of old and new methods. *Med Eng Phys* **21**, 247-257.

Burden, A. M., Trew, M., & Baltzopoulos, V. (2003). Normalisation of gait EMGs: a re-examination. *J Electromyogr Kinesiol* **13**, 519-532.

Burgess, M. L., Robertson, R. J., Davis, J. M., & Norris, J. M. (1991). RPE, blood glucose, and carbohydrate oxidation during exercise: effects of glucose feedings. *Med Sci Sports Exerc* **23**, 353-359.

Burke, L. M., Angus, D. J., Cox, G. R., Cummings, N. K., Febbraio, M. A., Gawthorn, K., Hawley, J. A., Minehan, M. , Martin, D. T., & Hargreaves, M. (2000). Effect of fat adaptation and carbohydrate restoration on metabolism and performance during prolonged cycling. *J Appl Physiol* **89**, 2413-2421.

Burke, L. M., Hawley, J. A., Angus, D. J., Cox, G. R., Clark, S. A., Cummings, N. K., Desbrow, B., & Hargreaves, M. (2002). Adaptations to short-term high-fat diet persist during exercise despite high carbohydrate availability. *Med Sci Sports Exerc* **34**, 83-91.

Burke, L. M. & Hawley, J. A. (2002). Effects of short-term fat adaptation on metabolism and performance of prolonged exercise. *Med Sci Sports Exerc* **34**, 1492-1498.

Cafarelli, E. & Noble, B. J. (1976). The effect of inspired carbon dioxide on subjective estimates of exertion during exercise. *Ergonomics* **19**, 581-589.

Cafarelli, E. (1977). Peripheral and central inputs to the effort sense during cycling exercise. *Eur J Appl Physiol Occup Physiol* **37**, 181-189.

Cafarelli, E., Cain, W. S., & Stevens, J. C. (1977). Effort of dynamic exercise: influence of load, duration, and task. *Ergonomics* **20**, 147-158.

Cafarelli, E. & Bigland-Ritchie, B. (1979). Sensation of static force in muscles of different length. *Exp Neurol* **65**, 511-525.

Carey, A. L., Staudacher, H. M., Cummings, N. K., Stepto, N. K., Nikolopoulos, V., Burke, L. M., & Hawley, J. A. (2001). Effects of fat adaptation and carbohydrate restoration on prolonged endurance exercise. *J Appl Physiol* **91**, 115-122.

Carrasco, D. I., Delp, M. D., & Ray, C. A. (1999). Effect of concentric and eccentric muscle actions on muscle sympathetic nerve activity. *J Appl Physiol* **86**, 558-563.

Carter, J. B., Banister, E. W., & Blaber, A. P. (2003). Effect of endurance exercise on autonomic control of heart rate. *Sports Med* **33**, 33-46.

Carton, R. L. & Rhodes, E. C. (1985). A critical review of the literature on ratings scales for perceived exertion. *Sports Med* **2**, 198-222.

Ceci, R. & Hassmen, P. (1991). Self-monitored exercise at three different RPE intensities in treadmill vs field running. *Med Sci Sports Exerc* **23**, 732-738.

Chesley, A., Hultman, E., & Spriet, L. L. (1995). Effects of epinephrine infusion on muscle glycogenolysis during intense aerobic exercise. *Am J Physiol (Endocrinol Metab)* **268** , E127-E134.

Claassen, A., Lambert, E. V., Bosch, A. N., Rodger, M., St Clair Gibson, A., & Noakes, T. D. (2005). Variability in exercise capacity and metabolic response during endurance exercise after a low carbohydrate diet. *Int J Sport Nutr Exerc Metab* **15**, 97-116.

Coggan, A. R. & Coyle, E. F. (1987). Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion. *J Appl Physiol* **63**, 2388-2395.

Coggan, A. R. (1997). Plasma glucose metabolism during exercise: effect of endurance training in humans. *Med Sci Sports Exerc* **29**, 620-627.

Conwit, R. A., Stashuk, D., Tracy, B., McHugh, M., Brown, W. F., & Metter, E. J. (1999). The relationship of motor unit size, firing rate and force. *Clin Neurophysiol* **110**, 1270-1275.

Conwit, R. A., Stashuk, D., Suzuki, H., Lynch, N., Schrager, M., & Metter, E. J. (2000). Fatigue effects on motor unit activity during submaximal contractions. *Arch Phys Med Rehabil* **81**, 1211-1216.

Cooper, D. F., Grimby, G., Jones, D. A., & Edwards, R. H. (1979). Perception of effort in isometric and dynamic muscular contraction. *Eur J Appl Physiol Occup Physiol* **41**, 173-180.

Coyle, E. F., Coggan, A. R., Hemmert, M. K., & Ivy, J. L. (1986). Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol* **61**, 165-172.

Davies, C. T. & Sargeant, A. J. (1979). The effects of atropine and practolol on the perception of exertion during treadmill exercise. *Ergonomics* **22**, 1141-1146.

Davis, J. M. & Bailey, S. P. (1997). Possible mechanisms of central nervous system fatigue during exercise. *Med Sci Sports Exerc* **29**, 45-57.

Davis, M. R., Mellman, M., & Shamoon, H. (1992). Further defects in counterregulatory responses induced by recurrent hypoglycemia in IDDM. *Diabetes* **41**, 1335-1340.

Davis, S. N., Shavers, C., Costa, F., & Mosqueda-Garcia, R. (1996). Role of cortisol in the pathogenesis of deficient counterregulation after antecedent hypoglycemia in normal humans. *J Clin Invest* **98**, 680-691.

Davis, S. N., Shavers, C., Mosqueda-Garcia, R., & Costa, F. (1997). Effects of differing antecedent hypoglycemia on subsequent counterregulation in normal humans. *Diabetes* **46**, 1328-1335.

Davis, S. N., Galassetti, P., Wasserman, D. H., & Tate, D. (2000a). Effects of antecedent hypoglycemia on subsequent counterregulatory responses to exercise. *Diabetes* **49**, 73-81.

Davis, S. N., Mann, S., Galassetti, P., Neill, R. A., Tate, D., Ertl, A. C., & Costa, F. (2000b). Effects of differing durations of antecedent hypoglycemia on counterregulatory responses to subsequent hypoglycemia in normal humans. *Diabetes* **49**, 1897-1903.

De Luca, C. J. & Merletti, R. (1988). Surface myoelectric signal cross-talk among muscles of the leg. *Electroencephalogr Clin Neurophysiol* **69**, 568-575.

De Luca, C. J., Foley, P. J., & Erim, Z. (1996). Motor unit control properties in constant-force isometric contractions. *J Neurophysiol* **76**, 1503-1516.

De Luca, C. J. (1997). The use of surface electromyography in biomechanics. *J Appl Biomech* **13**, 135-163.

Dettmers, C., Fink, G. R., Lemon, R. N., Stephan, K. M., Passingham, R. E., Silbersweig, D., Holmes, A., Ridding, M. C., Brooks, D. J., & Frackowiak, R. S. (1995). Relation between cerebral activity and force in the motor areas of the human brain. *J Neurophysiol* **74**, 802-815.

Dimitrijevic, M. R., McKay, W. B., Sarjanovic, I., Sherwood, A. M., Svirtlih, L., & Vrbova, G. (1992). Co-activation of ipsi- and contralateral muscle groups during contraction of ankle dorsiflexors. *J Neurol Sci* **109**, 49-55.

Dunbar, C. C., Robertson, R. J., Baun, R., Blandin, M. F., Metz, K., Burdett, R., & Goss, F. L. (1992). The validity of regulating exercise intensity by ratings of perceived exertion. *Med Sci Sports Exerc* **24**, 94-99.

Durnin, J. V. & Womersley, J. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr* **32**, 77-97.

Edwards, R. H., Melcher, A., Hesser, C. M., Wigertz, O., & Ekelund, L. G. (1972). Physiological correlates of perceived exertion in continuous and intermittent exercise with the same average power output. *Eur J Clin Invest* **2**, 108-114.

Ekblom, B. & Goldbarg, A. N. (1971). The influence of physical training and other factors on the subjective rating of perceived exertion. *Acta Physiol Scand* **83**, 399-406.

El Sayed, M. S., Balmer, J., & Rattu, A. J. (1997). Carbohydrate ingestion improves endurance performance during a 1 h simulated cycling time trial. *J Sports Sci* **15**, 223-230.

Enoka, R. M., Robinson, G. A., & Kossev, A. R. (1989). Task and fatigue effects on low-threshold motor units in human hand muscle. *J Neurophysiol* **62**, 1344-1359.

Enoka, R. M. & Stuart, D. G. (1992). Neurobiology of muscle fatigue. *J Appl Physiol* **72**, 1631-1648.

Enoka, R. M. (1995). Morphological features and activation patterns of motor units. *J Clin Neurophysiol* **12**, 538-559.

Eston, R. G., Davies, B. L., & Williams, J. G. (1987). Use of perceived effort ratings to control exercise intensity in young healthy adults. *Eur J Appl Physiol Occup Physiol* **56**, 222-224.

Eston, R. G. & Williams, J. G. (1988). Reliability of ratings of perceived effort regulation of exercise intensity. *Br J Sports Med* **22**, 153-155.

Eston, R. G., Lamb, K. L., Parfitt, G., & King, N. (2005). The validity of predicting maximal oxygen uptake from a perceptually-regulated graded exercise test. *Eur J Appl Physiol* **94**, 221-227.

Eston, R. G., Faulkner, J. A., Mason, E. A., & Parfitt, G. (2006). The validity of predicting maximal oxygen uptake from perceptually-regulated graded exercise tests of different durations. *Eur J Appl Physiol* **97**, 535-541.

Farina, D. & Merletti, R. (2000). Comparison of algorithms for estimation of EMG variables during voluntary isometric contractions. *J Electromyogr Kinesiol* **10**, 337-349.

Farina, D., Cescon, C., & Merletti, R. (2002). Influence of anatomical, physical, and detection-system parameters on surface EMG. *Biol Cybern* **86**, 445-456.

Farina, D., Merletti, R., & Enoka, R. M. (2004). The extraction of neural strategies from the surface EMG. *J Appl Physiol* **96**, 1486-1495.

Febbraio, M., Lambert, D. L., Starkie, R. L., Proietto, S. J., & Hargreaves, M. (1998). Effect of epinephrine on muscle glycogenolysis during exercise in trained men. *J Appl Physiol* **84**, 465-470.

Febbraio, M. A., Chiu, A., Angus, D. J., Arkinstall, M. J., & Hawley, J. A. (2000). Effects of carbohydrate ingestion before and during exercise on glucose kinetics and performance. *J Appl Physiol* **89**, 2220-2226.

Felig, P., Cherif, A., Minagawa, A., & Wahren, J. (1982). Hypoglycemia during prolonged exercise in normal men. *N Engl J Med* **306**, 895-900.

Finsterer, J. (2001). EMG-interference pattern analysis. *J Electromyogr Kinesiol* **11**, 231-246.

Fitts, R. H. (1994). Cellular mechanisms of muscle fatigue. *Physiol Rev* **74**, 49-94.

Forster, C. D. & Macdonald, I. A. (1999). The assay of the catecholamine content of small volumes of human plasma. *Biomed Chromatogr* **13**, 215.

French, D. N., Kraemer, W. J., Volek, J. S., Spiering, B. A., Judelson, D. A., Hoffman, J. R., & Maresh, C. M. (2006). Anticipatory Responses of Catecholamines on Muscle Force Production. *J Appl Physiol* **(In Press)**.

Fuglevand, A. J., Zackowski, K. M., Huey, K. A., & Enoka, R. M. (1993). Impairment of neuromuscular propagation during human fatiguing contractions at submaximal forces. *J Physiol* **460**, 549-572.

Galassetti, P., Mann, S., Tate, D., Neill, R. A., Costa, F., Wasserman, D. H., & Davis, S. N. (2001a). Effects of antecedent prolonged exercise on subsequent counterregulatory responses to hypoglycemia. *Am J Physiol Endocrinol Metab* **280**, E908-E917.

Galassetti, P., Neill, A. R., Tate, D., Ertl, A. C., Wasserman, D. H., & Davis, S. N. (2001b). Sexual dimorphism in counterregulatory responses to hypoglycemia after antecedent exercise. *J Clin Endocrinol Metab* **86**, 3516-3524.

Galloway, S. D. & Maughan, R. J. (1997). Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Med Sci Sports Exerc* **29**, 1240-1249.

Gamberale, F. (1972). Perceived exertion, heart rate, oxygen uptake and blood lactate in different work operations. *Ergonomics* **15**, 545-554.

Gandevia, S. C. & McCloskey, D. I. (1978). Interpretation of perceived motor commands by reference to afferent signals. *J Physiol* **283**, 193-199.

Gandevia, S. C. (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* **81**, 1725-1789.

Gearhart, R. F., Jr., Becque, M. D., Palm, C. M., & Hutchins, M. D. (2005). Rating perceived exertion during short duration, very high intensity cycle exercise. *Percept Mot Skills* **100**, 767-773.

Giannesini, B., Cozzone, P. J., & Bendahan, D. (2003). Non-invasive investigations of muscular fatigue: metabolic and electromyographic components. *Biochimie* **85**, 873-883.

Glass, S. C., Knowlton, R. G., & Becque, M. D. (1994). Perception of effort during high-intensity exercise at low, moderate and high wet bulb globe temperatures. *Eur J Appl Physiol Occup Physiol* **68**, 519-524.

Goedecke, J. H., Christie, C., Wilson, G., Dennis, S. C., Noakes, T. D., Hopkins, W. G., & Lambert, E. V. (1999). Metabolic adaptations to a high-fat diet in endurance cyclists. *Metabolism* **48**, 1509-1517.

Goedecke, J. H., St Clair, G. A., Grobler, L., Collins, M., Noakes, T. D., & Lambert, E. V. (2000). Determinants of the variability in respiratory exchange ratio at rest and during exercise in trained athletes. *Am J Physiol Endocrinol Metab* **279**, E1325-E1334.

Goldberger, J. J. (1999). Sympathovagal balance: how should we measure it? *Am J Physiol* **276**, H1273-H1280.

Hagberg, M. (1981). Muscular endurance and surface electromyogram in isometric and dynamic exercise. *J Appl Physiol* **51**, 1-7.

Hakkinen, K. & Komi, P. V. (1983). Electromyographic and mechanical characteristics of human skeletal muscle during fatigue under voluntary and reflex conditions. *Electroencephalogr Clin Neurophysiol* **55**, 436-444.

Hampson, D. B., St Clair Gibson, A., Lambert, M. I., & Noakes, T. D. (2001). The influence of sensory cues on the perception of exertion during exercise and central regulation of exercise performance. *Sports Med* **31**, 935-952.

Hampson, D. B., St Clair Gibson, A., Lambert, M. I., Dugas, J. P., Lambert, E. V., & Noakes, T. D. (2004). Deception and perceived exertion during high-intensity running bouts. *Percept Mot Skills* **98**, 1027-1038.

Haskvitz, E. M., Seip, R. L., Weltman, J. Y., Rogol, A. D. , & Weltman, A. (1992). The effect of training intensity on ratings of perceived exertion. *Int J Sports Med* **13**, 377-383.

Hassmen, P. (1990). Perceptual and physiological responses to cycling and running in groups of trained and untrained subjects. *Eur J Appl Physiol Occup Physiol* **60**, 445-451.

Hawley, J. A., Brouns, F., & Jeukendrup, A. (1998). Strategies to enhance fat utilisation during exercise. *Sports Med* **25**, 241-257.

Helge, J. W., Richter, E. A., & Kiens, B. (1996). Interaction of training and diet on metabolism and endurance during exercise in man. *J Physiol* **492(1)**, 293-306.

Helge, J. W., Wulff, B., & Kiens, B. (1998). Impact of a fat-rich diet on endurance in man: role of the dietary period. *Med Sci Sports Exerc* **30**, 456-461.

Helge, J. W. (2002). Long-term fat diet adaptation effects on performance, training capacity, and fat utilization. *Med Sci Sports Exerc* **34**, 1499-1504.

Heller, S. R. & Cryer, P. E. (1991). Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. *Diabetes* **40**, 223-226.

Henneman, E., Somjen, G., & Carpenter, D. O. (1965). Functional significance of cell size in spinal motorneurons. *J Neurophysiol* **28**, 560-580.

Hetzler, R. K., Seip, R. L., Boutcher, S. H., Pierce, E., Snead, D., & Weltman, A. (1991). Effect of exercise modality on ratings of perceived exertion at various lactate concentrations. *Med Sci Sports Exerc* **23**, 88-92.

Holmberg, E. & Waldeck, B. (1980). On the possible role of potassium ions in the action of terbutaline on skeletal muscle contractions. *Acta Pharmacol Toxicol (Copenh)* **46**, 141-149.

Horstman, D. H. (1977). Exercise performance at 5 degrees C. *Med Sci Sports* **9**, 52.

Horstman, D. H., Morgan, W. P., Cymerman, A., & Stokes, J. (1979a). Perception of effort during constant work to self-imposed exhaustion. *Percept Mot Skills* **48**, 1111-1126.

Horstman, D. H., Weiskopf, R., & Robinson, S. (1979b). The nature of the perception of effort at sea level and high altitude. *Med Sci Sports* **11**, 150-154.

Horstman, D., Kowal, D., Vaughan, L., & Stivanelli, A. (1979c). The influence of previous physical experience on the perception of work effort. *Med Sci Sports* **11**, 79.

Howlett, K., Febbraio, M., & Hargreaves, M. (1999a). Glucose production during strenuous exercise in humans: role of epinephrine. *Am J Physiol (Endocrinol Metab)* **276**, E1130-E1135.

Howlett, K., Galbo, H., Lorentsen, J., Bergeron, R., Zimmerman-Belsing, T., Bulow, J., Feldt-Rasmussen, U., & Kjaer, M. (1999b). Effect of adrenaline on glucose kinetics during exercise in adrenalectomised humans. *J Physiol* **519**, 911-921.

Hunter, A. M., St Clair Gibson, A., Lambert, M., & Noakes, T. D. (2002a). Electromyographic (EMG) normalization method for cycle fatigue protocols. *Med Sci Sports Exerc* **34**, 857-861.

Hunter, A. M., St Clair Gibson, A., Derman, W. E., Lambert, M., Dennis, S. C., & Noakes, T. D. (2002b). The effect of selective beta1-blockade on EMG signal

characteristics during progressive endurance exercise. *Eur J Appl Physiol* **88**, 275-281.

Hunter, A. M., St Clair Gibson, A., Lambert, M. I., Nobbs, L., & Noakes, T. D. (2003a). Effects of supramaximal exercise on the electromyographic signal. *Br J Sports Med* **37**, 296-299.

Hunter, A. M., St Clair Gibson, A., Lambert, M., Dennis, S., Mullany, H., O'Malley, M. J., Vaughan, C. L., Kay, D., & Noakes, T. D. (2003b). EMG amplitude in maximal and submaximal exercise is dependent on signal capture rate. *Int J Sports Med* **24**, 83-89.

Hunter, S. K. & Enoka, R. M. (2003). Changes in muscle activation can prolong the endurance time of a submaximal isometric contraction in humans. *J Appl Physiol* **94**, 108-118.

Hutton, R. S., Enoka, R. M., & Suzuki, S. (1984). Activation history and constant errors in human force production. *Brain Res* **307**, 344-346.

Iellamo, F., Legramante, J. M. , Pigozzi, F., Spataro, A., Norbiato, G., Lucini, D., & Pagani, M. (2002). Conversion from vagal to sympathetic predominance with strenuous training in high-performance world class athletes. *Circulation* **105**, 2719-2724.

Ivanova, T., Garland, S. J., & Miller, K. J. (1997). Motor unit recruitment and discharge behavior in movements and isometric contractions. *Muscle Nerve* **20**, 867-874.

Ivy, J. L. (1999). Role of carbohydrate in physical activity. *Clin Sports Med* **18**, 469-84.

Jackson, A. W. & Dishman, R. K. (2000). Perceived submaximal force production in young adult males and females. *Med Sci Sports Exerc* **32**, 448-451.

Jameson, C. & Ring, C. (2000). Contributions of local and central sensations to the perception of exertion during cycling: effects of work rate and cadence. *J Sports Sci* **18**, 291-298.

Jansson, E., Hjemdahl, P., & Kaijser, L. (1982). Diet induced changes in sympatho-adrenal activity during submaximal exercise in relation to substrate utilization in man. *Acta Physiol Scand* **114**, 171-178.

Jansson, E., Hjemdahl, P., & Kaijser, L. (1986). Epinephrine-induced changes in muscle carbohydrate metabolism during exercise in male subjects. *J Appl Physiol* **60**, 1466-1470.

Jensen, C., Vasseljen, O., & Westgaard, R. H. (1993). The influence of electrode position on bipolar surface electromyogram recordings of the upper trapezius muscle. *Eur J Appl Physiol Occup Physiol* **67**, 266-273.

Jentjens, R. L., Cale, C., Gutch, C., & Jeukendrup, A. E. (2003). Effects of pre-exercise ingestion of differing amounts of carbohydrate on subsequent metabolism and cycling performance. *Eur J Appl Physiol* **88**, 444-452.

Jones, D. A. (1996). High-and low-frequency fatigue revisited. *Acta Physiol Scand* **156**, 265-270.

Jones, L. A. & Hunter, I. W. (1982). Force sensation in isometric contractions: a relative force effect. *Brain Res* **244**, 186-189.

Jones, L. A. & Hunter, I. W. (1983a). Effect of fatigue on force sensation. *Exp Neurol* **81**, 640-650.

Jones, L. A. & Hunter, I. W. (1983b). Perceived force in fatiguing isometric contractions. *Percept Psychophys* **33**, 369-374.

Kadaba, M. P., Wootten, M. E., Gainey, J., & Cochran, G. V. (1985). Repeatability of phasic muscle activity: performance of surface and intramuscular wire electrodes in gait analysis. *J Orthop Res* **3**, 350-359.

Kamen, G. & Caldwell, G. E. (1996). Physiology and interpretation of the electromyogram. *J Clin Neurophysiol* **13**, 366-384.

Kamon, E., Pandolf, K., & Cafarelli, E. (1974). The relationship between perceptual information and physiological responses to exercise in the heat. *J Hum Ergol (Tokyo)* **3**, 45-54.

Kang, J., Robertson, R. J., Goss, F. L., DaSilva, S. G., Visich, P., Suminski, R. R., Utter, A. C., & Denys, B. C. (1996). Effect of carbohydrate substrate availability on ratings of perceived exertion during prolonged exercise of moderate intensity. *Percept Mot Skills* **82**, 495-506.

Kang, J., Chaloupka, E. C., Mastrangelo, M. A., Donnelly, M. S., Martz, W. P., & Robertson, R. J. (1998). Regulating exercise intensity using ratings of perceived exertion during arm and leg ergometry. *Eur J Appl Physiol Occup Physiol* **78**, 241-246.

Kang, J., Hoffman, J. R., Walker, H., Chaloupka, E. C., & Utter, A. C. (2003). Regulating intensity using perceived exertion during extended exercise periods. *Eur J Appl Physiol* **89**, 475-482.

Karlsson, J., Bonde-Petersen, F., Henriksson, J., & Knuttgen, H. G. (1975). Effects of previous exercise with arms or legs on metabolism and performance in exhaustive exercise. *J Appl Physiol* **38**, 763-767.

Karlsson, J. S., Ostlund, N., Larsson, B., & Gerdle, B. (2003). An estimation of the influence of force decrease on the mean power spectral frequency shift of the EMG during repetitive maximum dynamic knee extensions. *J Electromyogr Kinesiol* **13**, 461-468.

Karlsson, S. & Gerdle, B. (2001). Mean frequency and signal amplitude of the surface EMG of the quadriceps muscles increase with increasing torque--a study using the continuous wavelet transform. *J Electromyogr Kinesiol* **11**, 131-140.

Kay, D., St Clair Gibson, A., Mitchell, M. J., Lambert, M. I., & Noakes, T. D. (2000). Different neuromuscular recruitment patterns during eccentric, concentric and isometric contractions. *J Electromyogr Kinesiol* **10**, 425-431.

Kay, D., Marino, F. E., Cannon, J., St Clair Gibson, A., Lambert, M. I., & Noakes, T. D. (2001). Evidence for neuromuscular fatigue during high-intensity cycling in warm, humid conditions. *Eur J Appl Physiol* **84**, 115-121.

Kayser, B., Narici, M., Binzoni, T., Grassi, B., & Cerretelli, P. (1994). Fatigue and exhaustion in chronic hypobaric hypoxia: influence of exercising muscle mass. *J Appl Physiol* **76**, 634-640.

Kellis, E. & Baltzopoulos, V. (1996). Gravitational moment correction in isokinetic dynamometry using anthropometric data. *Med Sci Sports Exerc* **28**, 900-907.

Kiens, B. & Richter, E. A. (1996). Types of carbohydrate in an ordinary diet affect insulin action and muscle substrates in humans. *Am J Clin Nutr* **63**, 47-53.

Kirkendall, D. T. (1990). Mechanisms of peripheral fatigue. *Med Sci Sports Exerc* **22**, 444-449.

Kjaer, M., Secher, N. H., & Galbo, H. (1987). Physical stress and catecholamine release. *Baillieres Clin Endocrinol Metab* **1**, 279-298.

Kjaer, M., & Galbo, H. (1988). Effect of physical training on the capacity to secrete epinephrine. *J Appl Physiol* **64**, 11-16.

Kjaer, M. (1989). Epinephrine and some other hormonal responses to exercise in man: with special reference to physical training. *Int J Sports Med* **10** , 2-15.

Kjaer, M. (1998). Adrenal medulla and exercise training. *Eur J Appl Physiol Occup Physiol* **77**, 195-199.

Kjaer, M., Howlett, K., Langfort, J., Zimmerman-Belsing, T., Lorentsen, J., Bulow, J., Ihlemann, J., Feldt-Rasmussen, U., & Galbo, H. (2000). Adrenaline and glycogenolysis in skeletal muscle during exercise: a study in adrenalectomised humans. *J Physiol* **528** Pt 2, 371-378.

Kjaer, M. & Lange, K. (2000). Adrenergic regulation of energy metabolis In *Sports Endocrinology*, eds. Warren, M. P. & Constantini, N. W., pp. 181-188. Humana Press Inc., New Jersey.

Klausen, K., Knuttgen, H. G., & Forster, H. V. (1972). Effect of pre-existing high blood lactate concentration on maximal exercise performance. *Scand J Clin Lab Invest* **30**, 415-419.

Klausen, K., Secher, N. H., Clausen, J. P., Hartling, O., & Trap-Jensen, J. (1982). Central and regional circulatory adaptations to one-leg training. *J Appl Physiol* **52**, 976-983.

Knudson, D. V. & Johnston, D. (1993). Comparison of EMG normalization methods in a sit-to-stand movement. *Journal of Human Movement Studies* **25**, 39-50.

Knutson, L. M., Sodberg, G. L. , Ballantyne, B. T., & Clarke, W. R. (1994). A study of various normalization procedures for within day electromyographic data. *J Electromyogr Kinesiol* **4**, 47-59.

Komesaroff, P. A. & Funder, J. W. (1994). Differential glucocorticoid effects on catecholamine responses to stress. *Am J Physiol* **266**, E118-E128.

Kostka, C. E. & Cafarelli, E. (1982). Effect of pH on sensation and vastus lateralis electromyogram during cycling exercise. *J Appl Physiol* **52**, 1181-1185.

Kreisman, S. H., Mew, N. AH., Arsenault, M., Nessim, S. J., Halter, J. B., Vranic, M., & Marliss, E. B. (2000). Epinephrine infusion during moderate intensity exercise increases glucose production and uptake. *Am J Physiol Endocrinol Metab* **278**, E949-E957.

Kreisman, S. H., Halter, J. B., Vranic, M., & Marliss, E. B. (2003). Combined infusion of epinephrine and norepinephrine during moderate exercise reproduces the glucoregulatory response of intense exercise. *Diabetes* **52**, 1347-1354.

Kuipers, H., Fransen, E. J., & Keizer, H. A. (1999). Pre-exercise ingestion of carbohydrate and transient hypoglycemia during exercise. *Int J Sports Med* **20**, 227-231.

Kukulka, C. G. & Clamann, H. P. (1981). Comparison of the recruitment and discharge properties of motor units in human brachial biceps and adductor pollicis during isometric contractions. *Brain Res* **219**, 45-55.

Kumar, S. & Simmonds, M. (1994). The accuracy of magnitude production of submaximal precision and power grips and gross motor efforts. *Ergonomics* **37**, 1345-1353.

Kvetnansky, R., Fukuhara, K., Pacak, K., Cizza, G., Goldstein, D. S., & Kopin, I. J. (1993). Endogenous glucocorticoids restrain catecholamine synthesis and release at rest and during immobilization stress in rats. *Endocrinology* **133**, 1411-1419.

Lambert, E. V., Speechly, D. P., Dennis, S. C., & Noakes, T. D. (1994). Enhanced endurance in trained cyclists during moderate intensity exercise

following 2 weeks adaptation to a high fat diet. *Eur J Appl Physiol Occup Physiol* **69**, 287-293.

Lambert, E. V., St Clair Gibson, A., & Noakes, T. D. (2005). Complex systems model of fatigue: integrative homoeostatic control of peripheral physiological systems during exercise in humans. *Br J Sports Med* **39**, 52-62.

Leal-Cerro, A., Gippini, A., Amaya, M. J., Lage, M., Mato, J. A., Dieguez, C., & Casanueva, F. F. (2003). Mechanisms underlying the neuroendocrine response to physical exercise. *J Endocrinol Invest* **26**, 879-885.

Lewis, S., Thompson, P., Areskog, N. H., Vodak, P., Marconyak, M., DeBusk, R., Mellen, S., & Haskell, W. (1980). Transfer effects of endurance training to exercise with untrained limbs. *Eur J Appl Physiol Occup Physiol* **44**, 25-34.

Linnamo, V., Moritani, T., Nicol, C., & Komi, P. V. (2003). Motor unit activation patterns during isometric, concentric and eccentric actions at different force levels. *J Electromyogr Kinesiol* **13**, 93-101.

Lollgen, H., Ulmer, H. V., Gross, R., Wilbert, G., & von Nieding, G. (1975). Methodical aspects of perceived exertion rating and its relation to pedalling rate and rotating mass. *Eur J Appl Physiol Occup Physiol* **34**, 205-215.

Lollgen, H., Ulmer, H. V., & von Nieding, G. (1977). Heart rate and perceptual response to exercise with different pedalling speed in normal subjects and patients. *Eur J Appl Physiol Occup Physiol* **37**, 297-304.

Maisetti, O., Guevel, A., Legros, P., & Hogrel, J. Y. (2002). SEMG power spectrum changes during a sustained 50% Maximum Voluntary Isometric Torque do not depend upon the prior knowledge of the exercise duration. *J Electromyogr Kinesiol* **12**, 103-109.

Marino, F. E. (2004). Anticipatory regulation and avoidance of catastrophe during exercise-induced hyperthermia. *Comp Bio Physiol* **(Pt B) 139**, 561-569.

Marsh, A. P. & Martin, P. E. (1993). The association between cycling experience and preferred and most economical cadences. *Med Sci Sports Exerc* **25**, 1269-1274.

Marsh, A. P. & Martin, P. E. (1998). Perceived exertion and the preferred cycling cadence. *Med Sci Sports Exerc* **30**, 942-948.

Masuda, K., Masuda, T., Sadoyama, T., Inaki, M., & Katsuta, S. (1999). Changes in surface EMG parameters during static and dynamic fatiguing contractions. *J Electromyogr Kinesiol* **9**, 39-46.

Mathiassen, S. E., Winkel, J., & Hagg, G. M. (1995). Normalization of surface EMG amplitude from upper trapezuis muscle in ergonomic studies - a review. *J Electromyogr Kinesiol* **5**, 197-226.

Mazzeo, R. S. (1991). Catecholamine responses to acute and chronic exercise. *Med Sci Sports Exerc* **23**, 839-845.

McDermott, J. C., Elder, G. C., & Bonen, A. (1987). Adrenal hormones enhance glycogenolysis in nonexercising muscle during exercise. *J Appl Physiol* **63**, 1275-1283.

McDermott, J. C., Elder, G. C., & Bonen, A. (1991). Non-exercising muscle metabolism during exercise. *Pflugers Arch* **418**, 301-307.

Megeney, L. A., Elder, G. C., Tan, M. H., & Bonen, A. (1992). Increased glucose transport in nonexercising muscle. *Am J Physiol* **262**, E20-E26.

Merletti, R., Lo Conte, L. R., & Orizio, C. (1991). Indices of muscle fatigue. *J Electromyogr Kinesiol* **1**, 20-33.

Merletti, R. & Roy, S. (1996). Myoelectric and mechanical manifestations of muscle fatigue in voluntary contractions. *J Orthop Sports Phys Ther* **24**, 342-353.

Merletti, R. & Lo Conte, L. R. (1997). Surface EMG signal processing during isometric contractions. *J Electromyogr Kinesiol* **7**, 241-250.

Micheal, E. D. & Eckhardt, L. (1972). The selection of hard work by trained and non-trained subjects. *Med Sci Sports* **4**, 107-110.

Mihevic, P. M. (1981). Sensory cues for perceived exertion: a review. *Med Sci Sports Exerc* **13**, 150-163.

Mikkelsen, U. R., Gissel, H., Fredsted, A., & Clausen, T. (2006). Excitation-induced cell damage and beta2-adrenoceptor agonist stimulated force recovery in rat skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* **290**, R265-R272.

Milner-Brown, H. S., Stein, R. B., & Yemm, R. (1973). The orderly recruitment of human motor units during voluntary isometric contractions. *J Physiol* **230**, 359-370.

Mirka, G. A. (1991). The quantification of EMG normalization error. *Ergonomics* **34**, 343-352.

Mora-Rodriguez, R. & Coyle, E. (2000). Effects of plasma epinephrine on fat metabolism during exercise: interactions with exercise intensity. *Am J Physiol Endocrinol Metab* **278**, E669-E676.

Morgan, W. P. (1973). Psychological factors influencing perceived exertion. *Med Sci Sports* **5**, 97-103.

Morgan, W. P., Hirta, K., Weitz, G. A., & Balke, B. (1976). Hypnotic perturbation of perceived exertion: ventilatory consequences. *Am J Clin Hypn* **18**, 182-190.

Moritani, T., Nagata, A., & Muro, M. (1982). Electromyographic manifestations of muscular fatigue. *Med Sci Sports Exerc* **14**, 198-202.

Moritani, T., Muro, M., Kijima, A., Gaffney, F. A., & Parsons, D. (1985). Electromechanical changes during electrically induced and maximal voluntary contractions: surface and intramuscular EMG responses during sustained maximal voluntary contraction. *Exp Neurol* **88**, 484-499.

Moritani, T., Muro, M., & Nagata, A. (1986). Intramuscular and surface electromyogram changes during muscle fatigue. *J Appl Physiol* **60**, 1179-1185.

Moritani, T. & Muro, M. (1987). Motor unit activity and surface electromyogram power spectrum during increasing force of contraction. *Eur J Appl Physiol Occup Physiol* **56**, 260-265.

Moritani, T. & Yoshitake, Y. (1998). 1998 ISEK Congress Keynote Lecture: The use of electromyography in applied physiology. International Society of Electrophysiology and Kinesiology. *J Electromyogr Kinesiol* **8**, 363-381.

Morrison, S., Sleivert, G. G., & Cheung, S. S. (2004). Passive hyperthermia reduces voluntary activation and isometric force production. *Eur J Appl Physiol* **91**, 729-736.

Mullany, H. (2000). POO [2 2] Computer Program.

Mullany, H., O'Malley, M., St Clair Gibson, A., & Vaughan, C. (2002). Agonist-antagonist common drive during fatiguing knee extension efforts using surface electromyography. *J Electromyogr Kinesiol* **12**, 375-384.

Neary, P. J. & Wenger, H. A. (1986). The effects of one- and two-legged exercise on the lactate and ventilatory threshold. *Eur J Appl Physiol Occup Physiol* **54**, 591-595.

Nikolopoulos, V., Arkinstall, M. J., & Hawley, J. A. (2004). Reduced neuromuscular activity with carbohydrate ingestion during constant load cycling. *Int J Sport Nutr Exerc Metab* **14**, 161-170.

Noakes, T. D. (1998). Maximal oxygen uptake: "classical" versus "contemporary" viewpoints: a rebuttal. *Med Sci Sports Exerc* **30**, 1381-1398.

Noakes, T. D. (2004). Linear relationship between the perception of effort and the duration of constant load exercise that remains. *J Appl Physiol* **96**, 1571-1572.

Noakes, T. D. & St Clair Gibson, A. (2004). Logical limitations to the "catastrophe" models of fatigue during exercise in humans. *Br J Sports Med* **38**, 648-649.

Noakes, T. D., St Clair Gibson, A., & Lambert, E. V. (2004). From catastrophe to complexity: a novel model of integrative central neural regulation of effort and fatigue during exercise in humans. *Br J Sports Med* **38**, 511-514.

Noble, B. J., Metz, K. F., Pandolf, K. B., & Cafarelli, E. (1973). Perceptual responses to exercise: a multiple regression study. *Med Sci Sports* **5**, 104-109.

Noble, B. J., Maresh, C. M., Allison, T. G., & Drash, A. (1979). Cardio-respiratory and perceptual recovery from a marathon run. *Med Sci Sports* **11**, 239-243.

Noble, B. J. (1982). Clinical applications of perceived exertion. *Med Sci Sports Exerc* **14**, 406-411.

Noble, B. J., Borg, G. A., Jacobs, I., Ceci, R., & Kaiser, P. (1983). A category-ratio perceived exertion scale: relationship to blood and muscle lactates and heart rate. *Med Sci Sports Exerc* **15**, 523-528.

Nordlund, M. M., Thorstensson, A., & Cresswell, A. G. (2004). Central and peripheral contributions to fatigue in relation to level of activation during repeated maximal voluntary isometric plantar flexions. *J Appl Physiol* **96**, 218-225.

Nybo, L. & Nielsen, B. (2001). Perceived exertion is associated with an altered brain activity during exercise with progressive hyperthermia. *J Appl Physiol* **91**, 2017-2023.

Nybo, L. (2003). CNS fatigue and prolonged exercise: effect of glucose supplementation. *Med Sci Sports Exerc* **35**, 589-594.

O' Keefe, K. A., Keith, R. E., Wilson, G. D., & Blessing, D. L. (1989). Dietary carbohydrate intake and endurance exercise performance of trained female. *Nutr Res* **9**, 819-830.

Ogita, F., Stam, R. P., Tazawa, H. O., Toussaint, H. M., & Hollander, A. P. (2000). Oxygen uptake in one-legged and two-legged exercise. *Med Sci Sports Exerc* **32**, 1737-1742.

Ori, Z., Monir, G., Weiss, J., Sayhouni, X., & Singer, D. H. (1992). Heart rate variability. Frequency domain analysis. *Cardiol Clin* **10**, 499-537.

Palmer, G. S., Dennis, S. C., Noakes, T. D., & Hawley, J. A. (1996). Assessment of the reproducibility of performance testing on an air-braked cycle ergometer. *Int J Sports Med* **17**, 293-298.

Pandolf, K. B., Cafarelli, E., Noble, B. J., & Metz, K. F. (1972). Perceptual responses during prolonged work. *Percept Mot Skills* **35**, 975-985.

Pandolf, K. B. & Noble, B. J. (1973). The effect of pedalling speed and resistance changes on perceived exertion for equivalent power outputs on the bicycle ergometer. *Med Sci Sports* **5**, 132-136.

Pandolf, K. B., Burse, R. L., & Goldman, R. F. (1975). Differentiated ratings of perceived exertion during physical conditioning of older individuals using leg-weight loading. *Percept Mot Skills* **40**, 563-574.

Pandolf, K. B., Kamon, E., & Noble, B. J. (1978). Perceived exertion and physiological responses during negative and positive work in climbing a laddermill. *J Sports Med Phys Fitness* **18**, 227-236.

Pandolf, K. B. (1978). Influence of local and central factors in dominating rated perceived exertion during physical work. *Percept Mot Skills* **46**, 683-698.

Pandolf, K. B. (1983). Advances in the study and application of perceived exertion. *Exerc Sport Sci Rev* **11**, 118-158.

Passonneau, J. V. & Lauderdale, V. R. (1999). A comparison of three methods of glycogen measurement in tissues. *Anal Biochem* **60**, 405-412.

Paterson, S. & Marino, F. E. (2004). Effect of deception of distance on prolonged cycling performance. *Percept Mot Skills* **98**, 1017-1026.

Pederson, P. K. & Welch, H. G. (1977). Oxygen breathing, selected physiological variables and perception of effort during submaximal exercise. In *Physical Work and Effort*, ed. Borg, G. A., pp. 217-221. Pergamon Press, Solna, Sweden.

Perini, R., Orizio, C., Baselli, G., Cerutti, S., & Veicsteinas, A. (1990) The influence of exercise inetnsity on the power spectrum of heart rate variability. *Eur J Appl Physiol Occup Physiol* **61**, 143-148.

Perini, R., Orizio, C., Milesi, S., Biancardi, L., Baselli, G., & Veicsteinas, A. (1993). Body position affects the power spectrum of heart rate variability during dynamic exercise. *Eur J Appl Physiol Occup Physiol* **66**, 207-213.

Perini, R., & Veicsteinas, A. (2003). Heart rate variability and autonomic activity at rest and during exercise in various physiological conditions. *Eur J Appl Physiol* **90**, 317-325.

Phinney, S. D., Bistrian, B. R., Evans, W. J., Gervino, E., & Blackburn, G. L. (1983). The human metabolic response to chronic ketosis without caloric restriction: preservation of submaximal exercise capability with reduced carbohydrate oxidation. *Metabolism* **32**, 769-776.

Pichon, A. P., de Bisschop, C., Roulaud, M., Denjean, A., & Papelier, Y. (2004). Spectral analysis of heart rate variability during exercise in trained subjects. *Med Sci Sports Exerc* **36**, 1702-1708.

Pincivero, D. M., Gear, W. S., Moyna, N. M., & Robertson, R. J. (1999). The effects of rest interval on quadriceps torque and perceived exertion in healthy males. *J Sports Med Phys Fitness* **39**, 294-299.

Pincivero, D. M., Coelho, A. J., & Erikson, W. H. (2000a). Perceived exertion during isometric quadriceps contraction. A comparison between men and women. *J Sports Med Phys Fitness* **40**, 319-326.

Pincivero, D. M. & Gear, W. S. (2000b). Quadriceps activation and perceived exertion during a high intensity, steady state contraction to failure. *Muscle Nerve* **23**, 514-520.

Pincivero, D. M., Coelho, A. J., Campy, R. M., Salfetnikov, Y., & Bright, A. (2001). The effects of voluntary contraction intensity and gender on perceived exertion during isokinetic quadriceps exercise. *Eur J Appl Physiol* **84**, 221-226.

Pincivero, D. M., Coelho, A. J., Campy, R. M., Salfetnikov, Y., & Suter, E. (2003a). Knee extensor torque and quadriceps femoris EMG during perceptually-guided isometric contractions. *J Electromyogr Kinesiol* **13**, 159-167.

Pincivero, D. M., Dixon, P. T., & Coelho, A. J. (2003b). Knee extensor torque, work, and EMG during subjectively graded dynamic contractions. *Muscle Nerve* **28**, 54-61.

Pincivero, D. M., Coelho, A. J., & Campy, R. M. (2004). Gender differences in perceived exertion during fatiguing knee extensions. *Med Sci Sports Exerc* **36**, 109-117.

Pitsiladis, Y. P. & Maughan, R. J. (1999). The effects of exercise and diet manipulation on the capacity to perform prolonged exercise in the heat and in the cold in trained humans. *J Physiol* **517 (Pt 3)**, 919-930.

Potvin, J. R. (1997). Effects of muscle kinematics on surface EMG amplitude and frequency during fatiguing dynamic contractions. *J Appl Physiol* **82**, 144-151.

Poulus, A. J., Docter, H. J., & Westra, H. G. (1974). Acid-base balance and subjective feelings of fatigue during physical exercise. *Eur J Appl Physiol Occup Physiol* **33**, 207-213.

Prusaczyk, W. K., Cureton, K. J., Graham, R. E., & Ray, C. A. (1992). Differential effects of dietary carbohydrate on RPE at the lactate and ventilatory thresholds. *Med Sci Sports Exerc* **24**, 568-575.

Rainoldi, A., Nazzaro, M., Merletti, R., Farina, D., Caruso, I., & Gaudenti, S. (2000). Geometrical factors in surface EMG of the vastus medialis and lateralis muscles. *J Electromyogr Kinesiol* **10**, 327-336.

Rainoldi, A., Melchiorri, G., & Caruso, I. (2004). A method for positioning electrodes during surface EMG recordings in lower limb muscles. *J Neurosci Methods* **134**, 37-43.

Ray, C. A. (1993). Muscle sympathetic nerve responses to prolonged one-legged exercise. *J Appl Physiol* **74**, 1719-1722.

Ray, C. A. (1999). Sympathetic adaptations to one-legged training. *J Appl Physiol* **86**, 1583-1587.

Robertson, R. J., Gillespie, R. L., McCarthy, J., & Rose, K. D. (1979a). Differentiated perceptions of exertion: part I. mode of integration of regional signals. *Percept Mot Skills* **49**, 683-689.

Robertson, R. J., Gillespie, R. L., McCarthy, J., & Rose, K. D. (1979b). Differentiated perceptions of exertion: part II. relationship to local and central physiological responses. *Percept Mot Skills* **49**, 691-697.

Robertson, R. J., Gilcher, R., & Metz, K. (1979c). Central circulation and work capacity after red blood cell reinfusion under normoxia and hypoxia in woman. *Med Sci Sports* **11**, 98.

Robertson, R. J. (1982). Central signals of perceived exertion during dynamic exercise. *Med Sci Sports Exerc* **14**, 390-396.

Robertson, R. J., Falkel, J. E., Drash, A. L., Swank, A. M., Metz, K. F., Spungen, S. A., & LeBoeuf, J. R. (1986). Effect of blood pH on peripheral and central signals of perceived exertion. *Med Sci Sports Exerc* **18**, 114-122.

Robertson, R. J., Stanko, R. T., Goss, F. L., Spina, R. J., Reilly, J. J., & Greenawalt, K. D. (1990). Blood glucose extraction as a mediator of perceived exertion during prolonged exercise. *Eur J Appl Physiol Occup Physiol* **61**, 100-105.

Robertson, R. J. & Noble, B. J. (1997). Perception of physical exertion: methods, mediators, and applications. *Exerc Sport Sci Rev* **25**, 407-452.

Rube, N. & Secher, N. H. (1991). Effect of training on central factors in fatigue following two- and one-leg static exercise in man. *Acta Physiol Scand* **141**, 87-95.

Saito, M., Iwase, S., & Mano, T. (1986). Different responses of muscle sympathetic nerve activity to sustained and rhythmic handgrip exercises. *Japanese J Physiol* **36**, 1053-1057.

Saito, M., Mano, T., & Iwase, S. (1989). Sympathetic nerve activity related to local fatigue sensation during static contraction. *J Appl Physiol* **67**, 980-984.

Saito, M., & Mano, T. (1991). Exercise mode affects muscle sympathetic nerve responsiveness. *Japanese J Physiol* **41**, 143-151.

Saltin, B., Nazar, K., Costill, D. L., Stein, E., Jansson, E., Essen, B., & Gollnick, D. (1976). The nature of the training response; peripheral and central adaptations of one-legged exercise. *Acta Physiol Scand* **96**, 289-305.

Sargeant, A. J. & Davies, C. T. (1973). Perceived exertion during rhythmic exercise involving different muscle masses. *J Hum Ergol (Tokyo)* **2**, 3-11.

Sargeant, A. J. & Davies, C. T. (1977). Forces applied to cranks of a bicycle ergometer during one- and two-leg cycling. *J Appl Physiol* **42**, 514-518.

Sasaki, H., Hotta, N., & Ishiko, T. (1991). Comparison of sympatho-adrenal activity during endurance exercise performed under high and low carbohydrate conditions. *J Sports Med Phys Fitness* **31**, 407-412.

Seals, D. R. & Enoka, R. M. (1989). Sympathetic activation is associated with increases in EMG during fatiguing exercise. *J Appl Physiol* **66**, 88-95.

Sherman, W. M. & Leenders, N. (1995). Fat loading: the next magic bullet? *Int J Sport Nutr* **5 Suppl**, S1-12.

Sidney, K. H. & Shephard, R. J. (1977). Perception of exertion in the elderly, effects of aging, mode of exercise and physical training. *Percept Mot Skills* **44**, 999-1010.

Simonsen, J. C., Sherman, W. M., Lamb, D. R., Dernbach, A. R., Doyle, J. A., & Strauss, R. (1991). Dietary carbohydrate, muscle glycogen, and power output during rowing training. *J Appl Physiol* **70**, 1500-1505.

Skinner, J. S., Borg, G. A., & Buskirk, E. R. (1969). Physiological and perceptual reactions to exertion of young men differing in activity and body size. In *Exercise and Fitness* pp. 53-66. The Athletic Institute, Chicago.

Skinner, J. S., Hutsler, R., Bergsteinova, V., & Buskirk, E. R. (1973). Perception of effort during different types of exercise and under different environmental conditions. *Med Sci Sports* **5**, 110-115.

Skrinar, G. S., Ingram, S. P., & Pandolf, K. B. (1983). Effect of endurance training on perceived exertion and stress hormones in women. *Percept Mot Skills* **57**, 1239-1250.

Solomonow, M., Baten, C., Smit, J., Baratta, R., Hermens, H., D'Ambrosia, R., & Shoji, H. (1990). Electromyogram power spectra frequencies associated with motor unit recruitment strategies. *J Appl Physiol* **68**, 1177-1185.

St Clair Gibson, A., Lambert, M. L., & Noakes, T. D. (2001a). Neural control of force output during maximal and submaximal exercise. *Sports Med* **31**, 637-650.

St Clair Gibson, A., Lambert, E. V., Lambert, M. I., Hampson, D. B., & Noakes, T. D. (2001b). Exercise and Fatigue-Control Mechanisms. *Int Sport Med J* 2, 1-14.

St Clair Gibson, A., Schabort, E. J., & Noakes, T. D. (2001c). Reduced neuromuscular activity and force generation during prolonged cycling. *Am J Physiol Regul Integr Comp Physiol* **281**, R187-R196.

St Clair Gibson, A., Baden, D. A., Lambert, M. I., Lambert, E. V., Harley, Y. X., Hampson, D., Russell, V. A., & Noakes, T. D. (2003). The conscious perception of the sensation of fatigue. *Sports Med* 33, 167-176.

St Clair Gibson, A. & Noakes, T. D. (2004). Evidence for complex system integration and dynamic neural regulation of skeletal muscle recruitment during exercise in humans. *Br J Sports Med* **38**, 797-806.

Stallknecht, B., Lorentsen, J., Enevoldsen, L. H., Bulow, J., Biering-Sorensen, F., Galbo, H., & Kjaer, M. (2001). Role of the sympathoadrenergic system in adipose tissue metabolism during exercise in humans. *J Physiol* **536**, 283-294.

Stamford, B. A. & Noble, B. J. (1974). Metabolic cost and perception of effort during bicycle ergometer work performance. *Med Sci Sports* **6**, 226-231.

Stamford, B. A. (1976). Validity and reliability of subjective ratings of perceived exertion during work. *Ergonomics* **19**, 53-60.

Starling, R. D., Trappe, T. A., Parcell, A. C., Kerr, C. G., Fink, W. J., & Costill, D. L. (1997). Effects of diet on muscle triglyceride and endurance performance. *J Appl Physiol* **82(4)**, 1185-1189.

Stegeman, D. F., Blok, J. H., Hermens, H. J., & Roeleveld, K. (2000). Surface EMG models: properties and applications. *J Electromyogr Kinesiol* **10**, 313-326.

Stepto, N. K., Carey, A. L., Staudacher, H. M., Cummings, N. K., Burke, L. M., & Hawley, J. A. (2002). Effect of short-term fat adaptation on high-intensity training. *Med Sci Sports Exerc* **34**, 449-455.

Stevens, J. C. & Mack, J. D. (1959). Scales of apparent force. *J Exp Psychol* **58**, 405-413.

Suzuki, H., Conwit, R. A., Stashuk, D., Santarsiero, L., & Metter, E. J. (2002). Relationships between surface-detected EMG signals and motor unit activation. *Med Sci Sports Exerc* **34**, 1509-1517.

Swank, A. & Robertson, R. J. (1989). Effect of induced alkalosis on perception of exertion during intermittent exercise. *J Appl Physiol* **67**, 1862-1867.

Takaishi, T., Yasuda, Y., & Moritani, T. (1994). Neuromuscular fatigue during prolonged pedalling exercise at different pedalling rates. *Eur J Appl Physiol Occup Physiol* **69**, 154-158.

Takaishi, T., Yasuda, Y., Ono, T., & Moritani, T. (1996). Optimal pedaling rate estimated from neuromuscular fatigue for cyclists. *Med Sci Sports Exerc* **28**, 1492-1497.

Tax, A. A., Denier van der Gon JJ, Gielen, C. C., & van den Tempel, C. M. (1989). Differences in the activation of m. biceps brachii in the control of slow isotonic movements and isometric contractions. *Exp Brain Res* **76**, 55-63.

Taylor, A. D., Bronks, R., Smith, P., & Humphries, B. (1997). Myoelectric evidence of peripheral muscle fatigue during exercise in severe hypoxia: some references to m. vastus lateralis myosin heavy chain composition. *Eur J Appl Physiol Occup Physiol* **75**, 151-159.

Tesch, P. A., Dudley, G. A., Duvoisin, M. R., Hather, B. M., & Harris, R. T. (1990). Force and EMG signal patterns during repeated bouts of concentric or eccentric muscle actions. *Acta Physiol Scand* **138**, 263-271.

Thickbroom, G. W., Phillips, B. A., Morris, I., Byrnes, M. L., & Mastaglia, F. L. (1998). Isometric force-related activity in sensorimotor cortex measured with functional MRI. *Exp Brain Res* **121**, 59-64.

Thomas, G. D., & Segal, S. S. (2004). Neural control of muscle blood flow during exercise. *J Appl Physiol* **97**, 731-738.

Tihanyi, J., Apor, P., & Fekete, G. (1982). Force-velocity-power characteristics and fiber composition in human knee extensor muscles. *Eur J Appl Physiol Occup Physiol* **48**, 331-343.

Tortora, G. T., & Grabowski, S. (1993). *Principles of anatomy and physiology*, 7th ed. Biological Sciences Textbooks, Inc., A & P Textbooks, New York.

Tucker, R., Rauch, L., Harley, Y. X., & Noakes, T. D. (2004). Impaired exercise performance in the heat is associated with an anticipatory reduction in skeletal muscle recruitment. *Pflugers Arch* **448**, 422-430.

Ulmer, H. V. (1996). Concept of an extracellular regulation of muscular metabolic rate during heavy exercise in humans by psychophysiological feedback. *Experientia* **52**, 416-420.

Utter, A., Kang, J., Nieman, D., & Warren, B. (1997). Effect of carbohydrate substrate availability on ratings of perceived exertion during prolonged running. *Int J Sport Nutr* **7**, 274-285.

Utter, A. C., Kang, J., Nieman, D. C., Williams, F., Robertson, R. J., Henson, D. A. , Davis, J. M., & Butterworth, D. E. (1999). Effect of carbohydrate ingestion and hormonal responses on ratings of perceived exertion during prolonged cycling and running. *Eur J Appl Physiol Occup Physiol* **80**, 92-99.

Utter, A. C., Kang, J., Nieman, D. C., Vinci, D. M., McAnulty, S. R., Dumke, C. L., & McAnulty, L. (2003). Ratings of perceived exertion throughout an ultramarathon during carbohydrate ingestion. *Percept Mot Skills* **97**, 175-184.

Utter, A. C., Kang, J., Nieman, D. C., Dumke, C. L., McAnulty, S. R., Vinci, D. M., & McAnulty, L. S. (2004). Carbohydrate supplementation and perceived exertion during prolonged running. *Med Sci Sports Exerc* **36**, 1036-1041.

Victor, R. G., Seals, D. R., & Mark, A. L. (1987). Differencial control oh heart rate and sympathetic nerve activity during dynamic exercise: insight from direct intraneural recordings in humans. *J Clin Invest* **79**, 508-516.

Viitasalo, J. H. & Komi, P. V. (1977). Signal characteristics of EMG during fatigue. *Eur J Appl Physiol Occup Physiol* **37**, 111-121.

Wallin, B. G., & Fagius, J. (1988). Peripheral sympathetic neural activity in conscious humans. *Ann Rev Physiol* **50**, 565-576.

Watt, M. J., Howlett, K. F., Febbraio, M. A., Spriet, L. L., & Hargreaves, M. (2001). Adrenaline increases skeletal muscle glycogenolysis, pyruvate dehydrogenase activation and carbohydrate oxidation during moderate exercise in humans. *J Physiol* **534**, 269-278.

Watt, M. J. & Hargreaves, M. (2002). Effect of epinephrine on glucose disposal during exercise in humans: role of muscle glycogen. *Am J Physiol Endocrinol Metab* **283**, E578-E583.

Wendling, P. S., Peters, S. J., Heigenhauser, G. J. F., & Spriet, L. L. (1996). Epinephrine infusion does not enhance net muscle glycogenolysis during prolonged aerobic exercise. *Can J Appl Physiol* **21**, 271-284.

Westgaard, R. H. & de Luca, C. J. (1999). Motor unit substitution in long-duration contractions of the human trapezius muscle. *J Neurophysiol* **82**, 501-504.

Womack, C. J., Davis, S. E., Weltman, J. Y., Blumer, J., Barrett, E. J., Gaesser, G. A., & Weltman, A. (1998). The effect of training and epinephrine infusion on ratings of perceived exertion (RPE). *Int J Sports Med* **19**, 121-124.

Woods, S., Bridge, T., Nelson, D., Risse, K., & Pincivero, D. M. (2004). The effects of rest interval length on ratings of perceived exertion during dynamic knee extension exercise. *J Strength Cond Res* **18**, 540-545.

Yang, J. F. & Winter, D. A. (1983). Electromyography reliability in maximal and submaximal isometric contractions. *Arch Phys Med Rehabil* **64**, 417-420.

Yang, J. F. & Winter, D. A. (1984). Electromyographic amplitude normalization methods: improving their sensitivity as diagnostic tools in gait analysis. *Arch Phys Med Rehabil* **65**, 517-521.

Young, J. B., Rosa, R. M., & Landsberg, L. (1984). Disociation of sympathetic nervous system and adrenal medullary responses. *Am J Physiol* **247**, E35-E40.

CHAPTER 9

APPENDICES

9.1 CATECHOLAMINE METHOD (AS IMPLEMENTED AT UCT)

CHEMICALS REQUIRED

EGTA - [ethylene glycol-bis (B-aminoethyl ether) N,N,N,N,-tetraacetic acid (Sigma E4378)]

Glutathione- reduced form 98-100% (Y-Glu-Cys_Gly;GSH) (Sigma G4251)

Adrenaline bitartrate (A),

Norepinephrine bitartrate (NA),

3,4-dihydroxybenzylamine hydrochloride (DHBA),

Sodium acetate,

Disodium EDTA

Sodium lauryl sulfate (sodium dodecyl sulfate)

Glacial acetic acid

Diphenylboric acid ethanolamine complex (DPBEA)

Tetraoctylammonium bromide (ToABr)

Octan-1-ol

Methanol (MeOH)

n-Heptane,

Ammonium hydroxide

Ammonium chloride

Dopamine

All water used throughout should be of HPLC electrochemical grade.

All chemicals should be HPLC grade

Solvent Safety Usage

The analyst must read and understand the risk assessment (including the separate one for pregnancy if appropriate) and all relevant MSD sheets before commencing work.

Gloves must be worn at all times when handling biological samples.

Analyst must be vaccinated against hepatitis B.

Methanol to be dispensed in fume cupboard and gloves worn.

Adrenaline and Noradrenaline are very toxic- gloves to be worn at all times when handling.

Glacial acetic acid - gloves to be worn. Must be dispensed in a fume hood.

REAGENT AND STANDARD PREPARATION

Preparation of EGTA/glutathione preservative

Weigh 4.75g EGTA and 3.00g Glutathione into a beaker, preferably in a fume cupboard, add 50ml water and stir. Solution will be cloudy white.

Neutralise with 1 molar sodium hydroxide to pH 6-7 and the solution will become clearer.

Aliquot solution into 0.5ml or 1ml portions and freeze at -20C.

Defrost as needed, remaining solution can be re-frozen if required

400mM acetic acid

Put approx 400ml of deionised water into a 1000ml volumetric flask, and carefully add 23ml of glacial acetic acid. Mix thoroughly, and make up to volume with deionised water.

Caution- always adds concentrated acids to water and not water to acid.

Ammonia/Ammonium chloride/DPBEA (ammonia buffer) - extraction solution 1

Prepare a 2M ammonium chloride solution (10.698g/100ml) **solution a**

Prepare a 2M ammonium hydroxide solution (approx 11ml sg=0.88 per 100ml). **solution b**

Adjust pH of the ammonium chloride solution (solution a) to 8.8 using the 2M ammonium hydroxide solution (solution b) to give **solution c**

The above solution (c) is then used to prepare a 0.2% w/v solution of diphenylboric acid ethanolamine complex DPBEA. (0.2g/100ml) with 0.5% w/v EDTA (0.5g/100ml).

adjust to pH8.8 if required. (extraction solution 1)

Heptane solution (extraction solution 2)

Weigh out 1.75g (equivalent to 0.35% w/v) of Tetraoctylammonium bromide (TOABr) into a small beaker and dissolve in 5ml (1% final volume) octan-1-ol. (This will take a long time in the sonic bath).

Transfer quantitatively to 500ml volumetric flask using n-heptane and make up to volume with n-heptane.

Mobile Phase

Weigh into a beaker 9.02g sodium acetate, 0.372g disodium EDTA and 100mg sodium dodecyl sulphate washing out weighing boats with deionised water.

Add approx 800ml of deionised water and place beaker in magnetic stirrer until all solids have dissolved

Adjust pH to 5.2 using acetic acid

Make to 1000ml with deionised water.

1000 ml buffer plus **200 ml MeOH** makes up the mobile phase (smaller aliquots can be use and filtered daily)

Filter under vacuum through a 0.45µm filter, and sonicate for 5 mins. before use.

Stored on instrument at room temperature.

Do not recycle the mobile phase.

Standards

The catecholamine standards are prepared as 1 X 10-3 M stock solutions.

0.0033g adrenaline bitartrate made up to 10ml with 400mM acetic acid. (A)

0.0032g norepinephrine bitartrate made up to 10ml with 400mM acetic acid(NA)

0.0022g DHBA made up to 10ml with 400mM acetic acid.

(0.0019g Dopamine made up to 10ml with 400mM acetic acid.)-occasional use only.

These are then serially diluted to give working solutions of 1 X 10-7 M solutions.

All solutions are stored at below 5^{o} C.

STANDARD CALIBRATION CURVE

A calibration curve should be run periodically to check the linearity of the system. Solutions are prepared as below.

pipette 1ml of 1x10-3 M standard into a 100ml volumetric flask and make up to volume with 400mM acetic acid = **1x10-5M concentration**

pipette 1ml of 1x10-5 M standard into a 10ml volumetric flask and make up to volume with 400mM acetic acid = **1x10-6M concentration**

Dilute the above solution (1 x 10-6) to give solutions of concentrations as below

100µl standard into 10ml acetic acid to give 1 x10-8 concentration

200µl standard into 10ml acetic acid to give 2 x10-8 concentration

400µl standard into 10ml acetic acid to give 4 x10-8 concentration

600µl standard into 10ml acetic acid to give 6 x10-8 concentration

800µl standard into 10ml acetic acid to give 1 x10-8 concentration

1000µl standard into 10ml acetic acid to give 1 x10-8 concentration

Inject each of the above solutions and plot a graph of concentration against peak height. This should give a straight line with an R^2 >0.99

SAMPLE COLLECTION/PREPARATION

Add 75µl of EGTA/Glutathione preservative to lithium/heparin tubes, then add 10ml whole blood. Mix and then centrifuge at 3000rpm for 10mins. Take plasma off and store at -80C until analysis. Blood to be stored on ice while waiting for centrifuge.

STORAGE PRIOR TO DISPATCH

Please try and freeze samples with the tube upright.

Please store samples in bags preferable by project

Samples from these studies must be stored in a -80 freezer.

EXTRACTION PROCEDURE

Remove samples and Quality control sample from freezer to thaw. When thawed, mix well and centrifuge at 3000rpm for 5min prior to use in extraction.

Label 1 scintillation vial insert and 2 eppendorf tubes for each sample/blank/std to be analysed. (Each run will typically contain 2 or 3 QC's, 3 individual standards, a blank and a mixed standard with the samples)

Into a scintillation vial pipette

500µl sample

100µl 1x10-7M DHBA (internal std)

250µl ammonia buffer (extraction solution 1)

1ml heptane solution (extraction solution 2)

Cap and vortex mix in 20 short bursts.

Centrifuge at 3000rpm for 5mins.

Using a Gilson pipette, carefully remove 750µl of top organic layer into an eppendorf tube, discarding the lower layer.

Add 380µl octan-1-ol and 40µl of 400mM acetic acid

Cap and vortex vigorously ensuring that the acid droplet is well dispersed.

Centrifuge at 3000rpm for 5 mins.

Using a flat-tipped Hamilton syringe, carefully remove as much as possible of the acid droplet from base of tube and dispense into second tube.

Centrifuge at 3000rpm for 5 mins.

Store on ice/-80C until injected.

blank extraction- as above with no sample and 500µl ammonia buffer rather than 250µl

mixed std extraction - as above except 20µl adrenaline std and 20 µl noradrenaline std instead of 500µl sample, and 500µl ammonia buffer rather than 250µl.

HPLC parameters.

Waters 1525 Binary HPLC Pump

Waters 2465 Electrochemical detector

Waters Breeze software

Pump flow rate: 0.40ml/min and pressure about 2000-3000 psi.

Column- Agilent Extended C18 5µm 4.6 x 250mm

Salt-Bridge Ag/AgCl reference electrode

Potential set at 0.8V

Detector output was measured with Breeze software.

CALCULATIONS

Results are calculated from peak heights using an internal standard to establish recovery using an Excell Spreadsheet

9.2 BORG'S RPE SCALE

6	No exertion at all
7	
	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

(Borg, 1973)

9.2.1 Borg's RPE Scale Instructions

While exercising we want you to rate your perception of exertion, i.e., how heavy and strenuous the exercise feels to you. The perception of exertion depends mainly on the strain and fatigue in your muscles and on your feeling of breathlessness or aches in the chest.

Look at this rating scale; we want you to use this scale from 6 to 20, where 6 means "no exertion at all" and 20 means "maximal exertion".

9 corresponds to "very light" exercise. For a normal, healthy person it is like walking slowly at his or her own pace for some minutes.

13 on the scale is "somewhat hard" exercise, but it still feels OK to continue.

17 "very hard" is very strenuous. A healthy person can still go on, but he or she really has to push him or herself. It feels very heavy, and the person is very tired.

19 on the scale is an extremely strenuous exercise level. For most people this is the most strenuous exercise they have ever experienced.

Try to appraise your feeling of exertion as honestly as possible, without thinking about what the actual physical load is. Don't underestimate it, but don't overestimate it either. It is your own feeling of effort and exertion that's important, not how it compares to other people. What other people think is not important either. Look at the scale and the expressions and then point to a number. Any questions?

9.3 BORG'S CATEGORY-RATIO RPE SCALE

0	Nothing at all
0.3	
0.5	Extremely weak
1	Very weak
1.5	
2	Light / Weak
2.5	
3	Moderate
4	
5	Heavy / Strong
6	
7	Very strong
8	
9	
10	EXTREMELY STRONG "Max P"
11	
•	Highest possible / Absolute maximum

(Borg, 1982)

9.3.1 Borg's CR10 Scale Instructions

Basic instruction: 10, "Extremely strong – Max P" is the main anchor. It is the strongest perception (P) you have ever experienced. It may be possible, however, to experience or to imagine something even stronger. Therefore,"Absolute maximum" is placed somewhat further down the scale without a fixed number and marked with a dot "•". If you perceive an intensity stronger than 10, you may use a higher number.

Start with a *verbal expression* and then choose a *number.* If your perception is "Very weak" point to 1; if "Moderate" say 3; and so on. It is very important that you answer what *you* perceive and not what you believe you ought to answer. Be as honest as possible and try not to overestimate or underestimate the intensities.

Scaling perceived exertion: We want you to rate your perceived (P) exertion, that is, how heavy and strenuous the exercise feels to you. This depends mainly on the strain and fatigue in your muscles and on your feeling of breathlessness or aches in the chest. But you must only attend to your subjective feelings and not to the physiological cues or what the actual physical load is.

1	is "very light" like walking slowly at your own pace for several minutes
3	is not especially hard; it feels fine, and it is no problem to continue
5	you are tired, but you don't have any great difficulties
7	you can still go on but have to push yourself very much. You are very tired
10	this is as hard as most people have ever experienced before in their lives
•	this is "Absolute maximum," for example, 11 or 12 or higher

Scaling pain: What are your worst experiences of pain? If you use 10 as the strongest exertion you have ever experienced or can think of, how strong would you say that your three worst pain experiences have been?

10 "Extremely strong – Max P" is your main point of reference. It is anchored in your previously experienced worst pain, which you just described, the "Max P".

- Your worst pain experienced, the "Max P," may not be the highest possible level. There may be pain that is still worse. If that feeling is somewhat stronger, you will point higher.

9.4 EMG ANALYSIS: POO PROGRAM (MATLAB 4.0)

```
function [] = poo(file01, file02, file03, file04, file05, file06, file07, file08, file09, file10, file11, file12,
file13, file14, file15, file16, file17, file18, file19, file20, file21)
%
%
%POO  Flexcomp .pdf File EMG Processing Tool Ver 2.1 for MATLAB 4.0
%
%  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 - it just bears no responsibility for failing to do so...)
%
%  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
%  a rough 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.
%  Alternativel 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 MORMALISATION 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
%
```

```
% See also PLOP
%
% Both POO and PLOP come, like in nature, as is. They take no responsibilty for any mess
% they may cause.

% 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
%
% 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.
%
clear global
global i timemax

% A silly array of variable names - this is all because I can't be arse how to take
% commands of variable argument number from matlab4 - indeed the presence of varargin
% in matlab5 suggests that everything is wank
names(1,:) = 'file01';
names(2,:) = 'file02';
names(3,:) = 'file03';
names(4,:) = 'file04';
names(5,:) = 'file05';
names(6,:) = 'file06',
names(7,:) = 'file07',
```

```
names(8,:) = 'file08';
names(9,:) = 'file09';
names(10,:) = 'file10';
names(11,:) = 'file11';
names(12,:) = 'file12';
names(13,:) = 'file13';
names(14,:) = 'file14',
names(15,:) = 'file15';
names(16,:) = 'file16';
names(17,:) = 'file17';
names(18, ) = 'file18';
names(19,:) = 'file19';
names(20,:) = 'file20';
names(21,:) = 'file21';

% Check the number of arguments
if(nargin==0)
  error('Correct usage:  poo file.pdf <file2.pdf> <file3.pdf> <file4.pdf>...')
end

% Input
% Zero pad the strings to get them all the same length so they can be bunged into the
% one matrix
for nn = 1:nargin
  name_length(nn) = length(eval(names(nn,:)));
end

for nn = 1:nargin
  need(nn) = max(name_length)-name_length(nn);
end


for nn = 1:nargin
  filez(nn,:) = [eval(names(nn,:)),blanks(need(nn))];
end
```

```
% 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

disp(' ');
disp('*****************************************************************************************************')
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.');
disp('*****************************************************************************************************')

disp(' ')

% Start a loop that will loop between files
for i = 1:nargin

% 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.
EMGdata = 0.20757511668611e-6 * fread(fid(i),'short');


% Find out the total number of samples, along with other silly 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
figure
plot(time,EMGdata)
xlabel('time (secs)')
ylabel('amplitude (volts)')
title(['File no. ',num2str(i),': ',filez(i,:)])
set(gca,'XLim',[0 timemax(i)])
end


disp(' ')
disp('**********************************************************************************************************')


% Here we clear all variables except the things we need.  We do this out of memory
% considerations
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.
        % All comments marking statements executed when i==1 only are marked N.
        if(i==1)

        % N Blurb for user
        disp(' ')
        disp('The file(s) have been displayed on separate figures. Specify the amount of data in')
        disp('seconds from the left hand side of the screen that you wish to process. Maximum
time')
        disp('allowed is the shortest EMG data set.')
        disp('Hitting return will select just the displayed EMG for processing.')
        disp(' ')
        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(' ')
  disp('**********************************************************************************************************')
          disp('Only plotted data will be processed')
  else
```

```
    mode = 1;
          disp(' ')

disp('**********************************************************************************************************')
          disp(' ')
          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
  % postion in the title of plot and the wee buttons on the bottom
  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')
    disp('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(axislimits(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(' ')
      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(' ')
      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(' ')
      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

  % 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('*************************************************************************************************************')
  disp(' ')
  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
  % postion in the title of plot and again, that daft 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')
    disp('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(axislimits(1)),"" to
"",num2str(axislimits(2)),"" secs displayed, now at "",num2str(jiggerypokery2(1)),"" secs.""])");'),
                pause


        % D Now find out what data was selected

    % D This just gets the start and stop of the x-axis on the plot
    vv(:,i) = get(gca, 'XLim')';

    % D A time selection is at this stage always specified
        vv(2,i) = vv(1,i) + tselection;

    % D 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.')
      disp('Please select an earlier start to the data sequence.')
    else
      we_are_not_happy = 0;
    end


end % end of while loop

% Close the figure
close
```

```
end % 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 liklihood 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));


% The 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

% contact mark.omalley@ucd.ie

% Low passfilter 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
%arrea = 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)), '.'])
disp(' ')


% 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  % End of all the file looping - NO LOOPS FROM HERE ON OUT


if (nargin>1)

  % Using the cumulative power spectra, find the spectral shift for each file in comparison
  % with the first


disp('*************************************************************************************************************')

disp('*************************************************************************************************************')


%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% This is the corrected version of the zig.m file.

% Find the length of the data streams up to 500, 100 and 350 Hz respectively in
% the amplitude spectra - this will be the same for all files
        h500 = round((n/2)*500/(1984/2));
        h100 = round((n/2)*100/(1984/2));
        h350 = round((n/2)*350/(1984/2));

        % Calculate the cumulutative amplitude spectrum for all data samples
  for count = 1:nargin
    totalpowerin500Hz = sum(amplitude(1:h500,count));
    for nn = 1:h500
      q(count,nn) = sum(amplitude(1:nn,count))/totalpowerin500Hz;
    end
```

```
end

% Now for 100 Hz to 350 Hz in the first data file, find the corresponding frequencies
% in the other data files below which the same amount of amplitude is contained

for count = 1:nargin;
for nn = 1:h350-h100
  [c,d] = min(abs(q(1,nn+h100-1)-q(count,:)));
  k(nn,count) = d*(1984/2)/(n/2);
end
end

% Finally divide these frequencies by the original frequencies to find by how much they
% have shifted (on average) in the range 100 to 350 Hz

f = (h100:h350-1)*(1984/2)/(n/2);
for count = 1:nargin
  ratio(count) = mean(k(1:h350-h100,count)./f');
end

% This is end the corrected version of the zig.m file.
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Output all the data in tabular format to the workspace
disp(' ')
disp(' ')
disp('Basic Normalised Results')
disp(' ')
disp('****************************************************************************************************')
disp(['Normalisation file (', deblank(filez(1,:)), '),   RMS:   ', num2str(RMS(1)/RMS(1)),',   frequency compression:  ', num2str(100-ratio(1)*100),'%.'])
for nn = 2:nargin
  disp(['Data file no. ',num2str(nn-1),' (', deblank(filez(nn,:)), '), RMS: ', num2str(RMS(nn)/RMS(1)),',  frequency compression:  ', num2str(100-ratio(nn)*100),'%.'])
end
disp(' ')
```

```
% Output all this data to an ascii file and more
disp('*************************************************************************************************')
disp('*************************************************************************************************')
disp(' ')
outputn  = input('Specify a file name for the detailed summary information (default = c:\results.txt):
','s');
disp(' ')
disp(' ')



time = clock;
hours = num2str(time(4));
mins = num2str(time(5));


if(isempty(outputn))
  outputn = 'c:\results.txt';
end

fidd = fopen(outputn,'w');

fprintf(fidd,'POO Ver 2.1 Flexcomp EMG Summary Information.');
fprintf(fidd,['\nThis file was created on ', date, ' at ', hours,':',mins,'hrs.']);
fprintf(fidd,'\n\nNormalisation file: %s, data taken from %f to %f
secs.',deblank(filez(1,:)),vv(1,1),vv(2,1));
for nn = 2:nargin
fprintf(fidd,'\nComparison file no. %d: %s, data taken from %f to %f secs, normalised RMS %f ,
frequency compression %f %%.',nn-1,deblank(filez(1,:)),vv(1,nn),vv(2,nn),RMS(nn)/RMS(1),100-
ratio(nn)*100);
end

end  % That's the end of the comparisons and outputting in general



% Close everything
clear all;
clear global;
```

```
fclose('all');
```

9.5 PUBLISHED PAPERS

West SJ, Goedecke JH, van Niekerk L, Collins M, St Clair Gibson A, MacDonald IA, Noakes TD, Lambert EV (2006). Effects of elevated plasma adrenaline levels on substrate metabolism, effort perception and muscle activation during low-to-moderate intensity exercise. *Pflugers Arch – Eur J Physiol 451: 727-737.*

Havemann L, **West SJ**, Goedecke JH, MacDonald IA, St Clair Gibson A, Noakes TD, Lambert EV (2006). Fat adaptation followed by carbohydrate loading compromises high-intensity sprint performance. *J Appl Physiol 100(1): 194-202.*

West SJ, Smith L, Lambert EV, Noakes TD, St Clair Gibson (2005). Submaximal force production during perceptually guided isometric exercise. *Eur J Appl Physiol 95(5-6): 537-542.*

Pflugers Arch Eur J Physiol (2006) 451: 727–737
DOI 10.1007/s00424-005-1516-1

ENDOCRINOLOGY

Sacha J. West · Julia H. Goedecke · Lizl van Niekerk
Malcolm Collins · Alan St Clair Gibson
Ian A. Macdonald · Timothy D. Noakes
Estelle V. Lambert

Effects of elevated plasma adrenaline levels on substrate metabolism, effort perception and muscle activation during low-to-moderate intensity exercise

Received: 26 July 2005 / Accepted: 2 September 2005 / Published online: 8 October 2005

Abstract The aim of this study was to differentiate the role of raised plasma adrenaline (Adr) concentrations from sympathoadrenal activation associated with moderate-intensity exercise, on muscle activation, cardiopulmonary responses, fuel metabolism, and ratings of perceived exertion (RPE) during low-intensity exercise. Two groups of subjects (MOD, $n = 6$; LOW, $n = 7$) cycled on two occasions for 90 min. MOD cycled at 68% VO_{2max} with saline infusion, and at 34% VO_{2max} with Adr infusion. LOW cycled twice at 34% VO_{2max}, with either Adr or saline infusion. Infusions (0.015 g Adr/kg/min) started at 15 min and increased plasma [Adr] somewhat higher than during exercise at 68% VO_{2max} (~1.9 vs. 1.4 nM, at 75 min). Mean plasma glucose and lactate concentrations during LOW were significantly higher with Adr than saline infusion (5.1 ± 0.6 vs.4.4 ± 0.3 mmol/l, $P < 0.01$ and 2.1 ± 0.8 vs. 1.3 ± 0.5 mmol/l, $P < 0.01$, respectively). Elevated [Adr], without increased exercise intensity, did not alter glycogenolysis. There were also no effects of Adr infusion at 34% VO_{2max} on heart rate, oxygen consumption, [FFA], respiratory exchange ratio, intramuscular triglyceride utilization, muscle activation or RPE. In conclusion, elevated [Adr] similar to those found during moderate-intensity exercise increased plasma glucose and lactate availability, but did not alter intramuscular fuel utilization, effort perception or muscle activation.

Keywords Electromyographic activity · Intramuscular triglyceride · Glycogen · Sympathetic nervous system · Substrate availability

S. J. West · J. H. Goedecke (✉) · L. van Niekerk · M. Collins
A. St Clair Gibson · T. D. Noakes · E. V. Lambert
Department of Human Biology, UCT/MRC Research Unit for Exercise Science and Sports Medicine, University of Cape Town, P.O. Box 115, 7725 Newlands, South Africa
E-mail: julia@sports.uct.ac.za
Tel.: +27-21-6504573
Fax: +27-21-6867530

I. A. Macdonald
Queen's Medical Centre, University of Nottingham Medical School, Nottingham, UK

Introduction

An increase in exercise intensity is associated with increased activation of the sympathetic nervous system (SNS), as well as the sympathoadrenal response, resulting in an increase in endogenous adrenaline (Adr) and noradrenaline (NorAdr) secretion [23]. There is indirect evidence that it is the increased circulating catecholamine concentrations that are responsible for the major sympathoadrenergic effects on energy metabolism during exercise [25, 36].

Previous studies have attempted to differentiate the role of circulating [Adr] from that of general sympathoadrenal response in regulating the metabolic sequelae during exercise of different intensities. These studies have typically infused Adr to levels at least twice the normal range measured during exercise. Collectively, these studies have demonstrated that Adr infusion increases the mobilization of fuels, resulting in elevated circulating glucose and lactate concentrations during dynamic exercise [7, 11, 14, 24, 39, 40]. The elevated plasma glucose levels with Adr infusion can be explained by an increase in hepatic glucose output [14, 15] and a possible attenuation in the exercise-induced increase in peripheral glucose uptake [15, 39]. Watt et al. [39] postulated that the reduction in glucose disposal with Adr infusion might be attributed to an increase in glycogenolysis [11, 20, 35, 39], resulting in an accumulation of glucose-6-phosphate, with the consequent inhibition of hexokinase and glucose phosphorylation [39].

However, not all studies have demonstrated an increase in glycogenolysis with Adr infusion during moderate (65% VO_{2max}) [40] and high-intensity exercise

(80–85% VO_{2max}) [7, 24], despite significant increases in glycogen phosphorylase activity [24]. Discrepancy in the findings of these studies may relate to the Adr concentrations achieved with infusion, the intensity and duration of the exercise bout, and the subjects' health, training and nutritional status.

Nonetheless, when plasma Adr levels are increased above those typically associated with the corresponding exercise intensity, a mismatch between the mobilisation and oxidation of fuels has been described. Kjaer et al. [24] found that, in parallel to the increase in glycogen phosphorylase activity stimulated by Adr infusion, an increase in skeletal muscle hormone sensitive lipase (HSL) activity was also found. However, there are no studies of which we are aware, that have measured the changes in intramuscular triglyceride (IMTG) utilisation in response to Adr infusion. Mora-Rodriguez and Coyle [29], using isotope techniques, demonstrated a mismatch between mobilisation and oxidation of FFA's with Adr infusion. They found that whole-body lipolysis increased progressively throughout exercise with graded Adr infusions (0.96–3.44 nmol/l) during low-intensity (25% VO_{2max}) exercise, resulting in an increase in plasma FFA concentrations, with evidence of subsequent re-esterification.

Kjaer et al. [24] postulated that Adr secretion during exercise is not entirely dependent on the metabolic demand of the muscle, but is the result of, and acts as part of a feed-forward control mechanism, mobilising both intra- and extra-muscular fuel stores. Conversely, muscle activation levels and work rate in response to increasing exercise intensity, and the associated increase in plasma [Adr] with SNS activation, results in the mobilisation of fuel stores to match oxidation [29].

To our knowledge, no previous studies have examined the effects of Adr infusion on perceived exertion and muscle activation levels during a single bout of exercise. We hypothesised that increased [Adr] sufficient to alter fuel utilisation, heart rate and/or ventilation would be associated with changed effort perception and muscle activation levels [13, 28]. Indirect evidence for this comes from studies using adrenergic blockade during exercise of moderate intensity [1, 8, 16, 21]. In these studies, adrenergic blockade resulted in an increase in effort perception and EMG activity, despite reductions in heart rate and VO_2 [9, 16]. Further, in models of anaesthetised animals, SNS activation preserved muscle contractility in previously fatigued muscles in situ [19].

These studies establish a relationship between sympathetic activation and muscle activation levels and contractility, which may be independent of workload. However, a similar relationship has not been established for the humoral effects of increased [Adr] otherwise associated with SNS activation. Therefore, the aim of this study was to evaluate the humoral role of raised circulating Adr levels, similar to those concentrations found during moderate-intensity exercise, on circulating substrate availability, intramuscular substrate utilization, muscle activation and effort perception during prolonged, low-intensity exercise.

Methods

Subjects and preliminary testing

Subject selection

Thirteen endurance trained male cyclists between the ages of 18 and 40 years were recruited to participate in the study. The study was approved by the Ethics and Research Committee of the Faculty of Health Sciences of the University of Cape Town. To participate in the study, subjects had to (i) be between the ages of 21 and 45 years; (ii) have completed a local 109 km cycle race in less than 4.5 h; (iii) have no known metabolic conditions that may adversely affect intermediary metabolism. Subjects were excluded if they were taking any medications for chronic conditions such as high-blood pressure or asthma (e.g. β-adrenergic receptor agonists or antagonists). The risks and benefits of the trial were carefully explained to the subjects and their informed consent was obtained in writing before participation in the trial.

Subjects were randomly assigned to one of two groups, MOD (moderate, $n=6$) and LOW (low, $n=7$). The MOD group were matched for circulating Adr levels found during moderate intensity exercise, either elicited by moderate exercise intensity (68% VO_{2max}) with saline infusion or low intensity exercise with Adr infusion. The LOW group were matched for exercise intensity and cycled on two occasions at a low exercise intensity (34% of VO_{2max}), with either saline or Adr infusion (to match MOD trial). Due to the invasiveness of the muscle biopsy procedure, the subjects did not serve as their own controls but were divided into two groups each receiving a total of four biopsies. The characteristics of the subjects are presented in Table 1. There were no significant differences in anthropometric measurements or VO_{2max} and peak power output, as described below, between groups.

Table 1 Subject characteristics

	MOD group ($n=6$)	*LOW group* ($n=7$)
Age (year)	28 ± 10	27 ± 10
Weight (kg)	75.6 ± 9.8	76.4 ± 9.8
Sum seven skinfolds (mm)	31 ± 6	42 ± 17
Body fat (%)	15.1 ± 1.6	15.1 ± 4.7
PPO (W)	302 ± 48	329 ± 48
VO_{2max} (ml/kg/min)	56.2 ± 4.1	58.6 ± 4.1

Values are means ± SD. *PPO* peak power output, VO_{2max} maximum oxygen consumption

Anthropometry

The subjects' mass, height and sum of seven skinfolds (triceps, biceps, subscapular, supra-iliac, abdominal, thigh and calf) were measured. Percentage body fat was estimated using the equations of Durnin and Womersley [10].

VO_2 peak and peak power output test

All subjects performed an incremental workload test to exhaustion on a cycle ergometer (Lode, Groningen, Holland) to determine their maximal oxygen consumption (VO_{2max}) and their peak power output (PPO). Work rates started at 3.33 W/kg body mass, after 150 s the workload increased by 50 W and then by 25 W every 150 s until the subject was exhausted. Exhaustion was defined as a > 10% reduction in pedalling frequency, or a respiratory exchange ratio (RER) of > 1.10, or both. PPO was defined as the highest exercise intensity, the subject completed for 150 s in W, plus the fraction of time spent in the final workload multiplied by 25 W.

During the incremental exercise test, oxygen uptake (VO_2), CO_2 production (VCO_2), and ventilation volume (V_E) were measured over 30 s intervals by use of a breath-by-breath oxycon alpha analyzer (Jaeger, Wuerzburg, Netherlands). The reliability of the oxycon alpha analyser was tested on a weekly basis by burning absolute ethanol [99% analytical reagent, Associated Chemical Enterprises (Pty), Glenvista, South Africa] as a reference. The pneumotach was calibrated before each test with a Hans Rudolph 3-litre syringe (Vacumed, Ventura, CA, USA) and the analysers were calibrated with room air and a 4% CO_2–96% N_2 gas mixture. This information was used to adjust the work rate in the experimental trial to correspond to either 34 or 68% of VO_{2max}.

Experimental protocol

The subjects were required to complete two 90 min exercise trials in randomised order, separated by at least 1 week. The MOD ($n = 6$) group cycled at 68% of VO_{2max} with saline infusion, and at 34% of VO_{2max} with Adr infusion (0.015 μg/kg/min). The LOW group ($n = 7$) cycled on two occasions at 34% of VO_{2max}, during which either saline or Adr (0.015 μg/kg/min) were infused. The exercise intensities chosen for the present study (34 and 68% of VO_{2max}) correspond to 25 and 50% of PPO, respectively, which are typically used for low- (low enough not to stimulate SNS activation) and moderate-intensity exercise [29, 40]. The infusion rate (0.015 μg/kg/min) was designed to elevate plasma [Adr] to levels matching those observed during exercise at 65% of VO_{2max} [29]. Muscle biopsies were taken prior to and at the end of the 90 min exercise, bout for the determination of muscle glycogen and triglyceride (TG) content. The following measurements were taken at 15 min intervals during the 90 min cycle: gas exchange, heart rate, effort perception, electromyographic (EMG) activity and blood samples for the determination of plasma catecholamines, glucose, lactate and free fatty acid (FFA) concentrations.

Resting measurements

The day prior to each experimental trial, the subjects were requested to abstain from any strenuous exercise and to follow the same diet. In addition, the subjects were required to avoid caffeine 12 h before the trial. On the subsequent day, the subjects arrived at the laboratory between 7:00 and 7:30 AM after an overnight fast (10–12 h). A muscle sample was obtained from the belly of the vastus lateralis muscle of the left leg, midway between the greater trochanter and femoral epicondyles along the line of the femur, before the start and immediately on completion of the exercise trials by means of the percutaneous needle biopsy technique. The muscle sample was rapidly frozen in liquid nitrogen (N_2) and stored at −80°C for subsequent analysis of glycogen and triglyceride content.

After the muscle biopsy was taken, a 20-gauge Teflon cannula (Jelco; Johnson and Johnson, Halfway House, South Africa) was placed in each arm and connected to a three-way stop-cock (Uniflex; Mallinckrodt Medical, Hennef-Sieg, Germany). One arm was used for blood sampling during the trial, while the other arm was used to infuse either saline or Adr during the trial. Thereafter, the subjects' peak isometric force was assessed on the lower right limb and EMG activity was measured as described below.

Subjects were then required to rest for at least 40 min to minimize the effects of the muscle biopsy and venipuncture. During this time period, the subjects were instructed on the use of the 15-point Borg-scale for the ratings of perceived exertion (RPE), as described below [2].

Five minutes before the start of exercise, subjects sat on the bike while resting oxygen consumption (VO_2) and carbon dioxide production (VCO_2) was measured for 5 min using the oxycon alpha analyzer (Jaeger, Wuerzburg, Netherlands), and RER calculated. Immediately thereafter, a 10-ml resting blood sample was drawn and placed in the appropriate tubes for subsequent analysis of plasma Adr, NorAdr, glucose, lactate and serum FFA concentrations. Heart rate was recorded continuously by means of a Polar™ heart rate monitor (Polar Electro, Kempele, Finland).

Exercise trials

Subjects then started cycling on the stationary cycle ergometer (Lode, Groningen, Holland) at a workload corresponding to either 34 or 68% of VO_{2max} for 90 min, maintaining a pedaling rate of 90 rpm

throughout both trials. Fifteen minutes into the exercise period, Adr or saline was infused at a constant rate (0.015 μg/kg/min) until the end of the trial [29]. All infusions were controlled using a calibrated automatic syringe pump (Travenol Laboratories, Hooksett, NJ, USA). Immediately after the infusion started and at 15-min intervals throughout each cycle trial, VO_2 and VCO_2 were recorded for 5 min, and blood samples were drawn, as previously described. In addition, RPE and EMG activity (as described below) were recorded at 15 min intervals throughout the exercise trial.

EMG activity

Electromyography (EMG) activity was measured from each subject's right vastus lateralis muscle. The electrode positioning was standardized for each subject and the electrode was not moved throughout the testing. The hair at the placement site was shaved and the skin scraped using industrial sandpaper. An alcohol swab removed any oil and dirt from the skin, which facilitated electrode adherence and conduction of the EMG signal. After preparation of the skin, a surface EMG triode electrode (1 cm radius, 1 cm inter-electrode distance, incorporated into a self-adhesive pad, Thought Technology TriodeTM MIEPO100, Montreal, Canada) was placed on the skin over the belly of the vastus lateralis muscle, midway between the greater trochanter and femoral epicondyles along the line of the femur.

Subjects' peak isometric force was assessed on the lower right limb on a Kin-Com isokinetic dynamometer (Chattanooga Group Inc., Chattanooga, USA). The subjects' hips and upper bodies were firmly strapped to the seat. The arm position for each test was standardised with each subject crossing his arms over his chest. All isometric tests were conducted at 60° flexion, with 0° being the limb in full extension. The standardised warm-up included two isometric contractions of the knee extensors at 50% followed by two contractions at 85% of each subjects' subjective maximum. The isometric test included four maximum voluntary contractions (MVC) of 5 s each, separated by 5 s intervals. Subjects were verbally motivated to encourage them to achieve their maximum potential. EMG activity of the vastus lateralis was recorded during the MVC isometric force test and the MVC with the highest mean force was used for subsequent analyses.

The purpose of the MVC allows the investigation of muscle activation levels during the subsequent cycling trial to be expressed as a percentage of the MVC [18]. Normalising each subject's EMG muscle activity relative to their own maximal activity also excludes confounding factors such as electrode positioning, skin impedance and differences in percentage body fat.

The triode electrode was attached to the muscle 'belly' as described above and connected to a pre-amplifier. The amplifier was linked via fibre-optic cable to the Flexcomp/DSP EMG apparatus (Thought Technology, Montreal, Canada) and host computer. EMG activity was sampled at 1984 Hz, a high enough frequency for reliable data collection and quantitative data analyses [17]. Prior to EMG sampling, EMG signals from the electrode were band-pass filtered (20 500 Hz) and amplified using standard differential amplifiers (Thought Technology, Montreal, Canada, common mode rejection ratio >103 dB at 1 kHz, input impedance = one million MegOhms). The sampled EMG was passed through a 50 Hz line filter to remove interference from electrical sources to yield raw data. The 50 Hz filter was designed with a narrow stop-band to remove as little of the physiological signal as possible. Its use was necessary due to the intermittent simultaneous utilisation of motorised equipment in the laboratory, such as treadmills, etc., during data capture. Mullany et al. [31] demonstrated that the presence of the 50 Hz filter did not affect the statistical power of their conclusions with similar frequency analyses and fewer subjects in their protocol. Movement artefact was removed from the raw EMG signals with a high-pass second order Butterworth filter with a cut off frequency of 15 Hz, to produce filtered EMG data. The filtering procedures were performed using MATLAB™ software (The MathWorks Inc.).

Out of the four 5 s isometric maximal voluntary contractions, filtered EMG data were only processed from the contraction that yielded the greatest force output. The full 5 s of filtered EMG data during the 5 s MVC were processed during the contraction. In a similar manner, 5 s of filtered EMG data were processed from the 20 s of filtered EMG data collected during the cycling trial every 15 min. The filtered EMG data were analysed as follows: the EMG data from each trial was zero-meaned, the zero-meaned filtered EMG amplitude of each cycling trial was then determined by taking the RMS of the filtered EMG signal, normalised to the RMS of the EMG taken during the MVC contraction [30].

Perceived exertion

Subjects were educated on the use of Borg's "rate of perceived exertion" (RPE) scale [2]. Printed scale instructions were given to familiarise subjects as well as an explanation of each scale and a description of how the scale should be used. The Borg 15-point RPE scale was used to obtain the subjects overall perception of effort. Subjects were asked to focus on their subjective feelings and not the workload or physiological cues, and subsequently score their level of exertion on the 15-point scale.

Blood sampling and analysis

Blood samples (10 ml) were placed in tubes containing potassium oxalate and sodium fluoride (2 ml) for the subsequent determination of plasma glucose and lactate concentrations, and lithium heparin tubes (5 ml) for the

subsequent determination of plasma Adr and NorAdr concentrations. The remaining aliquot (3 ml) was placed into a tube containing gel and clot activator for the determination of serum FFA concentrations. The tubes were immediately placed on ice and, on completion of the trial, centrifuged at 3000 rpm and 4°C for 10 min. The supernatants were then transferred to microfuge tubes and stored at −20 or −80°C for subsequent analysis of metabolite and hormone concentrations, respectively.

Plasma glucose concentrations were determined by the glucose oxidase method using a glucose analyser (Glucose analyzer 2; Beckman Instruments, Fullerton, CA, USA). Lactate concentrations were measured on the same plasma samples by enzymatic colorimetric assays (Lactate PAP, bioMerieux, Lyon, France). Serum FFA concentrations were measured using an enzymatic colorimetric assay (Halfmicro test; Roche, Mannheim, Germany). Plasma catecholamines were measured using HPLC with electrochemical detection using the method described by Forster and Macdonald [12].

Intramuscular triglyceride and glycogen concentrations

A portion of the frozen muscle biopsy sample (~50 mg) was freeze-dried and dissected free of any visible fat or connective tissue. Muscle glycogen content was determined as glucose residues (glucose oxidaze method; Glucose Analyzer 2, Beckman Instruments, Fullerton, CA, USA) after hydrolysis of approximately half of the freeze dried muscle sample in 2 M HCl at 95°C for 3 h as previously described [32]. Glycerol concentrations were measured in the remaining portion of the freeze dried muscle using a commercial glycerol kit (Boehringer Mannheim, Mannheim, Germany) after the TG was lipolysed to glycerol and FFAs, as described by Kiens and Richter [22].

Statistical analysis

All data are presented as mean ± standard deviation. *T*-tests for independent samples were used to examine differences in subject characteristics between groups. Repeated-measures analyses of variance (ANOVA) were used to examine differences between trials over time for plasma metabolites and hormone concentrations, muscle substrate levels, RPE, gas exchange, heart rate and EMG activity. A Tukey's HSD post hoc test was performed to locate differences over time. An alpha level of < 0.05 was considered statistically significant.

In order to show statistically significant differences in for example, whole body lipolysis, such as those found in the study by Mora-Rodrigues and Coyle [29] (Ra,

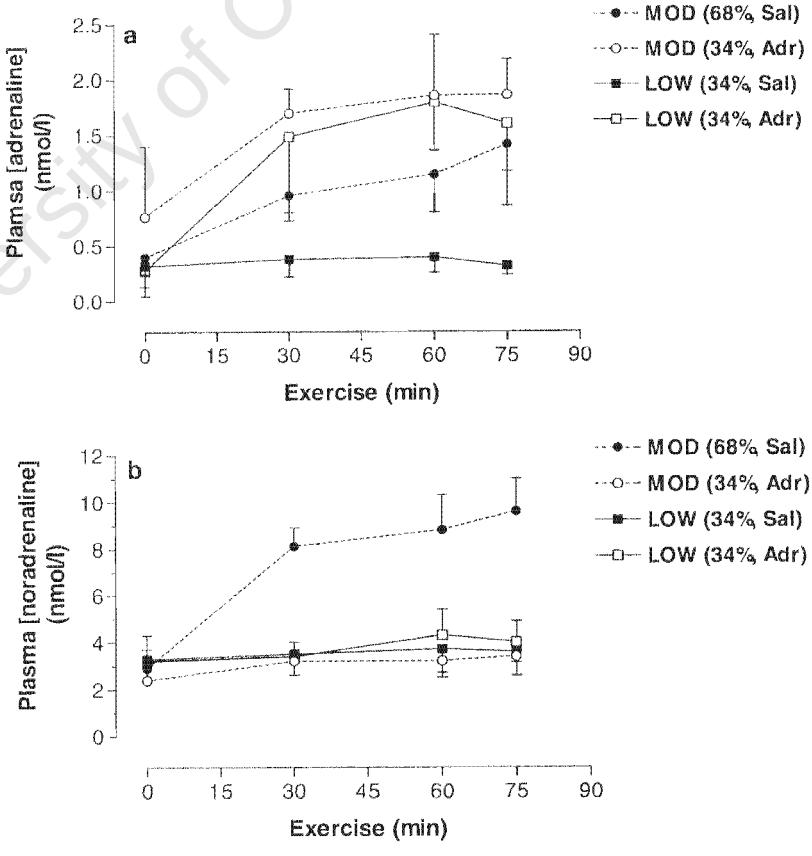


Fig. 1 a, b Plasma Adr and NorAdr concentrations during exercise at 34% VO_{2max}, with and without Adr infusion, and during exercise at 68% VO_{2max}. MOD trial, plasma Adr levels, $P < 0.001$ for both trial and time (≥30 min). LOW trial, plasma Adr levels, $P < 0.001$ for trial, time (≥30 min) and interaction effect. MOD trial, plasma noradrenaline (*NorAdr*) levels, $P < 0.001$ for trial, time (≥30 min) and interaction effect. LOW trial, plasma noradrenaline (*NorAdr*) levels, not significant

glycerol of 2 μmol kg $^{-1}$ min $^{-1}$ with a standard error of approximately 0.5), during Adr infusion, at an alpha level of 0.05, and with 80% power, a sample size of approximately 6–8 would be needed. To our knowledge, there are no previous studies examining changes in muscle activation in response to a similar perturbation, however unpublished data from our laboratory showed significant differences in muscle activation during sequential single leg exercise to fatigue, at a similar level of plasma [Adr] concentration (~15% difference in EMG, with a standard deviation of between 5 and 10%, at 30% double-leg VO_{2max}). To detect differences in muscle activation of similar magnitude in the present study, at an alpha level of 0.05, with 80% power, we would therefore require approximately 5–8 subjects per group. Thus, we felt the present study was adequately powered in order to show differences in the respective outcomes (Graphpad Instat V2.05, 1990–1994).

Results

Plasma adrenaline (Adr) and nonadrenaline (NorAdr) concentrations

Plasma Adr concentrations were significantly higher in both Adr infusion groups, when compared to saline infusion at both 68% and 34% of VO_{2max} (Fig. 1a, $P < 0.001$). Plasma NorAdr concentrations were higher during exercise at 68% VO_{2max} (Fig. 1b, $P < 0.001$), compared to 34% VO_{2max}, whether or not Adr was infused.

Intramuscular triacylglycerol and muscle glycogen content

The IMTG concentrations did not change significantly in both the MOD and LOW groups throughout the trials (Table 2). The glycogen concentrations decreased during the LOW saline trial ($P = 0.06$) and significantly decreased during exercise at 68% VO_{2max} ($P < 0.05$) (Table 3). Rates of muscle glycogen utilization were not significantly different during exercise at 34% VO_{2max} with Adr infusion.

Table 2 Intramuscular triacylglycerol concentrations (μmol/g dry wt) pre- and post-exercise at 34% VO_{2max}, with and without Adr infusion, and at 68% VO_{2max}

	Pre	*Post*
MOD group ($n = 6$)		
68% Saline	56.76 ± 46.58	30.87 ± 14.23
34% Adr	40.25 ± 18.13	42.98 ± 22.79
LOW group ($n = 7$)		
34% Saline	49.82 ± 30.91	34.81 ± 15.18
34% Adr	29.51 ± 11.87	36.40 ± 13.36

Values are means ± SD. MOD group, NS. LOW group, $P = 0.07$ for trial × time

Table 3 Muscle glycogen content (μmol/g dry wt.) pre- and post-exercise at 34% VO_2 max, with and without Adr infusion, and at 68% VO_{2max}

	Pre	*Post*
MOD group ($n = 6$)		
68% saline	406 ± 134	194 ± 36
34% Adr	369 ± 106	287 ± 79
LOW group ($n = 7$)		
34% saline	503 ± 117	371 ± 119
34% Adr	449 ± 111	413 ± 84

Values are means ± SD. MOD group, $P < 0.01$ for time. LOW group, $P = 0.06$ for time and $P = 0.07$ for trial × time

Plasma metabolite concentrations

Plasma glucose, lactate and serum FFA concentrations in response to exercise at 34% VO_{2max}, with and without Adr infusion, and during exercise at 68% VO_{2max} are presented in Fig. 2a–c. Adr infusion was associated with increased circulating plasma glucose and lactate concentrations during low-intensity exercise ($P < 0.001$). Plasma glucose concentrations declined to ~4 mmol/l during moderate-intensity exercise bout, but increased to ~5.3 mmol/l with Adr infusion ($P < 0.05$). In contrast, moderate-intensity exercise (68% VO_{2max}) resulted in a significantly greater increase in plasma lactate concentrations compared to the low-intensity bout, even with Adr infusion ($P < 0.001$). There was however also a significant time × trial interaction effect for serum FFA concentrations during moderate-intensity exercise. Serum FFA concentrations progressively increased during exercise at 68% VO_{2max}, however, during exercise at 34% VO_{2max} with Adr infusion, there was no further increase in serum FFA concentrations after 60 min of exercise. In contrast, there were no differences in serum FFA concentrations in response to Adr infusion during low-intensity exercise.

Heart rate, gas exchange and effort perception

Heart rate was significantly higher during the exercise at 68 than 34% VO_{2max} ($P < 0.001$), but was not altered by Adr infusion (Fig. 3a). There were also no effects of Adr infusion on VO_2, V_E or RER during low-intensity exercise (34% VO_{2max}) (Fig. 3b, c). However, VO_2, V_E and RER were significantly higher during exercise at 68% than 34% VO_{2max} ($P < 0.05$). RPE progressively increased during moderate-intensity exercise (68% VO_{2max}) ($P < 0.05$), but was similar during exercise at 34% VO_{2max} with or without Adr infusion (Fig. 3d).

Electromyography

Normalised EMG amplitude was significantly higher ($P < 0.05$) during exercise at 68% VO_{2max} compared to

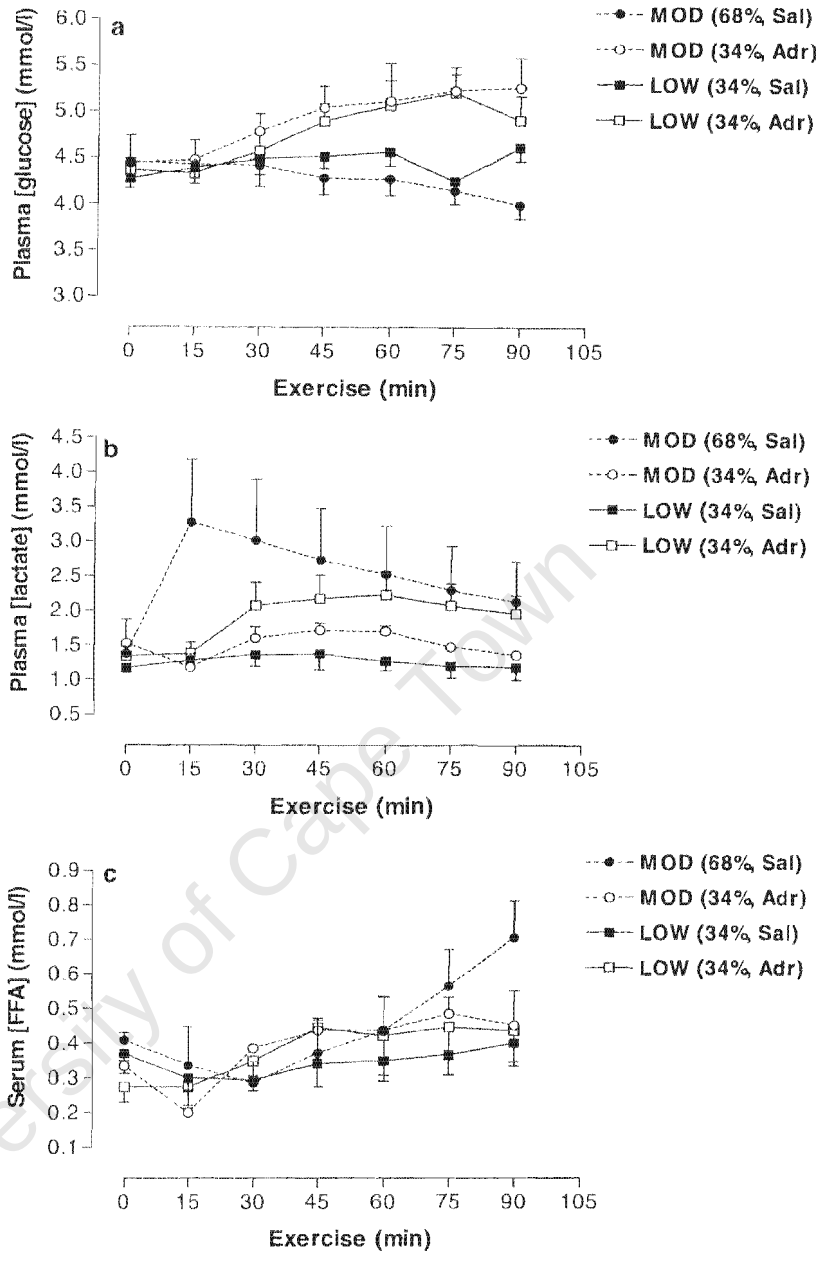


Fig. 2 a–c Plasma glucose, lactate and serum free fatty acid (*FFA*) concentrations during exercise at 34% VO_{2max}, with and without Adr infusion, and during exercise at 68% VO_{2max}. MOD trial, plasma glucose levels, $P < 0.05$ for trial, $P < 0.001$ for trial × time effect (≥45 min). LOW trial, plasma glucose levels, $P < 0.001$ for trial × time effect (≥45 min). MOD trial, plasma lactate, $P < 0.05$ for time (15, 30 and 45 min), $P < 0.01$ for trial × time effect. LOW trial, plasma lactate, $P < 0.01$ for trial, time (≥30 min) and interaction effect. MOD trial, serum FFA levels, $P < 0.001$ for time, $P < 0.05$ for trial × time effect (90 min). LOW trial, serum FFA levels, not significant

34% VO_{2max} (~20 ± 9 vs. 12 ± 5%, respectively), but was not altered by Adr infusion during low-intensity exercise (34% VO_{2max}) (Fig. 4).

Discussion

The purpose of the study was to investigate the role of raised circulating Adr levels, similar to those found during moderate-intensity exercise, on endogenous substrate utilisation and circulating substrate availability, as well as muscle activation and effort perception during prolonged low-intensity exercise. To our knowledge, this is the first study of this nature to examine the humoral effects of Adr on effort perception and muscle activation levels, as well as muscle glycogen and triglyceride utilization, measured using the biopsy technique, during prolonged, low-to-moderate intensity exercise.

The design of this study was such that it allowed one to differentiate between the humoral effects of physiological levels of Adr, from the exercise response itself. In contrast to most studies that have examined the role of Adr during high-intensity exercise, and which have infused Adr to levels two to six times that of normal exercising levels while keeping the exercise intensity constant, the trained subjects in this study cycled at a low intensity (34% VO_{2max}) and Adr was infused to

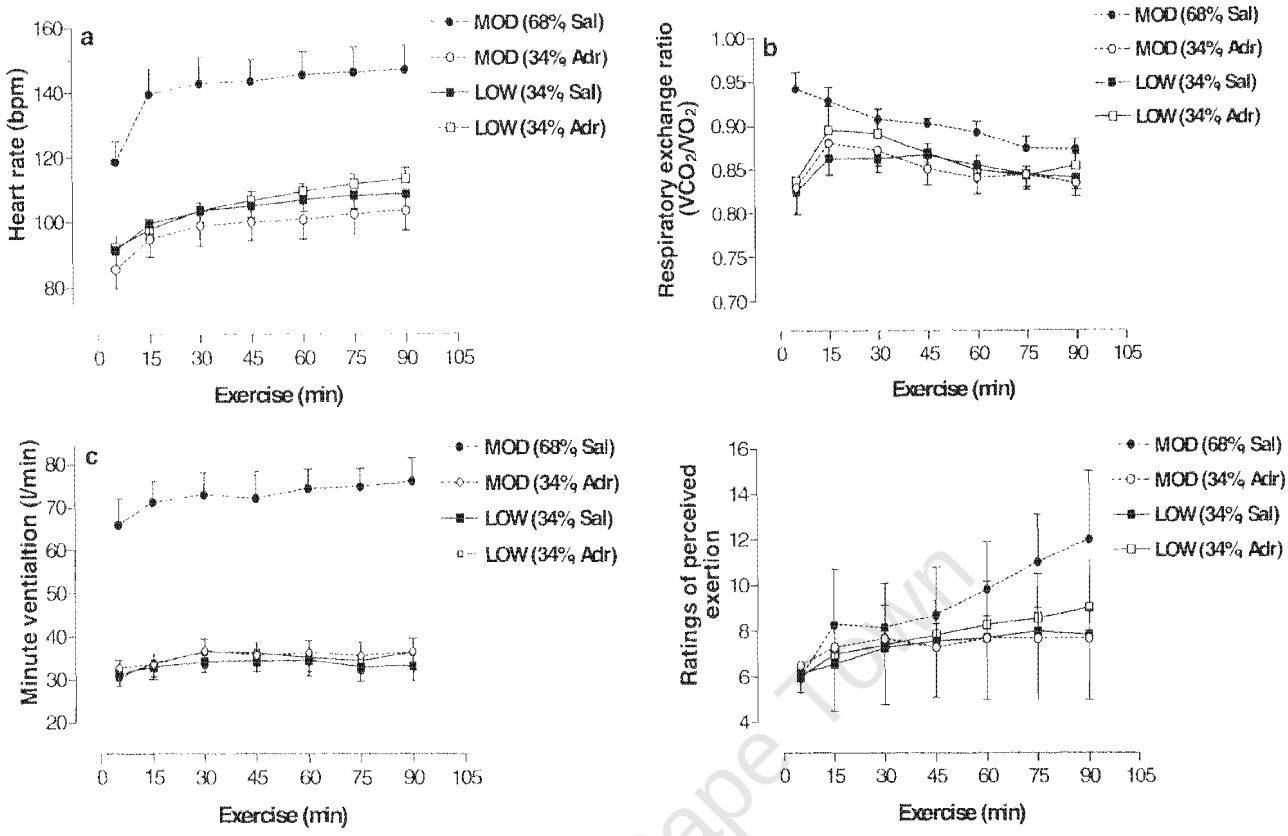


Fig. 3 a–d Heart rate, respiratory exchange ratio, minute ventilation and ratings of perceived exertion during exercise at 34% VO_{2max} with and without Adr infusion, and during exercise at 68% VO_{2max}. MOD trial, heart rate, $P < 0.001$ for trial and time, $P < 0.05$ for trial × time effect (≥5 min). LOW trial, heart rate, $P < 0.001$ for time (≥30 min). MOD trial, RER, $P < 0.01$ for trial and time (≥45 min), $P < 0.001$ for interaction effect. LOW trial, RER, $P < 0.01$ for time (15, 30 min). MOD trial, minute ventilation, $P < 0.001$ for trial and time (≥5 min). MOD trial, RPE, $P < 0.05$ for trial, $P < 0.001$ for time (≥15 min) and interaction effect. LOW trial, RPE, $P < 0.001$ for time (≥30 min)

levels to match those measured during moderate-intensity exercise (68% VO_{2max}).

Similar to the findings of previous studies [7, 11, 14, 24, 26, 39, 40], we found that Adr infusion resulted in an increase in the mobilisation of fuels, such that plasma glucose and lactate concentrations were elevated (Fig. 2a, b). The increase in plasma glucose concentration with Adr infusion is most likely related to an increase in hepatic glucose production [14, 15, 26] and possibly, to alterations in the metabolic clearance rate of glucose [15, 26]. For example, Howlett et al. [15] and Watt et al. [39] demonstrated a decrease in glucose uptake and metabolic clearance rate when Adr was infused to levels of ~4–5 nmol/l during exercise in adrenalectomised patients and moderately-trained athletes, respectively. In contrast, Kreisman et al. [26] found an increase in the rate of disappearance and the metabolic clearance rate of glucose when Adr was infused to supraphysiological levels (~9 nmol/l) in trained athletes. Despite the discrepancy in these findings, Adr infusion was associated with a greater increase in the rate of appearance than the rate of disappearance of glucose in both these studies, leading to elevated plasma glucose concentrations during exercise. These findings, therefore demonstrate that the increase in Adr during moderate- and high-intensity exercise results in a mismatch between the mobilisation and oxidation of glucose.

The reduction in glucose disposal and the consequent increase in plasma glucose concentrations, as well as the increase in plasma lactate concentrations with Adr infusion have been attributed, in part, to an increase in muscle glycogenolysis [37, 39]. Watt et al. [39] found that Adr infusion (~5 nmol/l) during 20 min of moderate-intensity exercise (58% VO_{2max}) resulted in an increase in muscle glycogenolysis and muscle glucose 6-phosphate and lactate content. Watt et al. [39] postulated that the elevated muscle glucose 6-phosphate, being a potent inhibitor of hexokinase, would reduce glucose phosphorylation, providing a possible explanation for the reduction in glucose disposal with Adr infusion [39]. Similar increases in muscle glycogenolysis [11, 20, 35], lactate and glucose 6-phosphate levels [35] have previously been demonstrated with Adr infusion.

In contrast, we failed to demonstrate an increase in muscle glycogenolysis with Adr infusion (Table 3). In the present study, the subjects exercised at a low

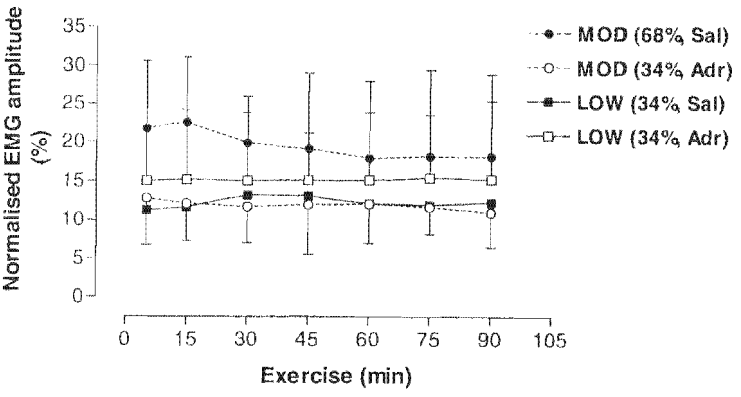


Fig. 4 Normalised EMG amplitude during exercise at 34% VO_{2max} with and without Adr infusion, and during exercise at 68% VO_{2max}. MOD trial, EMG amplitude, $P < 0.05$ for trial. LOW trial, EMG amplitude, not significant

intensity (34% VO_{2max}) and Adr was infused to match levels measured during moderate-intensity exercise (65% VO_{2max}). Plasma Adr concentrations increased to a maximum of ~1.8 nmol/l after 75 min of exercise, which is significantly lower than those used in previous studies. It is possible that these relatively low levels of Adr were not sufficient to stimulate glycogenolysis. However, during the MOD trial (68% VO_{2max}), which was associated with greater muscle contraction (Fig. 4), muscle glycogen stores decreased significantly during the 90 min trial, despite even lower plasma Adr levels (~1.3 nmol/l). Moreover, Febbraio et al. [11] demonstrated an increase in net muscle glycogen utilisation when plasma Adr concentrations were elevated to ~2 nmol/l after 40 min of exercise at 71% VO_{2max}. Differences in the training status or absorptive state of the subjects cannot explain the differences in these findings. Rather, differences in the exercise intensities employed in these studies (34% vs. 71% VO_{2max}) provide the most likely explanation. Although glycogen phosphorylase was seemingly activated by the Adr infusion (via cAMP) in both these studies, the higher exercise stimulus used in the MOD trial and the study of Febbraio et al. [11] would have resulted, amongst other factors [33], in a higher rate of ATP turnover and an associated increase in free AMP [34], and Pi concentrations [3, 5, 27] in the active muscle, stimulating glycogenolysis.

In contrast, the low intensity exercise stimulus in the present LOW trial would be associated with a lower ATP turnover rate and lower AMP and Pi levels, which would maintain phosphorylase activity and hence glycogenolysis at a lower level [3, 5, 27, 34]. The duration of the exercise bout could also explain the failure to demonstrate an increase in glycogenolysis with Adr infusion. Glycogen phosphorylase *a* activity tends to increase early in exercise and then progressively revert back to the inactive *b* form as the duration of the exercise bout increases [3, 6, 33].

In parallel to the increase in glycogen phosphorylase activity, Kjaer et al. [24] also found an increase in skeletal muscle HSL activity with Adr infusion. Unfortunately, Kjaer et al. [24] did not measure any other markers of fat metabolism. One might expect increased IMTG utilisation with elevated HSL activity; however, in the present study, IMTG content was unchanged and even tended to increase, following 90 min of moderate-intensity exercise with Adr infusion (Table 2). Although these findings must be interpreted with caution due to the large intra-assay variability associated with this technique [41], Mora-Rodriguez and Coyle [29], using a similar study design to ours, have reported comparable findings. In their study, Mora-Rodriguez and Coyle [29] examined the effects of graded Adr infusions (0.96–3.44 nmol/l) on fat metabolism during low-intensity exercise (25% VO_{2max}) using isotope techniques. They found that the graded Adr infusions increased whole-body lipolysis, but reduced fatty acid oxidation, resulting in an increase in plasma FFA concentrations, with subsequent reesterification [29].

Mora-Rodriguez and Coyle [29] showed that Adr infusions to low, mid and high levels (0.96, 1.92 and 3.44 nmol/l, respectively), resulted in corresponding increases in plasma FFA concentrations (0.57, 0.62, 0.89 mmol/l, respectively), which were higher than during the control trial (45% VO_{2max}) without Adr infusion (0.41 mmol/l) [29]. However, in the present study, plasma FFA concentrations during exercise did not increase in response to elevations in Adr concentrations and plateaued at ~0.4 mmol/l (Fig. 2c). Rather, plasma FFA levels increased throughout the MOD trial (68% VO_{2max}) to ~0.7 mmol/l, and corresponded to the progressive increase in plasma NorAdr levels (Fig. 1b). Changes in the rates of total fat oxidation, estimated from RER, paralleled these changes. It therefore appears that the higher exercise intensity (25 vs. 34% VO_{2max}) and the consequent elevation in plasma NorAdr levels (~4 vs. 2.2 nmol/l) were sufficient to attenuate the increase in lipolysis and plasma FFA levels in the present study compared to that of Mora-Rodriguez and Coyle [29]. Indeed, they demonstrated an attenuation in lipolysis and an increase in fat oxidation, corresponding to an increase in NorAdr levels, when the exercise intensity was increased from 25 to 45% VO_{2max}, despite identical plasma Adr levels [29]. This may also explain why plasma FFA levels were not elevated in response to Adr infusion in most other studies that have investigated the metabolic effects of Adr at higher exercise intensities [7, 11, 40].

As there were also no significant changes in muscle glycogen and triglyceride utilisation during exercise with Adr infusion, we did not expect to find changes in RER. However, when the exercise stimulus was sufficient to drive glycogenolysis (68% VO_{2max}), RER was increased (Fig. 3b). Similarly, trials in which increases in muscle glycogenolysis [11, 39] or glucose disposal [26] with Adr infusion were observed, reported increases in the rates of total carbohydrate oxidation during exercise [11, 39].

In the present study, no significant differences were found for heart rate, VO_2 and V_E during exercise with Adr infusion. We suggest that although the Adr infusion increased plasma Adr concentrations to those similar to moderate-intensity exercise, it was not sufficient to stimulate a cardiopulmonary response during the low-intensity exercise. Previous studies that have reported significant increases in heart rate with Adr infusion [11, 26, 38] utilised much higher exercise intensities ranging from 50 to 70% of VO_{2max}. Therefore, the effects of Adr infusion on the cardiopulmonary response may only manifest when there is a concomitant increase in SNS activation with higher exercise intensities. Furthermore, the Adr infusion in the present study resulted in less than a twofold increase in plasma Adr levels (Fig. 1a). In contrast, previous studies that have shown an effect on cardiopulmonary responses have produced increases in plasma Adr levels that ranged from approximately threefold [11] to tenfold [26, 38].

Similarly, Adr infusion was not associated with altered effort perception during low intensity exercise (Fig. 3d). This is in agreement with the findings of Womack et al [42], who found that RPE was similar during a constant-power exercise test after a 6 week training programme, despite Adr infusion sufficient to raise plasma Adr to supraphysiological levels (~13.2 mmol/l). Therefore, it appears that the rise in RPE is only a consequence of the increase in exercise intensity, and is not directly related to elevated plasma Adr concentrations.

Previous studies examining the relationship between RPE, muscle activation (EMG) and sympathetic activity during exercise found that RPE and EMG activity were augmented by the administration of a β-blocker [16]. It was suggested that the perceived exertion and neural output were increased to compensate for the reduced maximal exercise capacity associated with β blockade. In the present study there were no apparent changes in muscle activation levels as a consequence of any humoral effect of raised circulating [Adr] in the absence of an appropriate or matched increase in workload, or increased sympathetic activation. As expected, EMG amplitude was significantly greater at 68% VO_{2max} (moderate-intensity exercise), however Adr infusion had no effect on the contractility or the capacity of the muscle that was recruited to perform the exercise task. It is likely, therefore, that in the absence of direct SNS activation, the humoral effects of increased Adr levels are not associated with changes in effort perception and muscle activation.

In conclusion, Adr infusion, at levels similar and somewhat higher to those seen with prolonged, moderate exercise (68% VO_{2max}) was associated with increased circulating glucose and lactate concentrations. Increased circulating Adr, alone, did not alter the cardiovascular and respiratory responses to low-intensity exercise, or change substrate oxidation, effort perception or muscle activation levels. It appears that exercise intensity-dependent changes in neuromuscular and mechanical factors, humoral activation, substrate levels and blood flow in the active muscle, associated with actual work output and metabolic demand, are necessary afferent feedback for appropriate cardiopulmonary and peripheral responses as well as effort perception and muscle activation changes for moderate- and higher-intensity exercise.

Acknowledgements We thank all the subjects, who so willingly took part in this study. We are grateful to the staff of Lab B14 of the School of Biomedical Sciences, University of Nottingham Medical School for their assistance with the plasma catecholamine analysis. In addition, we would like to thank Judy Belonje for her expertise in analysing the blood and muscle samples and Doctor Hugh Mullany for his electromyography expertise and generous provision of EMG analysis software. This study was funded by NRF Mobility Award, the University of Cape Town, the Medical Research Council of South Africa, the Nellie Atkinson and Harry Crossley Staff Research Funds of the University of Cape Town, Bromor Foods Pty. Ltd. and the Technology and Human Resources for Industry Programme (THRIP).

References

1. Alway SE, Hughson RL, Green HJ, Patla AE, Frank JS (1987) Contractile properties of the human triceps surae following prolonged exercise and beta-blockade. Clin Physiol 7:151–163
2. Borg GA (1973) Perceived exertion: a note on "history" and methods. Med Sci Sports 5:90–93
3. Chasiotis D (1983) The regulation of glycogen phosphorylase and glycogen breakdown in human skeletal muscle. Acta Physiol Scand 518(Suppl):1–68
4. Chasiotis D (1988) Role of cyclic AMP and inorganic phosphate in the regulation of muscle glycogenolysis during exercise. Med Sci Sports Exerc 20:545–550
5. Chasiotis D, Sahlin K, Hultman E (1982) Regulation of glycogenolysis in human muscle at rest and during exercise. J Appl Physiol 53:708–715
6. Chasiotis D, Sahlin K, Hultman E (1983) Regulation of glycogenolysis in human muscle in response to adrenaline infusion. J Appl Physiol 54:45–50
7. Chesley A, Hultman E, Spriet LL (1995) Effects of adrenaline infusion on muscle glycogenolysis during intense aerobic exercise. Am J Physiol (Endocrinol Metab) 268:E127–E134
8. Cleroux J, van Nguyen P, Taylor AW, Leenen FH (1989) Effects of beta 1- vs beta 1 + beta 2-blockade on exercise endurance and muscle metabolism in humans. J Appl Physiol 66:548–554
9. Derman WE (1993) The effects of β-blockade on the physiological response to physical exercise and exercise training in man. Doctoral Thesis, University of Cape Town, Cape Town
10. Durnin JVGA, Womersley J (1974) Body fat assessed from total body density and its estimation from skinfold thickness measurement on 481 men and women aged 16–72 years. Br J Nutr 32:77–97

11. Febbraio M, Lambert DL, Starkie RL, Proietto SJ, Hargreaves M (1998) Effect of adrenaline on muscle glycogenolysis during exercise in trained men. J Appl Physiol 84:465–470
12. Forster CD, Macdonald IA (1999) The assay of the catecholamine content of small volumes of human plasma. Biomed Chromatogr 13:215
13. Hampson DB, St Clair GA, Lambert MI, Noakes TD (2001) The influence of sensory cues on the perception of exertion during exercise and central regulation of exercise performance. Sports Med 31:935–952
14. Howlett K, Febbraio M, Hargreaves M (1999) Glucose production during strenuous exercise in humans: role of adrenaline. Am J Physiol (Endocrinol Metab) 276:E1130–E1135
15. Howlett K, Galbo H, Lorentsen J, Bergeron R, Zimmerman-Belsing T, Bulow J, Feldt-Rasmussen U, Kjaer M (1999) Effect of adrenaline on glucose kinetics during exercise in adrenalectomised humans. J Physiol 519:911–921
16. Hunter AM, St Clair GA, Derman WE, Lambert M, Dennis SC, Noakes TD (2002) The effect of selective beta1-blockade on EMG signal characteristics during progressive endurance exercise. Eur J Appl Physiol 88:275–281
17. Hunter AM, St Clair GA, Lambert M, Dennis S, Mullany H, O'Malley MJ, Vaughan CL, Kay D, Noakes TD (2003) EMG amplitude in maximal and submaximal exercise is dependent on signal capture rate. Int J Sports Med 24:83–89
18. Hunter AM, St Clair GA, Lambert M, Noakes TD (2002) Electromyographic (EMG) normalization method for cycle fatigue protocols. Med Sci Sports Exerc 34:857–861
19. Jami L, Laporte Y, Scott JJ (1984) Some effects of sympathetic stimulation and isoprenaline on fatigued tetanic contractions of skeletal muscle in the cat. Brain Res 321:386–389
20. Jansson E, Hjemdahl P, Kaijser L (1986) Adrenaline-induced changes in muscle carbohydrate metabolism during exercise in male subjects. J Appl Physiol 60:1466–1470
21. Kaiser P, Tesch PA, Frisk-Holmberg M, Juhlin-Dannfelt A, Kaijser L (1986) Effect of beta 1-selective and non-selective beta-blockade on work capacity and muscle metabolism. Clin Physiol 6:197–207
22. Kiens B, Richter EA (1996) Types of carbohydrate in an ordinary diet affect insulin action and muscle substrates in humans. Am J Clin Nutr 63:47–53
23. Kjaer M (1989) Adrenaline and some other hormonal responses to exercise in man: with special reference to physical training. Int J Sports Med 10:2–15
24. Kjaer M, Howlett K, Langfort J, Zimmerman-Belsing T, Lorentsen J, Bulow J, Ihlemann J, Feldt-Rasmussen U, Galbo H (2000) Adrenaline and glycogenolysis in skeletal muscle during exercise: a study in adrenalectomised humans. J Physiol 528(Pt 2):371–378
25. Kjaer M, Lange K (2000) Adrenergic regulation of energy metabolism?. In: Warren MP, Constantini NW (eds) Sports endocrinology. Humana Press Inc, NJ, pp 181–188
26. Kreisman SH, Mew NAH, Arsenault M, Nessim SJ, Halter JB, Vranic M, Marliss EB (2000) Adrenaline infusion during moderate intensity exercise increases glucose production and uptake. Am J Physiol Endocrinol Metab 278:E949–E957
27. Laurent D, Petersen KF, Russell RR, Cline GW, Shulman GI (1998) Effect of adrenaline on muscle glycogenolysis and insulin-stimulated muscle glycogen synthesis in humans. Am J Physiol 274:E130–E138
28. Mihevic PM (1981) Sensory cues for perceived exertion: a review. Med Sci Sports Exerc 13:150–163
29. Mora-Rodriguez R, Coyle E (2000) Effects of plasma adrenaline on fat metabolism during exercise: interactions with exercise intensity. Am J Physiol Endocrinol Metab 278:E669–E676
30. Mullany H (2000) POO. [2.2]. Computer Program
31. Mullany H, O'Malley M, St Clair GA, Vaughan C (2002) Agonist-antagonist common drive during fatiguing knee extension efforts using surface electromyography. J Electromyogr Kinesiol 12:375–384
32. Passonneau JV, Lauderdale VR (1999) A comparison of three methods of glycogen measurement in tissues. Anal Biochem 60:405–412
33. Ren JM, Hultman E (1989) Regulation of glycogenolysis in human skeletal muscle. J Appl Physiol 67:2243–2248
34. Ren JM, Hultman E (1990) Regulation of phosphorylase a activity in human skeletal muscle. J Appl Physiol 69:919–923
35. Spriet LL, Ren JM, Hultman E (1988) Adrenaline infusion enhances muscle glycogenolysis during prolonged electrical stimulation. J Appl Physiol 64:1439–1444
36. Stallknecht B, Lorentsen J, Enevoldsen LH, Bulow J, Biering-Sorensen F, Galbo H, Kjaer M (2001) Role of the sympathoadrenergic system in adipose tissue metabolism during exercise in humans. J Physiol 536:283–294
37. Turner MJ, Howley ET, Tanaka H, Ashraf M, Bassett DR, Keefer DJ (1995) Effect of graded adrenaline infusion on blood lactate response to exercise. J Appl Physiol 79:1206–1211
38. Watt MJ, Hargreaves M (2002) Effect of adrenaline on glucose disposal during exercise in humans: role of muscle glycogen. Am J Physiol Endocrinol Metab 283:E578–E583
39. Watt MJ, Howlett KF, Febbraio MA, Spriet LL, Hargreaves M (2001) Adrenaline increases skeletal muscle glycogenolysis, pyruvate dehydrogenase activation and carbohydrate oxidation during moderate exercise in humans. J Physiol 534:269–278
40. Wendling PS, Peters SJ, Heigenhauser GJF, Spriet LL (1996) Adrenaline infusion does not enhance net muscle glycogenolysis during prolonged aerobic exercise. Can J Appl Physiol 21:271–284
41. Wendling PS, Peters SJ, Heigenhauser GJF, Spriet LL (1996) Variability of triacylglycerol content in human skeletal muscle biopsy samples. J Appl Physiol 81:1150–1155
42. Womack CJ, Davis SE, Weltman JY, Blumer J, Barrett EJ, Gaesser GA, Weltman A (1998) The effect of training and adrenaline infusion on ratings of perceived exertion (RPE). Int J Sports Med 19:121–124

J Appl Physiol 100: 194–202, 2006.
First published September 1, 2005; doi:10.1152/japplphysiol.00813.2005.

Fat adaptation followed by carbohydrate loading compromises high-intensity sprint performance

L. Havemann,[1] S. J. West,[1] J. H. Goedecke,[1] I. A. Macdonald,[2] A. St Clair Gibson,[1] T. D. Noakes,[1] and E. V. Lambert[1]

[1]*University of Cape Town/Medical Research Council Research Unit for Exercise Science and Sports Medicine, Department of Human Biology, University of Cape Town, South Africa; and* [2]*School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, United Kingdom*

Submitted 11 July 2005; accepted in final form 25 August 2005

Havemann, L., S. J. West, J. H. Goedecke, I. A. Macdonald, A. St Clair Gibson, T. D. Noakes, and E. V. Lambert. Fat adaptation followed by carbohydrate loading compromised high-intensity sprint performance. *J Appl Physiol* 100: 194–202, 2006. First published September 1, 2005; doi:10.1152/japplphysiol.00813.2005.—The aim of this study was to investigate the effect of a high-fat diet (HFD) followed by 1 day of carbohydrate (CHO) loading on substrate utilization, heart rate variability (HRV), effort perception [rating of perceived exertion (RPE)], muscle recruitment [electromyograph (EMG)], and performance during a 100-km cycling time trial. In this randomized single-blind crossover study, eight well-trained cyclists completed two trials, ingesting either a high-CHO diet (HCD) (68% CHO energy) or an isoenergetic HFD (68% fat energy) for 6 days, followed by 1 day of CHO loading (8–10 g CHO/kg). Subjects completed a 100-km time trial on *day 1* and a 1-h cycle at 70% of peak oxygen consumption on *days 3*, *5*, and *7*, during which resting HRV and resting and exercising respiratory exchange ratio (RER) were measured. On *day 8*, subjects completed a 100-km performance time trial, during which blood samples were drawn and EMG was recorded. Ingestion of the HFD reduced RER at rest ($P < 0.005$) and during exercise ($P < 0.01$) and increased plasma free fatty acid levels ($P < 0.01$), indicating increased fat utilization. There was a tendency for the low-frequency power component of HRV to be greater for HFD-CHO ($P = 0.056$), suggestive of increased sympathetic activation. Overall 100-km time-trial performance was not different between diets; however, 1-km sprint power output after HFD-CHO was lower ($P < 0.05$) compared with HCD-CHO. Despite a reduced power output with HFD-CHO, RPE, heart rate, and EMG were not different between trials. In conclusion, the HFD-CHO dietary strategy increased fat oxidation, but compromised high intensity sprint performance, possibly by increased sympathetic activation or altered contractile function.

muscle recruitment; rating of perceived exertion; heart rate variability; fat oxidation; endurance exercise

FATIGUE DURING ENDURANCE EXERCISE has been associated with, among other things, a depletion of muscle glycogen stores (2, 21). In an attempt to delay the onset of fatigue during endurance exercise, various nutritional strategies have focused on optimizing muscle glycogen stores before exercise and/or "sparing" muscle glycogen stores during exercise. A more recent nutritional strategy aimed at achieving this encompasses 5–6 days of fat loading, followed by 1 day of carbohydrate (CHO) loading before the event (3, 4, 6). This strategy has been shown to increase fat oxidation at rest and during exercise, in the fasted (3) and nonfasted state (4, 6), and even when CHOs are ingested during exercise (4, 6). This strategy has also been shown to increase muscle glycogen stores and reduce muscle glycogen utilization during exercise (3). However, despite this muscle glycogen-sparing effect, overall improvements in performance have not been demonstrated (3, 4, 6).

The effects of this particular dietary strategy on performance have only been tested under time-trial conditions (~25 min to 1 h) after prolonged submaximal steady-state exercise [2–4 h at 65–70% of peak oxygen consumption ($\dot{V}O_{2\,peak}$)] (3, 4, 6). The effects of 5–6 days of fat loading, followed by 1 day of CHO loading, have not been investigated during exercise that simulates race conditions, which includes high-intensity (>85% $\dot{V}O_{2\,peak}$) sprints. Because glycogen is the predominant fuel during high-intensity exercise (28), a nutritional strategy that not only stores muscle glycogen but also promotes glycogen sparing would most likely benefit endurance exercise that includes high-intensity exercise bouts. However, factors other than muscle glycogen content may also have an effect on exercise performance after a high-fat diet (HFD).

In fact, the ingestion of a HFD has been shown to increase sympathetic activation during exercise (17, 20, 29). Sasaki et al. (29) demonstrated an increase in sympathetic activation during exercise with 7 days of high-fat feeding (50% fat energy), which was associated with muscle glycogen depletion in the working muscle. Conversely, Helge et al. (17) demonstrated increased sympathetic activation during exercise with prolonged high-fat intake that persisted despite muscle glycogen restoration. Ingestion of the HFD was also associated with increased effort perception (18) and reduced endurance exercise capacity, possibly as a consequence of the increased sympathetic activation (17). Moreover, an increased effort perception has also been reported during high-intensity (~85% $\dot{V}O_{2\,peak}$) sprint bouts (16.0 ± 1.3 vs. 13.8 ± 1.8) (33) and during submaximal exercise at intensities between 30 and 90% $\dot{V}O_{2\,peak}$ (14.3 ± 2.5 vs. 12.6 ± 2.2) (27) after 3 days of high-fat compared with 3 days of high-CHO feeding. The coupling between the sympathetic activation with high-fat intake and the increase in effort perception during exercise requires further investigation.

The decrement in performance with increased sympathetic activation associated with high-fat feeding may be related to alterations in central drive. Using microneurography, Seals and Enoka (30) found that increased muscle sympathetic activation

Address for reprint requests and other correspondence: J. H. Goedecke, UCT/MRC Research Unit for Exercise Science and Sports Medicine, Dept. of Human Biology, Univ. of Cape Town, PO Box 115, Newlands 7725, South Africa (e-mail: juliag@sports.uct.ac.za).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

during fatiguing isometric handgrip exercise was associated with increased electromyograph (EMG) activity. To our knowledge, no other studies have investigated the effect of dietary manipulation, in particular high-fat feeding, on neural recruitment strategies, central regulation and "awareness of fatigue" during exercise. Therefore, the aim of the present study was to investigate the effect a HFD followed by 1 day of CHO loading on substrate utilization, heart rate variability as a proxy measure of sympathetic activation, effort perception, muscle recruitment, and performance during a 100-km cycling time trial, including high-intensity sprints, simulating race situations.

METHODS

Subjects and preliminary testing. Eight endurance-trained male cyclists participated in this study, which was approved by the Research and Ethics Committee of the Faculty of Medicine of the University of Cape Town. All subjects were free from known metabolic conditions and were currently not taking any medications for chronic conditions such as high blood pressure or stimulants for conditions such as asthma. The subjects were informed of the nature of the study and written, informed consent was obtained before the start of the study. Body weight and height were measured to the nearest decimal place. The percent body fat was determined from measurements of skinfold thickness, using the equations of Durnin and Womersley (10). The characteristics of the subjects are summarized in Table 1.

$\dot{V}O_{2\,peak}$ and peak power output (W_{peak}) were measured on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands) modified with toe clips and racing handlebars as described by Hawley and Noakes (16). Work rates were started at 3.33 W/kg body mass and increased first by 50 W and then by 25 W every 150 s until the subject was exhausted. W_{peak} was defined as the highest exercise intensity the subject completed for 150 s (in W), plus the fraction of time spent in the final workload. During the progressive exercise test, ventilation volume, oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured over 15-s intervals using a breath-by-breath Oxycon Alpha analyzer (Jaeger-Mijnhardt, Bunnik, The Netherlands). Heart rate was recorded continuously by means of a Polar heart rate monitor (Polar Electro, Kempele, Finland). Before each test, the gas meter was calibrated with a Hans Rudolph 3-liter syringe (Vacumed, Ventura, CA), and the analyzers were set with room air and a 4% carbon dioxide-96% nitrogen gas mixture. W_{peak} values were used to set the work rates in the experimental trials to correspond to 63% of each subject's W_{peak} (~70% $\dot{V}O_{2\,peak}$).

The subjects were further instructed to complete a 3-day dietary record consisting of 2 week days and 1 weekend day. These dietary records were analyzed with the Food Finder 3 program (Medtech, Medical Research Council, Tygerberg, South Africa) to determine the subjects' self-reported energy intake and macronutrient consumption. This dietary information was used as a guideline to devise the two experimental diets. To aid adherence to the diets, subjects were also required to indicate their food preferences.

Table 1. *Subject characteristics*

Characteristic	Mean (SD)	Range
Age, yr	26.0 (3.3)	22.0–32.0
Weight, kg	81.3 (9.6)	74.0–100.0
Height, m	1.80 (0.10)	1.65–1.91
Body fat, %	14.0 (2.8)	8.90–18.1
$\dot{V}O_{2\,peak}$, ml·kg^{-1}·min^{-1}	57.8 (5.5)	51.1–67.2
W_{peak}, W	361 (36)	290–419

Values are means (SD) for 8 subjects. $\dot{V}O_{2\,peak}$, peak oxygen uptake; W_{peak}, peak power output.

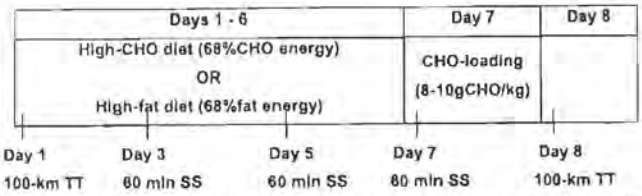


Fig. 1. Summary of diet and testing protocol. CHO, carbohydrate; SS, steady-state cycle at 63% of peak power output (W_{peak}); TT, 100-km time trial.

Study design. Each subject completed two trials in a randomized, single-blind, crossover design with a 2-wk washout period separating each trial. Each trial consisted of an 8-day diet, training, and testing period (Fig. 1). During the trials, subjects reported to the laboratory on *days 1, 3, 5, 7,* and *8* to undertake supervised training and testing.

Dietary manipulations. Subjects were required to ingest either a HFD (~68% energy from fat) for 6 days followed by 1 day of CHO loading (~90% energy from CHO), or an equal-energy CHO diet (68% energy from CHO) for 6 days followed by 1 day of CHO loading (~90% energy from CHO). A registered dietician formulated individualized menus. To control dietary intake, all the meals were prepacked and provided for the subjects together with a diary to record any deviations from the diet. Efforts were made to blind the diets by covertly manipulating the macronutrient compositions of the diets.

Exercise training sessions. On *days 1, 3,* and *5,* subjects reported to the laboratory after a 10- to 12-h fast and completed an exercise training session. On the first day of training (*day 1*), subjects completed a 100-km familiarization time trial on their own bicycles mounted on a Kingcycle trainer (EDS Portaprompt, High Wycombe, UK). The calibration and reliability of the Kingcycle has been described in detail previously (25). The time trial included five 1-km sprint distances after 10, 32, 52, 72, and 99 km, as well as four 4-km sprint distances after 20, 40, 60, and 80 km during which subjects were requested to cycle "as fast as possible." The familiarization time trial also served as a screening trial to see whether the subjects were adequately trained to complete the trial.

On *days 3* and *5,* subjects completed a 60-min steady-state cycle at 70% $\dot{V}O_{2\,peak}$ on a Iode bike, maintaining their cadence at 90 rpm. The steady-state training sessions on *days 3* and *5* were undertaken to ensure consistency in the subjects' training during the trial, as well as monitor their physiological and metabolic responses to the dietary interventions. During the steady-state cycle, heart rate was recorded continuously by means of a Polar heart rate monitor (Polar Electro) and $\dot{V}O_2$ and $\dot{V}CO_2$ values were measured for 4–5 min every 15 min (15, 30, 45, and 60 min), using the online computerized system (Oxycon Alpha Analyzer, Jaeger-Mijnhardt). Rate of perceived exertion (RPE) scores were also recorded at 15-min intervals, using the validated Borg 6–20 RPE scale (9). Printed scale instructions together with a verbal explanation of how the scale works, were given to the subjects before the trial to familiarize them with the operation of the scales.

Before exercise on all 3 days, $\dot{V}O_2$ and $\dot{V}CO_2$ values were measured while the subject was seated in a resting position for 15–20 min to determine the resting respiratory exchange ratio (RER), using the online computerized system, as previously described. Heart rate variability (HRV) was also recorded before exercise using a heart rate monitor (Body IQ, Cape Town, South Africa). HRV has been implicated as an indirect measure of autonomic nervous system activation (23). During the HRV test, heart rate measurements were recorded while subjects breathed rhythmically (12 breaths/min) for 5 min of supine lying, followed by 5 min of standing. Power spectrum analysis for low frequency (LF) (indicative of sympathetic activation) and high frequency (HF) (indicative of parasympathetic activation) was per-

formed based on the HRV interval, using MATLAB software (The MathWorks). The natural logarithm of LF and HF power, as well as the ratio of LF power to HF power was calculated from the power spectrum values.

Experimental trials. On *day 7*, subjects reported to the laboratory after a 10- to 12-h overnight fast. HRV, $\dot{V}o_2$ and $\dot{V}co_2$ values were measured at rest as described earlier. Subjects then completed a 60-min steady-state cycle at 70% $\dot{V}o_{2\,peak}$, during which heart rate was recorded continuously and $\dot{V}o_2$ and $\dot{V}co_2$ values were measured for 4–5 min every 15 min (15, 30, 45, and 60 min). Blood samples were drawn at rest and at 15-min intervals (15, 30, 45, and 60 min) during the constant-load exercise for the subsequent analysis of plasma glucose, lactate, free fatty acids (FFA) and catecholamine concentrations (see *Blood sampling and analysis*). In addition, electromyography (EMG) amplitude (*EMG measurements*) and RPE were recorded at 15-min intervals.

On *day 8*, CHO-loaded subjects reported to the laboratory after a 10- to 12-h overnight fast. HRV, $\dot{V}o_2$, and $\dot{V}co_2$ values were measured at rest as described earlier. After a 5-min warm-up, subjects completed a 100-km performance time trial. During the 100-km time trial, a blood sample was drawn at rest and again immediately before the 1-km sprints at 32, 52, 72, and 99 km for the subsequent analysis of plasma glucose, lactate, FFA, and catecholamine concentrations. EMG amplitude was recorded during the midpoint of each 1-km sprint (10.5, 32.5, 52.5, 72.5, and 99.5 km), each 4-km sprint (22, 42, 62, and 82 km), and at three nonsprint distances (5, 55, and 95 km) during the 100-km time trial. Power output and heart rate were measured continuously throughout the trial. RPE was recorded immediately before and after every sprint. The only feedback the subjects received during the 100-km time trial was their elapsed distance. During both trials, subjects ingested a 10% glucose polymer solution at regular intervals (200 ml every 20 min) to maintain plasma glucose concentrations.

Isometric maximal voluntary contraction. Before the exercise trial on *days 7* and *8*, subjects' peak isometric force was assessed on the lower right limb on a Kin-Com isokinetic dynamometer (Chattanooga Group, Chattanooga, TN). The subject's hips and upper body were firmly strapped to the seat. The arm position for each test was standardized with each subject crossing his arms over his chest. All isometric tests were conducted at 60° knee flexion, with the limb being in full extension at 0°. The angle of 60° flexion has been shown to be the angle of maximal isometric force generation (34). The standardized warm-up included two isometric contractions of the knee extensors at 50% followed by two contractions at 85% of each subject's subjective maximum. The isometric test included four maximum voluntary contractions (MVC) of 5 s each separated by 5-s intervals. Subjects were verbally motivated to encourage them to achieve their maximum potential. EMG amplitude of the vastus lateralis was recorded during the MVC isometric force test, and the MVC with the highest mean force was used for subsequent analyses. The purpose of the MVC was to allow measurement of muscle recruitment patterns during the subsequent cycling trials to be expressed as a percentage of the MVC. Normalizing each subject's EMG muscle activity relative to his own maximal activity also excluded confounding variables such as electrode positioning, skin impedance, and differences in percent body fat.

EMG measurements. Before the isometric MVC and the exercise trials, an EMG triode electrode (Triode MII:PO100, Thought Technology, Montreal, Canada) was placed over the belly of each subject's right vastus lateralis to measure muscle recruitment patterns during the isometric MVC, the steady-state cycle on *day 7* and the 100-km time-trial on *day 8*. The electrode positioning was standardized for each subject. The hair at the placement site was shaved off, and the skin was scraped using industrial sandpaper. An alcohol swab was used to remove any oil and dirt from the skin. To minimize interference, the electrode was taped onto the leg with self-adherent wrap and covered with cotton pads.

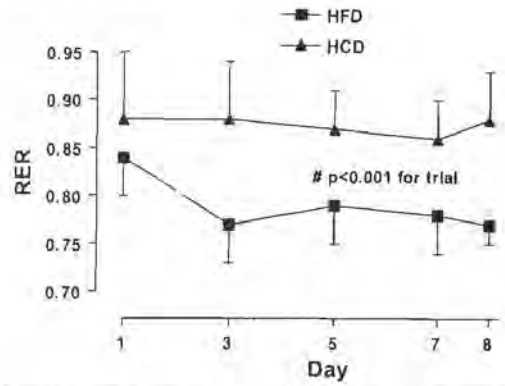


Fig. 2. Resting respiratory exchange ratio (RER) in response to the high-fat diet (HFD) and high-carbohydrate diet (HCD) interventions. Values are means (SD).

The triode electrode was attached to the muscle "belly" as described above and connected to a preamplifier. The amplifier was linked via fiber-optic cable to the Flexcomp/DSP EMG apparatus (Thought Technology) and host computer. EMG activity was sampled at 1,984 Hz, a high enough frequency for reliable data collection and quantitative data analyses (19). EMG signals from the electrode were band-pass filtered (20–500 Hz) and amplified using standard differential amplifiers (Thought Technology, common mode rejection ratio > 103 dB at 1 kHz, input impedance = 1×10^6 MΩ; adjustable gain up to 1,600). The sampled EMG was passed through a 50-Hz line filter to remove interference from electrical sources to yield raw data. Movement artifact was removed from the raw signals with a high-pass second-order Butterworth filter with a cut off frequency of 15 Hz. The means of the EMG signals were then removed, and the signals were full wave rectified. The signals were smoothed with a linear envelope using a low-pass second-order Butterworth filter with a cutoff frequency of 10 Hz. Filtering procedures were performed using MATLAB software (The MathWorks).

Of the four 5-s isometric maximal voluntary contractions from the right limb, EMG was only sampled from the contraction that yielded the greatest force output. The full 5 s of EMG data were sampled during this contraction. In a similar manner, 5 s of EMG were sampled from the 20 s of EMG data collected during the steady-state cycling trial every 15 min and during the 1-km, 4-km, and nonsprint distances of the 100-km time trial. The filtered EMG data was processed to yield mean amplitude data using specifically developed software (Mullany POO. [2.2] computer program, 2000; Ref. 24). EMG amplitude was calculated using the root mean square method.

Table 2. *Mean dietary intake during dietary treatments*

		CHO			Fat			Protein		
	Energy, cal	g	g/kg	%Energy	g	g/kg	%Energy	g	g/kg	%Energy
HFD	3,560 (246)	150 (14)*	1.85 (0.10)*	16.8 (0.6)*	270 (18)*	3.33 (0.16)*	68.2 (0.6)*	134 (8)	1.65 (0.09)	15.0 (0.5)
HCD	3,550 (206)	602 (32)*	7.48 (0.46)*	67.8 (0.7)	68 (6)*	0.83 (0.03)*	17.1 (0.6)*	134 (10)	1.66 (0.10)	15.1 (0.4)

Values are means (SD). CHO, carbohydrate; HFD, high-fat diet; HCD, high-carbohydrate diet. *Significant trial effect, ($P < 0.001$).

Table 3. *Mean resting heart rate variability in response to the HFD and HCD*

	Day 1	*Day 3*	*Day 5*	*Day 7*	*Day 8*	*P* Value
LF supine						
HFD	6.65 (0.73)	6.31 (0.75)	6.67 (1.10)	6.12 (1.13)	6.21 (0.73)	0.056 trial
HCD	5.78 (0.59)	6.26 (0.92)	5.87 (0.70)	5.27 (0.92)	6.07 (1.15)	
LF standing						
HFD	6.90 (1.55)	7.07 (0.90)	7.68 (0.84)	7.19 (0.89)	7.14 (0.63)	NS
HCD	7.04 (1.11)	6.92 (1.03)	7.18 (1.06)	6.98 (0.97)	7.03 (0.93)	

Values are means (SD) expressed as the natural logarithm. LF supine, low-frequency supine; LF standing, low-frequency standing. NS, not significant.

Blood sampling and analysis. Venous blood samples (~12 ml) were drawn during the steady-state cycle on *day 7* and during the 100-km time trial on *day 8* by inserting a flexible 20-gauge cannula into a forearm antecubital vein and attaching it to a three-way stopcock. The cannula was kept patent by flushing with 1 ml sterile saline after each blood sample. One aliquot (2 ml) was placed into a vacutainer containing potassium oxalate and sodium fluoride for subsequent analysis of glucose and lactate concentrations. Two aliquots (2 × 3 ml) were placed into vacutainers containing lithium heparin for analysis of plasma epinephrine and norepinephrine concentrations. The remaining aliquot (2 ml) was placed into a vacutainer containing gel and clot activator for determination of serum FFA (nonesterified) concentrations. All samples were kept on ice and then centrifuged at 3,000 rpm at 4°C for 10 min at the end of the trial. The supernatants were stored at −80°C (epinephrine and norepinephrine) and −20°C (glucose, lactate, insulin, and FFAs) for later analysis.

Plasma glucose concentrations were determined using the glucose oxidase method (Glucose analyzer 2, Beckman Instruments, Fullerton, CA). Plasma lactate and serum FFA concentrations were determined by spectrophotometric measurements (model 35, Beckman) using commercial kits (Lactate Pap, Bio-Merieux, Marcy-L'Etiole, France; and FFA Half-micro test, Boehringer, Mannheim, Germany). Plasma catecholamine concentrations were analyzed by high-performance liquid chromatography, according to the method described by Forster and MacDonald (12).

Statistical analysis. Values are presented as means (SD). An ANOVA with repeated measures and the Tukey post hoc analysis were performed using STATISTICA analysis software (version 6, Statsoft, Tulsa, OK). Statistical significance was accepted when $P < 0.05$.

RESULTS

Training and dietary control. All subjects followed the experimental diets, ingested the food that was provided during both trials, and achieved the recommended target of fat and CHO intakes. The mean dietary intakes during both trials are presented in Table 2. According to design, there was a significant difference ($P < 0.001$) between the CHO and fat contents of the HCD and HFD that were consumed. Although diets were blinded and covertly manipulated, subjects were able to distinguish that the diets were different but were unaware of their composition.

All the subjects attended all the training sessions, but two subjects only completed 45 min of the 60-min steady-state training session on *day 5*, after 4 days of high-fat intake. Although the remaining subjects successfully completed all the training sessions, four subjects experienced difficulties during the steady-state cycle on *day 3* and/or *day 5* on the HFD treatment, complaining of "tired" and "burning" legs or having difficulties in maintaining the training cadence at the defined workload.

Resting variables. The mean fasting RER was significantly lower with the HFD compared with the HCD trial ($P < 0.001$). RER decreased over 6 days of the high-fat intake (0.84 ± 0.04 *day 1* to 0.78 ± 0.04 *day 7*) and remained low (0.77 ± 0.02) on *day 8* despite 1 day of CHO loading on *day 7* (Fig. 2). In contrast, RER in the HCD trial did not change significantly over the 8 days.

The mean normalized HRV values for LF, reflecting sympathetic modulation for supine and standing, are presented in Table 3. No significant differences between trials were demonstrated for HF or LF-to-HF ratio (data not shown), but there was a tendency toward a significant trial effect ($P = 0.056$) for the LF supine values (Table 3). No significant differences in mean resting RPE or heart rate were found between the two diet treatments (data not shown).

RER, heart rate, and RPE during steady-state training rides. Mean exercising variables, including heart rate, RER, and RPE, measured at 15-min intervals during the 60-min steady-state cycle on *days 3*, *5*, and *7*, are presented in Table 4. Mean exercising RER on *days 3*, *5*, and *7* was significantly lower on the HFD compared with the HCD ($P < 0.05$). Conversely, mean exercising heart rate was significantly higher in response to the HFD treatment ($P < 0.05$) during the three steady-state rides. There was a tendency ($P = 0.063$) for mean effort perception during the three training rides to be higher when ingesting the HFD compared with the HCD.

Experimental steady-state cycle on day 7. Tables 5 and 6 summarize the metabolic responses during the steady-state cycle on *day 7*, after 6 days of high-fat intake. Mean RER was significantly lower ($P < 0.01$) on the HFD compared with HCD. Heart rate increased during the exercise bout ($P < 0.05$) and was significantly higher for HFD ($P < 0.05$) compared

Table 4. *Mean exercising values during the 60-min steady-state cycle on days 3, 5, and 7 in response to the HFD and HCD treatments*

	Day 3	*Day 5*	*Day 7*	*P* Value
RER				
HFD	0.85 (0.04)	0.86 (0.03)	0.87 (0.03)	
HCD	0.93 (0.04)	0.92 (0.01)	0.93 (0.02)	<0.05 for trial
Heart rate, beats/min				
HFD	153.0 (5.73)	152.0 (6.34)	151.0 (8.61)	
HCD	149.0 (7.33)	146.0 (7.23)	147.0 (8.19)	<0.05 for trial
RPE				
HFD	14.0 (1.17)	13.9 (1.21)	12.7 (0.58)	
HCD	13.2 (0.96)	12.6 (1.09)	12.6 (0.74)	0.063 for trial

Values are means (SD). RER, respiratory exchange ratio; RPE, rating of perceived exertion.

Table 5. *Mean exercising values during the 60-min steady-state cycle on day 7 in response to the HFD and HCD treatments*

	15 min	30 min	45 min	60 min	*P* Value
RER					
HFD	0.89 (0.03)	0.86 (0.03)	0.84 (0.02)	0.85 (0.03)	<0.005 for trial
HCD	0.96 (0.03)	0.91 (0.02)	0.91 (0.02)	0.91 (0.02)	<0.001 for time
Heart rate, beats/min					
HFD	149 (9)	152 (7)	156 (7)	158 (7)	<0.05 for trial
HCD	143 (11)	148 (8)	151 (6)	154 (8)	<0.001 for time
RPE					
HFD	12 (2)	13 (1)	14 (1)	14 (2)	
HCD	11 (2)	12 (1)	13 (1)	13 (1)	<0.001 for time
Normalized EMG amplitude, %					
HFD	36 (13)	29 (7)	27 (9)	25 (9)	
HCD	37 (8)	32 (1)	35 (13)	32 (8)	<0.01 for time

Values are means (SD). EMG, electromyograph.

with HCD. Despite the increase in heart rate after HFD, mean RPE was not different between trials. Similarly, there was no significant difference in normalized EMG amplitude in response to the two dietary interventions. The EMG amplitude did, however, decrease significantly during the exercise bout ($P < 0.01$) during both trials.

Euglycemia was maintained during the steady-state cycle during both trials, and plasma glucose concentrations were not different between the HFD and HCD. An interaction effect was demonstrated for plasma lactate concentrations ($P < 0.05$) in response to the dietary interventions, with the mean plasma lactate response being significantly lower ($P < 0.01$) after HFD compared with HCD. In contrast, plasma FFA concentrations were significantly higher ($P < 0.001$) at rest and during the steady-state cycle after the HFD compared with HCD. Plasma catecholamine concentrations were not different between trials.

Metabolic and performance data during 100-km time trial. Circulating blood levels, obtained immediately before the 1-km sprints at 10, 32, 52, 72, and 99 km during the 100-km time trial, are summarized in Table 7. Plasma glucose concentrations were not significantly different between the HFD-CHO and HCD-CHO and subjects remained euglycemic throughout the 100-km time trial after both diet treatments. Plasma FFA concentrations increased significantly during both trials ($P < 0.001$), but they were not different in response to the two diet interventions. Similarly plasma lactate concentrations increased during both trials ($P < 0.001$) and there was a tendency ($P = 0.069$) for the levels to be higher after the HCD-CHO compared with the HFD-CHO. Plasma catecholamine concentrations also increased significantly during both trials ($P < 0.001$) but were not different between the two dietary treatments.

Overall 100-km time trial performance was not significantly different between trials ($P = 0.23$); however, mean performance time was 3 min 44 s slower on the HFD-CHO compared with the HCD-CHO (Fig. 3). Performance of three of the eight subjects improved on the HFD-CHO, with no order effect observed ($P = 0.28$). Variables recorded during the 4-km sprints are summarized in Table 8. No between-trial differences were demonstrated during the 4-km sprints. Mean power output and sprint time recorded during 4-km sprints decreased significantly over time during both trials ($P < 0.01$). Conversely, RPE recorded immediately after 4-km sprints increased similarly over time ($P < 0.001$) in both trials.

In contrast to the overall and 4-km performance, mean power output recorded during the high-intensity 1-km sprints was significant lower ($P < 0.05$) after the HFD-CHO compared with the HCD-CHO treatment ($P < 0.05$ time × trial; Fig. 4). Consequently, 1-km sprint times tended to be slower ($P = 0.07$) after the HFD-CHO compared with the HCD-CHO. Mean heart rate was similar for both treatments (Fig. 5*A*). RPE

Table 6. *Mean circulating blood concentrations during the 60-min steady-state cycle on day 7 in response to the two dietary treatments*

	15 min	30 min	45 min	60 min	*P* Value
Plasma glucose, mmol/l					
HFD	3.9 (0.61)	4.3 (0.86)	4.3 (0.70)	4.0 (0.52)	NS
HCD	4.5 (0.78)	4.5 (0.78)	4.4 (0.78)	4.5 (0.91)	
Plasma lactate, mmol/l					
HFD	2.5 (0.94)	2.55 (1.05)	2.56 (0.98)	2.57 (0.90)	<0.05 time × trial
HCD	4.01 (1.28)	4.26 (1.31)	3.88 (1.28)	3.85 (1.31)	
Serum free fatty acids, mmol/l					
HFD	0.27 (0.09)	0.37 (0.11)	0.41 (0.12)	0.47 (0.12)	<0.005 for trial
HCD	0.20 (0.07)	0.24 (0.10)	0.29 (0.12)	0.35 (0.15)	<0.001 for time
Plasma epinephrine, nmol/l					
HFD		0.99 (0.22)	1.15 (0.28)	1.27 (0.27)	<0.001 for time
HCD		1.02 (0.08)	1.14 (0.22)	1.15 (0.08)	
Plasma norepinephrine, nmol/l					
HFD		8.42 (2.43)	8.81 (2.34)	10.36 (2.48)	<0.001 for time
HCD		8.10 (1.20)	8.55 (2.25)	9.48 (1.96)	

Values are means (SD).

Table 7. *Circulating blood concentrations during the 100-km time trial in response to the HFD-CHO and HCD-CHO treatments*

	10 km	32 km	52 km	72 km	99 km	*P* Value
Plasma glucose, mmol/l						
HFD-CHO	4.3 (0.47)	4.4 (0.70)	4.5 (0.66)	4.2 (0.35)	4.3 (0.43)	NS
HCD-CHO	4.4 (1.23)	4.7 (0.75)	4.7 (0.83)	4.4 (0.76)	4.4 (0.70)	
Serum free fatty acids, mmol/l						
HFD-CHO	0.31 (0.09)	0.28 (0.14)	0.35 (0.23)	0.46 (0.29)	0.78 (0.38)	
HCD-CHO	0.28 (0.12)	0.25 (0.12)	0.30 (0.18)	0.38 (0.16)	0.77 (0.35)	<0.001time
Plasma lactate, mmol/l						
HFD-CHO	1.18 (0.26)	4.69 (2.26)	4.51 (2.04)	4.04 (1.83)	2.95 (0.98)	0.069 trial
HCD-CHO	1.58 (0.37)	5.35 (3.45)	5.15 (3.33)	5.00 (2.87)	4.23 (2.19)	<0.001time
Plasma epinephrine, nmol/l						
HFD-CHO	0.25 (0.04)	0.91 (0.30)	1.10 (0.31)	1.46 (0.65)	2.86 (1.58)	
HCD-CHO	0.21 (0.05)	0.95 (0.38)	1.20 (0.58)	1.58 (0.28)	3.61 (1.49)	<0.001time
Plasma norepinephrine, nmol/l						
HFD-CHO	1.68 (0.49)	11.40 (6.13)	12.25 (6.93)	14.01 (7.33)	15.42 (8.48)	<0.001time
HCD-CHO	1.90 (0.53)	12.72 (7.99)	12.88 (6.17)	14.56 (4.98)	19.69 (7.47)	

Values are means (SD). HFD-CHO, 6-day high-fat diet + 1 day carbohydrate loading; HCD-CHO, 6-day high-carbohydrate diet + 1-day carbohydrate loading.

recorded immediately after the 1-km sprints rose progressively over time ($P < 0.01$) but was not different between treatments (Fig. 5*B*). Normalized EMG amplitude measured during the 1-km sprints was also similar for both treatments (Fig. 5*C*).

DISCUSSION

In this study, we examined the effects of 6 days of a high-fat intake, followed by 1 day of CHO loading, on substrate utilization, HRV, effort perception, muscle recruitment, and performance during endurance exercise. The study is unique in that it is the first study to investigate the effect of high-fat feeding, followed by CHO loading, on endurance exercise, including high-intensity sprints that simulate actual race situations. It was hypothesized that the potential glycogen-sparing effect of this dietary strategy (3) would be most beneficial for exercise that included high-intensity sprint bouts, where muscle glycogen is the predominant fuel (28). However, in contrast to our hypothesis, the HFD-CHO strategy actually compromised high-intensity 1-km sprint performance (Fig. 4). This is a novel finding and, to our knowledge, has not previously been reported.

The ingestion of a HFD for 6 days resulted in a shift in substrate metabolism toward a greater reliance on fat and a reduction in CHO oxidation. The increase in fat oxidation in the present study persisted despite 1 day of CHO loading on *day 7* as demonstrated by the lower resting RER (0.77 ± 0.02 vs. 0.88 ± 0.05, Fig. 2) and higher circulating FFA (Table 7) during exercise after HFD-CHO compared with HCD-CHO on *day 8*. These findings are consistent with the findings of Burke et al. (3, 4) and Carey et al. (6), who also demonstrated an increase in fat oxidation with short-term high-fat feeding that persisted even after restoration of CHO stores. Burke et al. (3) demonstrated that 1 day of rest and CHO loading was sufficient to restore muscle glycogen levels to above baseline levels in both dietary treatments (470 ± 24 to 554 ± 45 mmol/kg dry wt after HFD-CHO; 470 ± 24 to 608 ± 51 mmol/kg dry wt after HCD-CHO). Although muscle glycogen was not measured in our study, it is assumed that muscle glycogen levels were restored on *day 8* as a similar dietary strategy was used to that of Burke et al. (3) in which muscle glycogen levels were measured directly. The increase in fat oxidation with this dietary regime can therefore not be explained by low glycogen stores (36), and it may be related to changes in insulin sensitivity (13), increased fatty acid uptake into the muscle (5), and changes in skeletal muscle enzyme activities that favor fat oxidation (11, 13). In addition to an increase in fat oxidation, Burke et al. (3) have shown that the ingestion of a HFD-CHO resulted in a significant reduction in muscle glycogen utilization (~100 mmol/kg dry wt) during a 120-min cycle at ~70% maximal oxygen consumption with the HFD-CHO compared with the HCD-CHO dietary strategy.

Ingestion of a HFD for 6 days was associated with a significant increase in heart rate, as well as a tendency toward a higher effort perception during training on *days 3* and *5*. Six of the eight subjects complained of fatigue and difficulty in maintaining the defined workload during the steady-state cycle, with two subjects failing to complete the 60-min training session. Burke et al. (3) reported similar subject complaints while training on a HFD. The increased effort perception and heart rate may be attributed to low glycogen stores and an

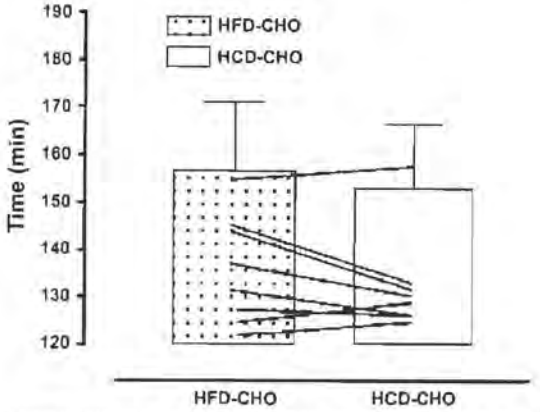


Fig. 3. Overall 100-km time trial performance in response to the 6-day HFD + 1-day CHO loading (HFD-CHO) and 6-day HCD diet + 1-day CHO loading (HCD-CHO) interventions. Solid lines, individual performance changes. Values are means (SD).

Table 8. *Variables measured during the 4-km sprints in response to the HFD-CHO and HCD-CHO treatments*

	20 km	40 km	60 km	80 km	*P* Value
Power, W					
HFD-CHO	289 (50)	291 (50)	279 (50)	268 (48)	<0.01 time
HCD-CHO	308 (56)	308 (61)	305 (62)	295 (55)	
Sprint time, s					
HFD-CHO	336 (26)	338 (26)	340 (24)	347 (29)	<0.05 time
HCD-CHO	327 (27)	330 (31)	328 (28)	335 (31)	
Heart rate, beats/min					
HFD-CHO	166 (6)	166 (8)	167 (6)	168 (7)	NS
HCD-CHO	166 (7)	166 (9)	164 (10)	166 (9)	
RPE					
HFD-CHO	16.6 (2.07)	17.6 (1.30)	18.4 (1.60)	18.8 (1.04)	<0.001 time
HCD-CHO	15.8 (2.66)	17.3 (2.05)	17.5 (2.14)	18.3 (1.75)	
Normalized EMG amplitude, %					
HFD-CHO	33.8 (12.6)	31.33 (11.0)	31.2 (13.4)	32.1 (11.7)	NS
HCD-CHO	31.3 (10.0)	27.7 (6.2)	31.2 (5.3)	29.4 (8.3)	

Values are means (SD).

increase in sympathetic activation (Tables 3 and 4) (17, 29). This has practical implications for athletes ingesting a low-CHO/high-fat diet, for example the Atkins diet, in terms of their ability to train at high intensities. Moreover, athletes that rely on heart rate to set their training loads may fail to achieve a desired power output and hence training stimulus.

Although the HFD-CHO dietary strategy was associated with an increase in fat oxidation and an apparent sparing of muscle glycogen stores on *day 8*, overall 100-km time trial (156 min 54 s for HFD-CHO vs. 153 min 10 s for HCD-CHO) and 4-km sprint performance times after the two dietary treatments were not significantly different. Similarly, Burke et al. (3) and Carey et al. (6) demonstrated no overall improvements in performance during a 7 kJ/kg time trial (lasting ~25 min) after a 2-h submaximal steady-state cycle (3) or a 1-h time trial after 4 h of constant-load exercise (6). However, in both studies, there were individual differences in performance. In the first study, time trial time was 8% faster in five of the seven subjects during the HFD-CHO compared with the HCD-CHO trials (3). Similarly, Carey et al. demonstrated improved performance in five of the seven subjects after the HFD. However, these studies did not simulate race conditions where high-intensity sprint bouts (>90% of W_{peak}) are integral to performance. Mean power output during the 25-min time trial in the two studies of Burke et al. (3, 4) after fat adaptation were 281 W (76% of W_{peak}) and 302 W (76% of W_{peak}), respectively, and mean power output during the 1-h time trial of Carey et al. (6) was 312 ± 15 (77.4% of W_{peak}). The present study is the first study that included high-intensity sprints (mean power output during 1-km sprints >90% of W_{peak}) with endurance exercise, simulating race conditions. In contrast to the original hypothesis, we found that HFD-CHO dietary strategy actually compromised high-intensity 1-km sprint power output. Power output during the 1-km high-intensity sprints was even compromised in the three subjects whose overall 100-km time-trial performance was improved on the HFD-CHO dietary strategy.

We postulated that this reduced performance might be related to changes in sympathetic activation associated with high-fat feeding, as we demonstrated an increase in LF power spectrum for HRV, suggestive of increased sympathetic activation after high-fat intake that persisted after 1 day of CHO loading. HRV has previously been shown to be a noninvasive, practical, and reliable measure of sympathetic modulation (14). In addition, heart rate was similar during the 1-km sprints (Fig. 5*A*) despite reduced 1-km power output after the HFD-CHO diet, suggesting increased sympathetic activation during the HFD-CHO trial. Previous research (29) has demonstrated an increase in sympathetic activation during exercise, as measured by plasma norepinephrine levels, with 7 days of high-fat feeding, which was associated with low muscle glycogen stores. However, Helge et al. (17) demonstrated that the increase in sympathetic activation during exercise with high-fat feeding persisted despite the restoration of muscle glycogen stores. An increase in sympathetic activation in response to a high-fat intake, as suggested by findings in the present study, has previously been associated with increased effort perception (17, 27, 33). The present study showed similar RPEs immediately after the 1-km sprints for both trials, despite reduced power output in the HFD-CHO trial, indicative of increased effort perception for less work produced (Fig. 5*B*).

Similarly, EMG amplitude was similar between treatments, and it failed to track the change in power output during the 1-km sprints. This suggests that the decrease in power output during the HFD-CHO trial was associated with a relative

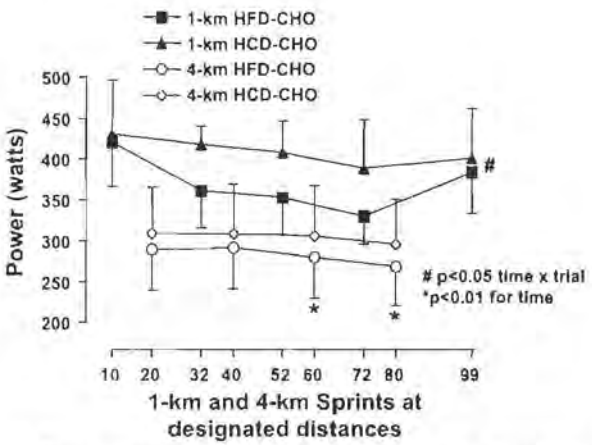


Fig. 4. Power output during the 1-km and 4-km sprints in response to the HFD-CHO and HCD-CHO interventions. Values are means (SD).

increase in muscle recruitment (Fig. 5*C*), indicating that subjects recruited a greater number of motor units for less power produced. In contrast to our findings, St Clair Gibson et al. (31) reported no effect of dietary manipulation on neuromuscular activity during a self-paced 100-km time-trial and showed similar reductions in power output and EMG activity during the 1-km or 4-km sprints between the placebo and CHO-loading exercise trials. It is not obvious how high-fat feeding increased muscle recruitment during the present study, but it may be related to the increased sympathetic activation associated with high-fat intake. It has previously been shown that an increase in sympathetic activation is associated with increases in EMG activity; however, this measure was during static and not dynamic exercise (30). It may also be possible that the increased muscle recruitment was a result of the development of peripheral fatigue due to altered contractile function and/or metabolic substrate perturbations (15).

Ingestion of the HFD-CHO may have compromised the ability to oxidize the available glycogen at a sufficient rate to fuel the high-intensity sprint bouts. The 1-km sprints were performed at an intensity of >90% of W_{peak}, during which muscle glycogen is the predominant fuel source (28). In contrast, power output during the 4-km sprints was performed at a lower intensity (~78–84% of W_{peak}) and was not affected by the high-fat intake. Therefore, the glycogen-sparing effect of the HFD-CHO strategy, which was thought to be beneficial for endurance performance, may in fact compromise high-intensity sprint performance. This may possibly be mediated by changes in pyruvate dehydrogenase (PDH) activity (7, 26). Indeed, studies investigating the effects of a high-fat intake for between 3 days and 3 wk on PDH activity demonstrated a decrease in the active form of PDH, suggesting reduced glycogenolysis and reduced CHO oxidation (7, 26). Furthermore, preliminary data from Stellingwerff et al. (32), using a similar HFD-CHO strategy to the present study, also demonstrated a decrease in mean active PDH activity during steady-state exercise. Further studies, however, are required to examine this hypothesis.

There was an increase in power output during the final 1-km sprint in both the HFD-CHO and HCD-CHO trials, which is indicative of a reserve capacity. This suggests the presence of a pacing strategy (35), even though subjects were verbally encouraged to exercise as hard as possible during each sprint. The EMG amplitude of the HFD-CHO and HCD-CHO trials did not track the increase in power output during the last 1-km sprint. In this trial, muscle recruitment was only measured from the vastus lateralis muscle. However, it is possible that the muscle recruitment of the entire quadriceps femoris was altered as subjects fatigued, and the maintenance or increase in power output during the last high-intensity sprint was the result of motor unit rotation and/or substitution (37) or additional recruitment of non measured synergistic muscles (1, 8).

In conclusion, ingestion of a HFD for 6 days, followed by 1 day of CHO-loading, increased fat oxidation, but it reduced high-intensity sprint power performance, which was associated with increased muscle recruitment, effort perception, and heart rate. The mechanisms associated with the decrement in performance are not clear, but they could possibly be related to increased sympathetic activation or altered contractile function and/or the inability to oxidize the available CHO during the high intensity sprints. Further research is required to investigate mechanisms associated with high-fat feeding and compromised high-intensity exercise performance.

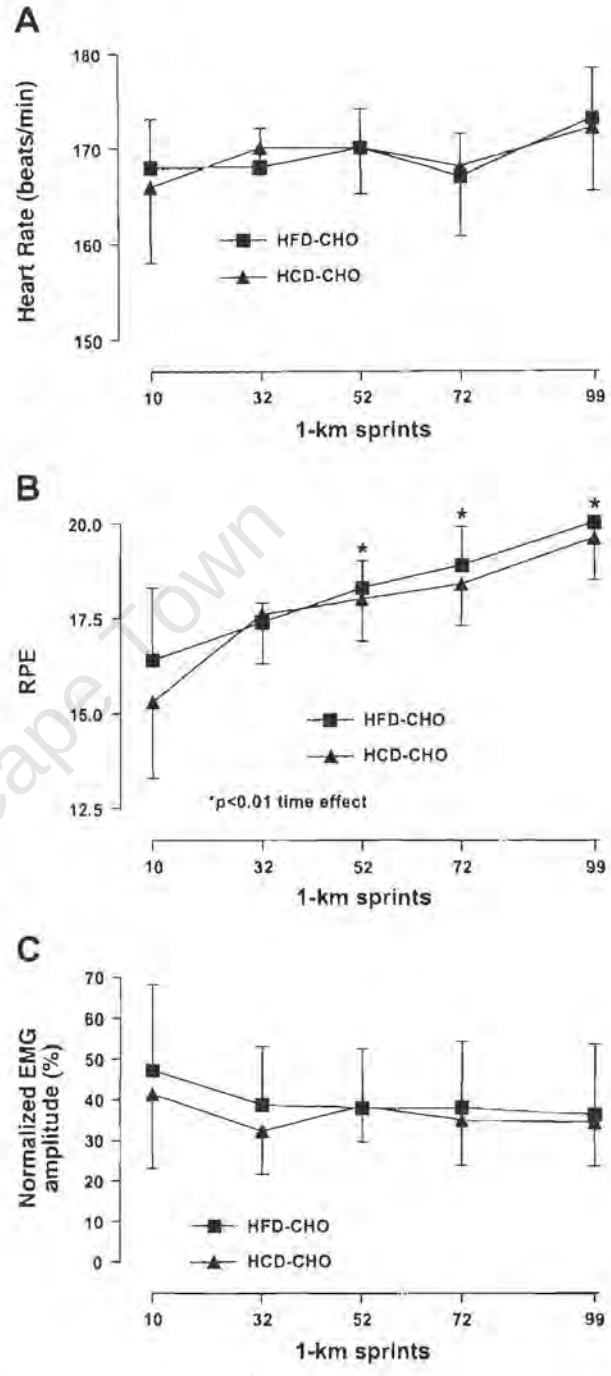


Fig. 5. Heart rate (A), ratings of perceived exertion (RPE; *B*), and normalized EMG amplitude (*C*) during the 1-km sprints in response to the HFD-CHO and HCD-CHO interventions.

ACKNOWLEDGMENTS

The authors thank the research volunteers for participation in this study. We are grateful to the staff of Lab B14 of the School of Biomedical Sciences, University of Nottingham Medical School for assistance with the plasma

Journal of Applied Physiology

catecholamine analysis. In addition, we thank Judy Belonje for expert technical assistance.

GRANTS

This study was funded by the National Research Foundation, the University of Cape Town, the Medical Research Council of South Africa, the Nellie Atkinson and Harry Crossley Staff Research Funds of the University of Cape Town, Bromor Foods Pty. Ltd., and the Technology and Human Resources for Industry Programme.

REFERENCES

1. **Akima H, Foley JM, Prior BM, Dudley GA, and Meyer RA.** Vastus lateralis fatigue alters recruitment of musculus quadriceps femoris in humans. *J Appl Physiol* 92: 679–684, 2002.
2. **Bergstrom J, Hermansen L, Hultman E, and Saltin B.** Diet, muscle glycogen and physical performance. *Acta Physiol Scand* 71: 140–150, 1967.
3. **Burke LM, Angus DJ, Cox GR, Cummins NK, Febbraio MA, Gawthorn K, Hawley JA, Minehan M, Martin DT, and Hargreaves M.** Effect of fat adaptation and carbohydrate restoration on metabolism and performance during prolonged cycling. *J Appl Physiol* 89: 2413–2421, 2000.
4. **Burke LM, Hawley JA, Angus DJ, Cox GR, Clark SA, Cummins NK, Desbrow B, and Hargreaves M.** Adaptations to short-term high-fat diet persist during exercise despite high carbohydrate availability. *Med Sci Sports Exerc* 34: 83–91, 2002.
5. **Cameron-Smith D, Burke LM, Angus DJ, Tunstall RJ, Cox GR, Bonen A, Hawley JA, and Hargreaves M.** A short-term, high-fat diet up-regulates lipid metabolism and gene expression in human skeletal muscle. *Am J Clin Nutr* 77: 313–318, 2003.
6. **Carey AL, Staudacher HM, Cummings NK, Stepto NK, Nikolopoulos V, Burke LM, and Hawley JA.** Effects of fat adaptation and carbohydrate restoration on prolonged endurance exercise. *J Appl Physiol* 91: 115–122, 2001.
7. **Cutler DL, Gray CG, Park SW, Hickman MG, Bell JM, and Kolterman OG.** Low-carbohydrate diet alters intracellular glucose metabolism but not overall glucose disposal in exercise-trained subjects. *Metabolism* 44: 1264–1270, 1995.
8. **Dimitrijevic MR, McKay WB, Sarjanovic I, Sherwood AM, Svirtlih L, and Vrbova G.** Co-activation of ipsi- and contralateral muscle groups during contraction of ankle dorsiflexors. *J Neurol Sci* 109: 49–55, 1992.
9. **Dunbar CC, Robertson RJ, Baun R, Blandin MF, Metz K, Burdett R, and Goss FL.** The validity of regulating exercise intensity by ratings of perceived exertion. *Med Sci Sports Exerc* 24: 94–99, 1992.
10. **Durnin JVGA and Womersley J.** Body fat assessed from total body density and its estimation from skinfold thickness measurement on 481 men and women aged 16–72 years. *Br J Nutr* 32: 77–97, 1974.
11. **Fisher EC, Evans WJ, Phinney SD, Blackburn GL, Bistrian BR, and Young VR.** Changes in skeletal muscle metabolism induced by eucaloric ketogenic diet. In: *Biochemistry of Exercise*, edited by Knuttgen HG, Vogel JA, and Poortsman J. Champaign, IL: Human Kinetics, 1983. p. 497–501.
12. **Forster CB and MacDonald IA.** The assay of catecholamine content of small volumes of human plasma. *Biomed Chromatogr* 13: 209–215, 1999.
13. **Goedecke JH, Christie G, Wilson G, Dennis SC, Noakes TD, Hopkins WG, and Lambert EV.** Metabolic adaptations to a high-fat diet in endurance cyclists. *Metabolism* 48: 1509–1517, 1999.
14. **Goldberger JJ.** Sympathovagal balance: how should we measure it? *Am J Physiol Heart Circ Physiol* 276: H1273–H1280, 1999.
15. **Hakkinen K and Komi PV.** Electromyographic and mechanical characteristics of human muscle during fatigue under voluntary and reflex conditions. *Electroencephalogr Clin Neurophysiol* 55: 436–444, 1983.
16. **Hawley JA and Noakes TD.** Peak sustained power output predicts $VO_{2\,max}$ and performance time trial in trained cyclists. *Eur J Appl Physiol* 65: 79–83, 1992.
17. **Helge JW, Richter EA, and Kiens J.** Interaction of diet and training on metabolism and endurance during exercise in man. *J Physiol* 492: 293–306, 1996.
18. **Helge JW.** Long-term diet adaptation effects on performance, training capacity, and fat utilization. *Med Sci Sports Exerc* 34: 1499–1504, 2002.
19. **Hunter AM, St Clair Gibson A, Lambert M, Dennis S, Mullany H, O'Malley MJ, Vaughan CL, Kay D, and Noakes TD.** EMG amplitude in maximal and submaximal exercise is dependent on signal capture rate. *Int J Sports Med* 24: 83–89, 2003.
20. **Jannson E, Hjemdahl P, and Kaijser L.** Diet induced changes in sympatho-adrenal activity during submaximal exercise in relation to substrate utilisation in man. *Acta Physiol Scand* 114: 171–178, 1982.
21. **Karlsson J and Saltin B.** Diet, muscle glycogen, and endurance performance. *J Appl Physiol* 31: 203–206, 1971.
22. **Mirka GA.** The quantification of EMG normalization error. *Ergonomics* 34: 343–352, 1991.
23. **Monir OZG, Weiss J, Sayhouni X, and Singer DH.** Heart rate variability: frequency domain analysis. *Cardiol Clin* 10: 499–538, 1992.
24. **Mullany H, O'Malley M, St Clair Gibson A, and Vaughan C.** Agonist-antagonist common drive during fatiguing knee extension efforts using surface electromyography. *J Electromyogr Kinesiol* 12: 375–384, 2002.
25. **Palmer GSSC, Noakes TD, and Hawley JA.** Assessment of the reproducibility of performance testing on an air-braked cycle ergometer. *Int J Sports Med* 17: 293–298, 1996.
26. **Peters SJ, St Amand TA, Howlett RA, Heigenhauser GJ, and Spriet LL.** Human skeletal muscle pyruvate dehydrogenase kinase activity increases after a low-carbohydrate diet. *Am J Physiol Endocrinol Metab* 275: E980–E986, 1998.
27. **Prusaczyk WK, Cureton KJ, Graham RE, and Ray CA.** Differential effects of dietary carbohydrate on RPE at the lactate and ventilatory thresholds. *Med Sci Sports Exerc* 24: 568–575, 1992.
28. **Romijn J, Coyle E, Sidossis L, Gastaldelli A, Horowitz J, and Endert E.** Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol Endocrinol Metab* 265: E380–E391, 1993.
29. **Sasaki H, Hotta N, and Ishiko T.** Comparison of sympatho-adrenal activity during endurance exercise performed under high- and low-carbohydrate diet conditions. *J Sports Med Phys Fitness* 31: 407–412, 1991.
30. **Seals DR and Enoka RM.** Sympathetic activation is associated with increases in EMG during fatiguing exercise. *J Appl Physiol* 66: 88–95, 1989.
31. **St Clair Gibson A, Schabort EJ, and Noakes TD** Reduced neuromuscular activity and force generation during prolonged cycling. *Am J Physiol Regul Integr Comp Physiol* 281: R187–R196, 2001.
32. **Stellingwerff T, Spriet LL, Watt MJ, Kimber NE, Hargreaves M, Hawley JA, and Burke LM.** Decreased skeletal muscle pyruvate dehydrogenase activation during cycling following short-term high-fat adaptation with carbohydrate restoration. *Med Sci Sports Exerc* 37: S3 (Abstract 56), 2005.
33. **Stepto NK, Carey AL, Staudacher HM, Cummins NK, Burke LM, and Hawley JA.** Effect of short-term fat adaptation on high-intensity training. *Med Sci Sports Exerc* 34: 449–455, 2002.
34. **Tihanyi J, Apor P, and Fekete G.** Force-velocity-power characteristics and fiber composition in human knee extensor muscles. *Eur J Appl Physiol* 48: 331–343, 1982.
35. **Ulmer HV.** Concept of an extracellular regulation of muscular metabolic rate during heavy exercise in humans by psychophysiological feedback. *Experientia* 52: 416–420, 1996.
36. **Welton SM, Bosch AN, Dennis SC, and Noakes TD.** Influence of muscle glycogen content on metabolic regulation. *Am J Physiol Endocrinol Metab* 274: E83–E88, 1998.
37. **Westgaard RH and de Luca CJ.** Motor unit substitution in long-duration contractions of the human trapezius muscle. *J Neurophysiol* 82: 501–504, 1999.

Eur J Appl Physiol (2005) 95: 537–542
DOI 10.1007/s00421-005-0004-9

ORIGINAL ARTICLE

Sacha J. West · Lynne Smith · Estelle V. Lambert
Timothy D. Noakes · Alan St Clair Gibson

Submaximal force production during perceptually guided isometric exercise

Accepted: 25 May 2005 Published online: 6 September 2005

Abstract The aim of this study was to examine submaximal isometric force production guided by perceptual feelings of exertion. Thirty young adults performed isometric knee extensions on an isokinetic dynamometer. Subjects performed five different tests; the first test was the same for all subjects (standard naïve test). During the standard naïve test, subjects were asked to randomly produce force at perceived contraction intensities (25%, 50% and 75% of their maximum voluntary contraction (MVC)), with 100% MVC performed as the final intensity. All intensities, including the 100% MVC, were randomly performed in the other four tests (control tests 1 and 2, post 20% MVC and post 100% MVC tests). Post 20% MVC and post 100% MVC tests included fatiguing isometric exercise at 20% and 100% MVC respectively, which were performed prior to the test protocol. Results show that absolute peak force increased with increasing intensity ($P < 0.001$) during all tests. During the standard naïve test, absolute peak force at 25% and 50% MVC was significantly lower ($P = 0.009$) compared to control test 2, post 20% MVC and 100% MVC tests, and relative peak force was lower at all intensities compared to all other tests ($P < 0.001$). Absolute and relative peak force was most accurate at 50% MVC (−12.06 N and −2.42%, respectively). Prior fatiguing isometric exercise did not affect the subsequent perceptual response range. In conclusion, isometric force was most accurate at 25% MVC but under-produced (perceptually overestimated) during higher contraction intensities preceding a maximal voluntary contraction (100% MVC). The ability to match absolute force with target contraction intensities was most accurate at 50% MVC during all five experimental conditions and poor at opposite ends of the force domain. Furthermore, prior fatiguing isometric exercise did not have an effect on the subsequent perceptual response range.

Keywords Isometric exercise · Maximum voluntary contraction · Perceived exertion · Fatigue

S. J. West (✉) · L. Smith · E. V. Lambert · T. D. Noakes
A. S. C. Gibson
MRC/UCT Research Unit for Exercise Science
and Sports Medicine, Department of Human Biology,
University of Cape Town, P.O. Box 115, Newlands,
7725 Cape Town, South Africa
E-mail: sachaw@sports.uct.ac.za
Tel.: +27-21-6504559
Fax: +27-21-6867530

Introduction

Perceived exertion can be defined as the "subjective intensity of effort, strain, discomfort, and/or fatigue that is experienced during physical exercise" (Robertson and Noble 1997). There is an increasing focus on the role of "effort perception" in the aetiology of fatigue during physical activity and in the limitations to work output (Hampson et al. 2001). The analysis of perceived exertion is strongly correlated to psycho-behavioural and physiological measurements of physical performance and work capacity but this relationship is not well understood. Research examining ratings of perceived exertion is therefore important for both theoretical analysis of physical performance and for application to rehabilitative medicine, sport and human and occupational behaviour (Borg 1982).

A variety of psycho-physiological methods have been used over the years to investigate "effort perception". Ratio scaling procedures, magnitude estimation and magnitude production, are common and valid procedures used in perceived exertion experiments involving both static and dynamic exercise (Pandolf 1983). Magnitude estimation requires the individual to subjectively rate the level of effort with a verbal or number score, whereas magnitude production, the individual is required to exercise to intensities determined by their sense of perceived exertion. Recent evidence has shown a close correlation between submaximal isometric contractions and perceived exertion measured via the Borg CR-10 scale (Pincivero et al. 2000). However, other studies have established that the ability of individuals to accu-

rately match voluntary muscle force to target contraction intensities in the absence of external feedback is inconsistent, especially at high perceived intensities where force output tends to be under produced (Cooper et al. 1979; Jackson and Dishman 2000; Kumar and Simmonds 1994; Pincivero et al. 2003a).

Many experimental designs have allowed individuals to experience a maximal voluntary contraction prior to the testing protocol enabling the individual to cognitively establish a perceptual range (Kumar and Simmonds 1994; Pincivero et al. 2001, 2003a, 2004). Using category scaling methodology, for example Borg's CR-10, to assess perceived exertion, low and high perceptual scale anchors are established which provides the individual with a perceptual range that is the same as the stimulus range (ie, exercise intensity), as well as subjectively matching minimal and maximal perceptual intensities between individuals (Robertson and Noble 1997). It has been suggested, however, that effort perception may be influenced by a maximal contraction being performed immediately prior a submaximal contraction resulting in perceptual underestimation (Hutton et al. 1984). Thus, prior experience of an MVC may in some models, provide anchors for the perceptual range of effort, or alternatively may actually alter the perceptual response range. This study therefore aims to examine the submaximal perceptual response range prior to experiencing a 100% MVC as an anchor.

The relationship between muscle fatigue and effort perception during exercise is still not well understood. Previous research has shown that when the maximum force generating capacity of the muscle is reduced, individuals' perceptual feelings of exertion during exercise are increased (Cafarelli and Bigland-Ritchie 1979; Pincivero and Gear 2000), thus altering the relationship between effort perception and the force generating capacity of the muscle. It is possible therefore, that the individuals' effort perception after performing a fatiguing contraction may be changed and confound their ability to estimate force (Enoka and Stuart 1992).

The aims of the present study therefore, using magnitude production, were to (1) investigate the submaximal perceptual response range prior to experiencing maximal force production as an anchor, (2) compare the absolute force to the target force production of varying relative intensities of MVC, and (3) examine the effect of prior fatiguing isometric exercise on the subsequent perceptual range and ability to replicate isometric force guided by perceptual feelings of exertion.

Methodology

Subject selection

Thirty healthy, active individuals (15 male and 15 female) were recruited to participate in the study. The subjects were young adults with a mean age of 24 ± 3 year, mean weight of 66.7 ± 12.1 kg and height of 170.4 ± 7.5 cm. The study was approved by the Ethics and Research Committee of the Faculty of Health Sciences, University of Cape Town. All subjects provided informed consent prior to participation.

All subjects were physically active at least three times a week and performed a mean of 9 ± 6 h/week physical activity. Exclusion criteria included any history or current signs of knee pathology that would influence exercise performance or be negatively affected by the exercise. Subjects were required to complete a questionnaire to establish age, training history and current training status, and previous and current injuries.

Experimental design

Subjects were required to visit the laboratory on five different occasions each separated by a rest day. The first visit followed a standard protocol (first visit was standard for all subjects), while the following fours visits were randomised. During all tests the subjects were blinded to the absolute force they were producing as this knowledge may have influenced their performance. This was achieved by placing a cover over the Kin-Com isokinetic dynamometer monitor displaying the force data.

A standardized warm up consisting of three stretches was performed before each test. The stretches were held for 30 s and included a standing quadriceps, hamstring and gastrocnemius stretch. Subjects' isometric force was assessed on the lower right limb using a Kin-Com isokinetic dynamometer (Chattanooga Group, Inc., Chattanooga, USA). Their hips and upper bodies were firmly strapped to the seat. The arm position for each test was standardized with each subject crossing his or her arms over the chest. All isometric tests were conducted at 60° knee flexion, with 0° being the limb in full extension. The angle of 60° flexion has been shown to be the angle of maximal isometric force generation (Tihanyi et al. 1982). Each subject performed four warm up isometric contractions of the knee extensors, at a voluntary force output below their maximum, for 5 s each separated by 10 s intervals. The same investigator performed all testing procedures for all the subjects, as well as the verbal encouragement.

The absolute peak isometric force in Newtons (N) for each required contraction intensity was measured on the Kin-Com isokinetic dynamometer which was calibrated before each day of testing. Peak force was the highest isometric force measured over 5 s. The relative peak isometric force expressed as a percentage was the absolute value normalised to the MVC at each perceived contraction intensity.

Experimental protocol

Standard naïve test

For the standard naïve test, by design, subjects were not initially exposed to a maximal voluntary contraction

(MVC), but were required to perform three voluntary isometric contractions at intensities of 25%, 50% and 75% corresponding to their naïve perception (no prior exposure) of a MVC. For example, subjects were asked to "contract their muscles at an intensity they felt was 25% of their MVC". The order of the three contraction intensities was randomised. The fourth voluntary isometric contraction was a maximal contraction (100% MVC). The investigator used verbal encouragement to facilitate the 100% MVC during the standard naïve test, and during the test days that followed. Each contraction was 5 s separated by 10 s intervals, or more if additional rest time was requested.

Control tests 1 and 2

Subjects were required to perform voluntary isometric contractions at the following perceptual intensities: 25%, 50%, 75% and 100% MVC. For example, subjects were asked to "contract their muscles at an intensity they felt was 25% of their MVC". The order of all contraction intensities was randomised. Each contraction was 5 s separated by 10 s intervals, or more if additional rest time was requested.

Post 20% MVC test

Prior to this testing session, the contraction intensity was calculated at 20% of each subject's 100% MVC performed during the standard test. Subjects then performed a voluntary isometric contraction at 20% of their MVC and were instructed to watch the monitor and maintain the isometric contraction at the indicated level on the monitor until volitional fatigue. Subjects were blinded to the absolute force they were generating during the fatiguing contraction. Subjects rested for 10 min thereafter before completing the same protocol used in the control tests.

Post 100% MVC test

Subjects performed a voluntary isometric contraction at 100% MVC until fatigued. For example, subjects were instructed to "contract their muscles maximally for as long as they could until volitional fatigue". Thereafter subjects rested for 10 min before completing the same protocol used in the control tests.

Statistical analysis

The statistical software package Statistica 6.1 (StatSoft, Inc., Tulsa, OK, USA) was used to analyse the data. All data are presented as means ± standard deviations. A two-way analysis of variance (ANOVA) with repeated measures was used to compare absolute and relative force output, as well as absolute and relative force errors, at the different perceptual intensities between the various experimental conditions, and to assess significant main effects and interactions. Where significant differences occurred, a Tukey's HSD post hoc analysis was used to examine the differences. Statistical significance was accepted when $P < 0.05$.

Results

Absolute peak force

The results demonstrate that absolute peak force increased with increasing contraction intensities at 25%, 50%, 75% and 100% MVC ($P < 0.001$) during the standard naïve test, both control tests and the post 20% and 100% MVC tests. Absolute force at 25% and 50% MVC was consistently lower during the standard naïve test where 100% MVC was the final intensity ($P = 0.009$) compared to control test 2, post 20% MVC and 100% MVC tests. At 75% MVC, force was also significantly lower ($P < 0.001$) during the standard naïve test compared to the post 100% MVC test. Maximum isometric force (100% MVC) was not different between experimental conditions (Table 1).

Relative peak force

The relative peak force was significantly lower during the standard naïve test, compared to control tests 1 and 2, and both the post 20% MVC and 100% MVC tests, at all contraction intensities ($P < 0.001$) (Table 2).

Table 1 The absolute peak force produced during the standard naïve test, control tests 1 and 2, post 20% MVC test and post 100% MVC test of the experimental sample ($N = 30$)

Contraction intensity (%)	Standard naïve test (Force in N)	Control test 1 (Force in N)	Control test 2 (Force in N)	Post 20% MVC test (Force in N)	Post 100% MVC test (Force in N)
25	152 ± 93[a]	197 ± 122	209 ± 100[b]	230 ± 101[c]	226 ± 87[c]
50	236 ± 131[a]	279 ± 133	294 ± 111[b]	308 ± 129[c]	315 ± 109[c]
75	324 ± 141[a]	351 ± 128	351 ± 133	366 ± 145	391 ± 129[c]
100	587 ± 148	599 ± 172	608 ± 172	587 ± 191	604 ± 173

All values are expressed as mean ± SD
Absolute Peak Force is reported in Newton's (N)
Main effects, intensity: $P = 0.00001$
Main effects, trial: $P = 0.0005$
Interaction effect: $P = 0.009$

[a] vs [b] Indicates a significant ($P < 0.01$) difference between the standard naïve test and control test 2
[a] vs [c] Indicates a significant ($P < 0.001$) difference between the standard naïve test, the post 20% MVC test and the post 100% MVC test

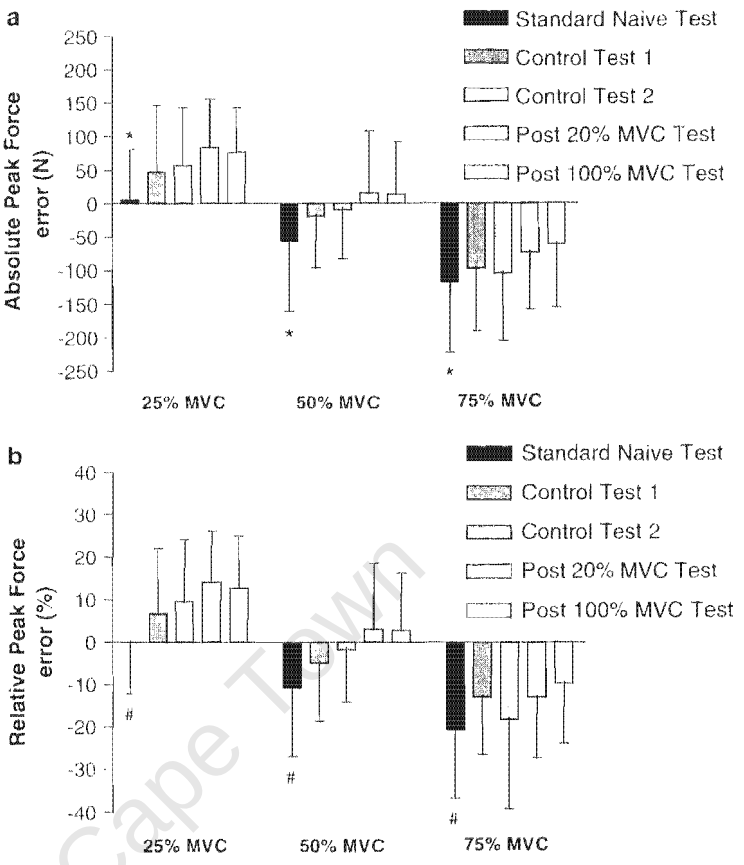


Fig. 1 The errors of the **a** absolute peak isometric force (N) and the **b** relative peak isometric force (%) at the target contraction intensities of 25, 50 and 75% MVC during all experimental conditions of the experimental sample. All values are expressed as mean ± SD. * $P < 0.001$ standard naive test vs control test 2, post 20% MVC and 100% MVC tests at all intensities (absolute peak isometric force) # $P < 0.001$ standard naïve test vs control tests 1 and 2, post 20% MVC and 100% MVC tests at all intensities (relative peak isometric force) (- represents underestimation of force, represents overestimation of force)

Peak force errors

Comparisons were made between the target contraction intensities and the subjects' absolute and relative peak isometric force, and the errors were then calculated (Fig. 1a, b). The absolute peak force errors of the standard naïve test were significantly different ($P < 0.001$) from control test 2, post 20% MVC and 100% MVC tests, at all intensities (Fig. 1a). The relative peak force errors of the standard naïve test was significantly different ($P < 0.001$) from control tests 1 and 2, post 20% MVC and 100% MVC tests at all intensities (Fig. 1b). Subjects consistently over produced isometric force (perceptually underestimated) at 25% MVC, except during the standard naïve test. Isometric force at 50% and 75% MVC intensities was lower on most occasions than equivalent percent values of the target contraction intensities (perceptual overestimation). Subjects tended to be less accurate at matching absolute force with the target contraction intensities at extreme ends of the force domain and the most accurate at 50% MVC (Table 3).

Discussion

The most important finding of the present study was that isometric force, preceding a maximal voluntary contraction (used as an anchor), was most accurate at the low perceptual intensity and significantly under produced (perceptually overestimated) at higher perceptual intensities. Subsequent exposure to a 100% MVC resulted in a better judgement of force during voluntary contractions at 50% and 75% MVC guided by the subjects' perceptual feelings of exertion. Matching isometric force with target contraction intensities tended to be less accurate at opposite ends of the force domain (25% and 75% MVC) during all five experimental conditions. In addition, the prior submaximal and maximal fatiguing isometric exercise did not affect the subjects' subsequent perceptual response range.

Absolute force produced during the standard naïve test, where the maximal voluntary contraction (100% MVC) was performed last, was the most accurate at 25% MVC but significantly lower than the isometric force produced at the 50% and 75% contraction intensities during the other four test days. It is unclear why subjects were able to accurately judge force at the low contraction intensity (25% MVC). These results do however suggest, that a perceptual range at higher contraction intensities was not established with no prior knowledge of one's maximal force generating capacity and therefore a dissociation exists between effort perception and force output resulting in an under production of force (perceptual overestimation) at submaximal contraction intensities (50% and 75% MVC). Horstman

Table 2 The relative peak isometric force at the target contraction intensities during the standard naïve test, control tests 1 and 2, post 20% MVC test and post 100% MVC test of the experimental sample ($N = 30$)

Contraction intensity (%)	Standard naïve test (%)	Control test 1 (%)	Control test 2 (%)	Post 20% MVC test (%)	Post 100% MVC test (%)
25	24.9 ± 12.1	31.6 ± 15.3	34.5 ± 14.5	39.1 ± 12.0	37.6 ± 12.2
50	39.1 ± 16.2	45.0 ± 13.6	48.1 ± 12.4	53.0 ± 15.4	52.7 ± 13.4
75	54.3 ± 16.0	62.0 ± 23.4	56.6 ± 20.6	62.0 ± 14.3	65.3 ± 14.2

All values are expressed as mean ± SD
Relative peak force is reported as a percentage (%) of the absolute peak force at each perceived contraction intensity

Main effects, intensity: $P = 0.00001$
Main effects, trial: $P = 0.00001$

et al. (1979c) suggest that although pre-exercise warm up may not influence the results, previous experience of the exercise may be necessary to accurately judge effort perception. In contrast, Hutton et al. (1984) proposed that transient postcontractile potentiation of spinal reflex pathways may summate with previously set motor commands or act independently to produce errors in effort perception.

The ability to accurately judge force based on perceptual feelings of exertion had a tendency to be poorer at opposite ends of the force domain where force was over produced at 25% MVC and consistently under produced at 75% MVC. The most accurate judgement of isometric force occurred at 50% MVC. The present finding is in agreement with previous research by Jones and Hunter (1982) who showed that forces under 40% MVC were consistently over produced, forces above 60% MVC under produced and the most accurate occurred around the middle of the force domain. Similar results were also found by Kumar and Simmonds (1994) who used a pinch grip, power grip and stoop lifting activity to measure accuracy and the reliability of effort perception during these activities. This study found a systematic bias at all submaximal force contractions except at 40% MVC. The force at 60% and 80% MVC was lower whereas at 20% MVC was higher than the objective values based on the MVC. Jackson and Dishman (2000) measured perceived submaximal force production using a chest press exercise in the order of 25%, 50%, 75% of maximum and a final MVC, and reported an over production of force at 25% MVC and lower forces produced at both 50% and 75% MVC when compared to the expected force production. It appears that the consistency of this result occurs whether the contraction intensities are presented in either a linear fashion (Jackson and Dishman 2000) or in a randomised manner (Jones and Hunter 1982; Kumar and Simmonds 1994; Pincivero et al. 2003a).

Table 3 The absolute and relative peak force errors during all five experimental conditions at the target contraction intensities of the experimental sample ($N = 30$)

Contraction intensity (%)	Absolute peak force error (Force in N)	Relative peak force error (Force as a %)
25	53.68	8.54
50	12.06	2.42
75	−91.20	−14.98

+ represents underestimation of force − represents overestimation of force

The reason for this perceptual over-and-underestimation is not immediately clear. It is possible that since the large musculature of the leg extension is more commonly involved in gross movements relating to power, it is relatively insensitive in identifying and performing the lower levels of force such as those required in fine movements thus resulting in perceptual underestimation (Jones and Hunter 1982). The reason for perceptual overestimation may be a teleological one, in that subjects subconsciously under produce force at higher intensities as a protective mechanism, in order to maintain a reserve capacity and therefore prevent mechanical and metabolic damage which may occur from maximal force generation. Another explanation for the perceptual inaccuracies may be the absence of an anchoring process, which allows the subjects to cognitively establish a perceptual range by a low and a high reference (Pincivero et al. 2003a, 2003b)

In the present study we introduced two different fatiguing protocols, a maximal 100% MVC and a submaximal 20% MVC fatigue test. Central and peripheral fatigue has been shown to develop during maximal and submaximal sustained voluntary muscle contractions (Bigland-Ritchie et al. 1983). Isometric exercise was used during the fatigue test to be consistent with the test protocol that followed. It has previously been found that muscle fatigue impairs the ability of subjects to make judgements of force (Gandevia and McCloskey 1978; Jones and Hunter 1983a), as well as increases effort perception (Jones and Hunter 1983b). Our results however, suggest that a previous bout of fatiguing isometric exercise did not impair the subjects' subsequent ability to match isometric force with target contraction intensities.

A possible explanation for the contradiction in results may be the difference in the methodology with regard to the fatigue tests. A 10 min rest interval was given to subjects in the present study after the fatiguing isometric exercise before performing the test protocol. Rest intervals of 40 s compared to 160 s (Pincivero

et al. 1999), as well as 1–3 min (Woods et al. 2004) between exercise bouts have shown no effect on perceived exertion, and therefore demonstrates, that perceived exertion may not be affected by the rest interval length, or may not be a sensitive indicator of muscle recovery. It has been concluded however, that rest intervals of 2 – 4 min are adequate for minimising muscle fatigue (Pincivero et al. 1999). The length of recovery time in the present study was therefore too long for the physical effects of the fatigue tests to have an affect on the perceptual range. In addition, the prior experience of the fatigue did not have an affect on the ability to match absolute force to the target contraction intensities in the present study.

In conclusion, subjects were most accurate at the low perceptual intensity but under-produced (perceptually overestimated) isometric force during the higher contraction intensities preceding a maximal voluntary contraction (100% MVC). Subsequent exposure to a maximal voluntary contraction enabled subjects to establish an improved perceptual range during voluntary contractions at 50% and 75% MVC that resulted in a better judgement of force guided by perceptual feelings of exertion. The ability of subjects to match absolute force with target contraction intensities during all five testing conditions was most accurate at 50% MVC and had a tendency to be poorer at opposites ends of the force domain. Furthermore, it was clearly observed that prior fatiguing isometric exercise did not change the subjects subsequent perceptual response range, irrespective of whether it was submaximal or maximal fatiguing exercise. Further work however, is required to investigate the cause of perceptual overestimation at the higher contraction intensities and the acute effects of fatigue on perceived exertion using the magnitude production method.

Acknowledgements The Medical Research Council of South Africa, the Nellie Atkinson and the Harry Crossley Staff Research Funds of the University of Cape Town, Discovery Health and the National Research Foundation of South Africa, through the THRIP initiative, provided financial assistance for this study

References

Bigland-Ritchie B, Johansson R, Lippold OC, Smith S, Woods JJ (1983) Changes in motoneurone firing rates during sustained maximal voluntary contractions. J Physiol 340:335–346

Borg GA (1982) Psychophysical bases of perceived exertion. Med Sci Sports Exerc 14:377–381

Cafarelli E, Bigland-Ritchie B (1979) Sensation of static force in muscles of different length. Exp Neurol 65:511–525

Cooper DF, Grimby G, Jones DA, Edwards RH (1979) Perception of effort in isometric and dynamic muscular contraction. Eur J Appl Physiol Occup Physiol 41:173–180

Enoka RM, Stuart DG (1992) Neurobiology of muscle fatigue. J Appl Physiol 72:1631–1648

Gandevia SC, McCloskey DI (1978) Interpretation of perceived motor commands by reference to afferent signals. J Physiol 283:193–199

Hampson DB, St Clair GA, Lambert MI, Noakes TD (2001) The influence of sensory cues on the perception of exertion during exercise and central regulation of exercise performance. Sports Med 31:935–952

Horstman D, Kowal D, Vaughan L, Stivanelli A (1979e) The influence of previous physical experience on the perception of work effort. Med Sci Sports Exerc 11:79

Hutton RS, Enoka RM, Suzuki S (1984) Activation history and constant errors in human force production. Brain Res 307:344–346

Jackson AW, Dishman RK (2000) Perceived submaximal force production in young adult males and females. Med Sci Sports Exerc 32:448–451

Jones LA, Hunter IW (1982) Force sensation in isometric contractions: a relative force effect. Brain Res 244:186–189

Jones LA, Hunter IW (1983a) Effect of fatigue on force sensation. Exp Neurol 81:640–650

Jones LA, Hunter IW (1983b) Perceived force in fatiguing isometric contractions. Percept Psychophys 33:369–374

Kumar S, Simmonds M (1994) The accuracy of magnitude production of submaximal precision and power grips and gross motor efforts. Ergonomics 37:1345–1353

Pandolf KB (1983) Advances in the study and application of perceived exertion. Exerc Sport Sci Rev 11:118–158

Pincivero DM, Gear WS (2000) Quadriceps activation and perceived exertion during a high intensity, steady state contraction to failure. Muscle Nerve 23:514–520

Pincivero DM, Gear WS, Moyna NM, Robertson RJ (1999) The effects of rest interval on quadriceps torque and perceived exertion in healthy males. J Sports Med Phys Fitness 39:294–299

Pincivero DM, Coelho AJ, Erikson WH (2000) Perceived exertion during isometric quadriceps contraction: A comparison between men and women. J Sports Med Phys Fitness 40:319–326

Pincivero DM, Coelho AJ, Campy RM, Salfetnikov Y, Bright A (2001) The effects of voluntary contraction intensity and gender on perceived exertion during isokinetic quadriceps exercise. Eur J Appl Physiol 84:221–226

Pincivero DM, Coelho AJ, Campy RM, Salfetnikov Y, Suter E (2003a) Knee extensor torque and quadriceps femoris EMG during perceptually-guided isometric contractions. J Electromyogr Kinesiol 13:159–167

Pincivero DM, Dixon PT, Coelho AJ (2003b) Knee extensor torque, work, and EMG during subjectively graded dynamic contractions. Muscle Nerve 28:54–61

Pincivero DM, Coelho AJ, Campy RM (2004) Gender differences in perceived exertion during fatiguing knee extensions. Med Sci Sports Exerc 36:109–117

Robertson RJ, Noble BJ (1997) Perception of physical exertion: methods, mediators, and applications. Exerc Sport Sci Rev 25:407–452

Tihanyi J, Apor P, Fekete G (1982) Force-velocity-power characteristics and fiber composition in human knee extensor muscles. Eur J Appl Physiol Occup Physiol 48:331–343

Woods S, Bridge T, Nelson D, Risse K, Pincivero DM (2004) The effects of rest interval length on ratings of perceived exertion during dynamic knee extension exercise. J Strength Cond Res 18:540–545