

**METABOLIC AND HORMONAL RESPONSES TO ALTERED
CARBOHYDRATE AVAILABILITY AND ITS EFFECT ON FATIGUE
DEVELOPMENT**

By

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Chapter 2 of this thesis represents research conducted as part of my first year of post-graduate research. The idea and supervision of this study were provided by Prof. Louise Burke (Head of Dept. Sports Nutrition, Australian Institute of Sport). Hence, the contents of chapter 2 as presented in this thesis, represent the work conducted and written by myself under the supervision of Prof. Louise Burke.

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As noted above, Chapter 2: Prelude formed part of my first year of post-graduate research (as part of my B.Sc(Med)(Hons)Exercise Science degree) and has been included as a prelude chapter (Chapter 2) as it formed the basis for the hypothesis of my PhD dissertation. Note that over and above chapter 2, there are 6 additional experimental chapters that form the body of this dissertation

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THESIS ABSTRACT

The main aims of the series of studies comprising this thesis were to investigate the effect of altered endogenous carbohydrate (CHO) availability, achieved primarily by pre-exercise dietary manipulation and antecedent exercise exposure, on interindividual variability in metabolic and hormonal responses to dynamic, steady-state exercise. Further, this thesis examined the impact of altered blood glucose availability on fatigue development during prolonged exercise. In this regard, it was hypothesized that endogenous CHO availability and the associated metabolic sequelae would impact on effort perception during exercise and fatigue development. It was further hypothesized that prior fatiguing exercise and local muscle glycogen depletion would result in a neural or humoral signal, or both, that would alter resistance to fatigue. Finally, it is proposed that antecedent exposure to hypoglycemia would reduce neuroendocrine and metabolic responses during subsequent (next-day) prolonged exercise, and that this would translate into reduced effort perception and improved exercise performance.

In the first study (**Chapter 2**) the effect of varying the glycemic index (GI) of the pre-exercise carbohydrate-rich meal, combined with CHO ingestion during exercise on metabolism and endurance exercise performance was examined. Varying the GI of the preexercise CHO-rich meal is known to alter substrate availability and hormonal responses (in particular insulin), and has been proposed to be of benefit to athletes in that it may improve endurance performance. In this study, it was hypothesized that a low GI preexercise meal would reduce postprandial hypoglycemia and hyperinsulinemia compared to a high GI meal, but when combined with the ingestion of relatively large amounts of CHO during exercise, would have no impact on exercise metabolism and performance. Seven, well-trained cyclists received, in a randomized, cross-over design, a low GI meal (pasta, LGI), or high GI meal (potato, HGI) providing 2 g CHO/kg body mass (BM) or artificially flavored jelly (placebo) 2 h before cycling at steady-state for 2 h at 70% of maximal oxygen consumption ($VO_{2\text{ max}}$). This was followed by a cycling time-trial (TT) consisting of time to complete 300 kJ of work. Subjects ingested a total of 24 ml/kg BM of a 10% glucose polymer solution labeled with [$U\text{-}^{14}\text{C}$]glucose immediately before and during the steady-state cycle. Despite differences in preexercise glucose, insulin and free fatty acid (FFA) concentrations between LGI and HGI trials, as soon as exercise commenced, these differences were negated. Varying the GI of the pre-exercise meal had no distinguishable effect on tracer-determined rate of exogenous CHO oxidation, or respiratory exchange ratio (RER); nor was exercise performance or rating of perceived exertion (RPE) altered. There was, however, a large range in variability in postprandial metabolic responses between individual subjects. It was concluded that when CHO is ingested during endurance exercise in amounts recommended by sports nutrition guidelines, then varying the GI of a preexercise CHO meal and the concomitant metabolic and hormonal responses it elicits have no effect on endurance (~2.5 h) exercise metabolism or performance.

The interindividual variation in postprandial glycemic and insulinemic responses observed in the first study, combined with that noted in the GI literature, raised a question over the validity of using the GI in predicting glycemic-insulinemic responses, particularly in a trained population group where insulin sensitivity (and other metabolic and hormonal responses) are likely to be altered. Determination of the GI of CHO-rich foods have traditionally only been done on sedentary population groups, and large intra- and interindividual variability in blood glucose responses have been noted. Therefore, the first study was extended to investigate the effect of training status on intra- and interindividual variability in glycemic response after ingestion of the GI reference food (white bread, providing 50 g of available CHO), when food- and methodologically-related factors are stringently controlled for (presented under **Addendum A**). This study indicated that training status (and exercise) have little impact on altering the large inter- and intra-individual variability in glycemic response seen after ingestion of the GI reference (white bread). The large intra- and interindividual variability highlights the need for further investigation surrounding the use of the GI as a credible, reproducible clinical tool for use in nutrition intervention.

The third study (**Chapter 3**) aimed to examine the role of local muscle glycogen depletion on fatigue development. A single limb exercise model was used to explore whether fatigue development, combined with local muscle glycogen depletion and the concomitant metabolic and neurohumoral responses elicited during exercise with the first limb affect exercise capacity of the second limb. To our knowledge, previous studies employing dynamic single limb protocols have not documented the effect on RPE and fatigue development when exercise is performed in a sequential manner (i.e. 1 leg immediately after the other). It was hypothesized that exercise and concomitant local muscle glycogen depletion will result in a neural or hormonal signal, or both, that would cause a reduction in exercise capacity of the second, previously rested limb. Six trained men performed a single trial consisting of cycling to exhaustion at 30% of peak sustainable workload with one leg at a time. At the point of exhaustion of each leg, a muscle biopsy was performed to assess muscle glycogen content. In an attempt to maintain plasma glucose concentrations during exercise, 300 ml of a 10% glucose polymer drink was ingested 30 min into the exercise time of each leg. Exercise time to fatigue was shorter with Leg 2 (55 ± 5 min) compared to Leg 1 (60 ± 6 min, $p=0.03$), whilst the muscle glycogen content at exhaustion in Leg 2 was higher in Leg 2 (51 ± 21 mmol/kg wet weight (w.w)) compared to Leg 1 (43 ± 25 mmol/kg w.w., $p=0.04$). In contrast to Leg 1, FFA concentrations during exercise with Leg 2 increased over time ($p<0.000$) and were higher than that of Leg 1 from at 45 min onwards ($p<0.05$), whilst RER-determined fat oxidation remained similar between legs. Epinephrine (0.7 ± 0.6 vs. 0.2 ± 0.2 nmol/L, $p=0.06$) and norepinephrine (4.1 ± 1.5 vs. 2.1 ± 0.4 nmol/L, $p=0.02$) concentrations were higher at the start of exercise with Leg 2 compared to Leg 1, respectively. Epinephrine concentrations remained higher at 30 min of exercise (1.3 ± 0.4 vs. 0.7 ± 0.4 nmol/L, $p=0.002$) in Leg 2 compared to Leg 1, respectively. In contrast, heart rate, VO_2 and

RER remained constant over time and similar between legs. The rate of increase in RPE was similar between legs when expressed as % of elapsed time. However, since the exercise time was shorter in Leg 2, the absolute rate of increase in RPE in leg 2 was faster. It was concluded that the difference in exercise capacity between legs was unrelated to muscle glycogen depletion or development of hypoglycemia, but may be related to increased sympathetic nervous system activation at the onset of exercise, and a faster absolute rate of increase in RPE.

Whilst the previous study examined the acute or instantaneous effect of local muscle glycogen depletion on exercise metabolism and performance during subsequent exercise, we sought to further examine the role of glycogen depletion, as achieved by glycogen-depleting exercise followed by a low CHO diet, on exercise metabolism and performance when combined either with or without exogenous CHO supplementation (Chapter 4). This allowed the opportunity to examine the effect of maintenance of glucose (fuel) supply compared to declining blood glucose concentrations on metabolism and exercise capacity in the face of reduced endogenous CHO stores. It was hypothesized that the declining blood glucose concentrations would reduce CHO oxidation and limit exercise capacity, whereas maintenance of euglycemia would maintain CHO oxidation and thereby enable subjects to complete the exercise task despite reduced muscle glycogen availability. In random order, 1 wk apart, 9 endurance-trained men underwent euglycemic (CI) or placebo (PI) clamps, whilst performing up to 150 min of cycling at 70% $\text{VO}_{2\text{max}}$, after following a low CHO diet for 48 h prior to the trial. The range in improvement in endurance capacity with glucose infusion was large ($28 \pm 26\%$, $p < 0.05$). The majority ($n=5$ of 9) subjects in CI failed to complete 150 min of exercise despite maintenance of euglycemia, whilst only 2 subjects in PI completed 150 min of exercise, despite being hypoglycemic (< 3.5 mmol/L). Total CHO oxidation, blood lactate and cortisol concentrations remained unaltered despite glucose infusion. Plasma glucose oxidation and insulin concentrations were elevated ($p < 0.05$) only after the 80 min timepoint in CI. Muscle glycogen concentrations were equally low at the start (~ 80 mmol/kg w.w.) and end (~ 40 mmol/kg w.w.) of exercise in both trials. Hence, despite a longer exercise time in CI, the absolute amount of muscle glycogen used was similar between trials, perhaps suggesting muscle glycogen sparing with glucose infusion. Serum FFA concentrations at exhaustion were strongly correlated to endurance time in both the PI ($r=0.87$) and CI ($r=0.83$; $p < 0.005$) trials.

It was concluded that the maintenance of glucose concentrations at 5 mmol/L in the CHO depleted state may have an ergogenic effect, however, the effect is highly variable between subjects and independent of changes in the rate of total CHO oxidation. Further, despite maintenance of euglycemia in a glycogen-depleted state, the majority of these endurance-trained subjects still could not complete 2.5 h of exercise, suggesting that some other aspect, most likely related to intra-hepatic or intra-muscular glycogen depletion and unrelated to fuel availability *per se*, could have contributed to fatigue development. These results suggests, but do not establish, that those

subjects with superior endurance capacity under glycogen-depleted conditions, are somewhat resistant to the potential ergolytic effects of declining blood glucose concentrations, and have a superior ability to utilize alternative fuel sources such as FFA's.

Recurrent exposure to hypoglycemia has been shown to reduce gluco-counterregulatory responses during subsequent exposures and reduce subjective awareness of hypoglycemia, termed "hypoglycemia unawareness". It is not uncommon for endurance athletes to develop hypoglycemia during prolonged exercise with insufficient exogenous CHO supplementation. Hypoglycemia unawareness may explain the superior endurance capacity of the 2 subjects in the previous study who had the longest endurance times despite being hypoglycemic. However, to our knowledge, this phenoma has not been investigated in healthy, well-trained individuals.

To explore the hypothesis of hypoglycemia unawareness and counterregulatory failure, the final study (Chapter 5) aimed to determine the effect of an antecedent exposure to two 80-min bouts of hypoglycemia (separated by 40 min of euglycemia), on the concomitant gluco-counterregulatory, symptomatic and cognitive function responses during each bout of hypoglycemia in healthy, well-trained men. Furthermore, the impact of antecedent exposure to hypoglycemia on next-day metabolic, neuroendocrine and cytokine (IL-6 in particular, Chapter 6) responses during prolonged exercise, and how these responses relate to exercise performance, RPE, and cognitive function were assessed. Particularly, it was hypothesized that antecedent exposure to hypoglycemia would reduce neuroendocrine and metabolic responses to prolonged exercise, and in addition, reduce effort perception and thereby improve exercise performance.

In this study, 10 healthy, trained men completed two, 2-day trials in random, counter-balanced order, repeated at least one month apart. On day 1, a hyperinsulinemic glucose clamp procedure was used to expose subjects to either 2 consecutive 80-min bouts of hypoglycemia (2.9 mmol/L) separated by 40 min of euglycemia (HYPO trial), on the other occasion, euglycemia was maintained throughout (5.2 mmol/L, EU trial). On day 2, subjects cycled for 90 min at 70% of $VO_{2\max}$, with a 10 kJ (~20 sec) sprint performed every 15 min after blood and RER sampling. At the end of 90 min, a cycling TT was performed consisting of time to complete 200 kJ of work. All experiments were performed after a 10-12 h overnight fast. Water (only) was ingested *ad libitum* during the experiments. Cortisol concentrations and symptomatic responses were significantly higher during the day 1 HYPO compared to EU trial, indicative of normal counterregulation and symptomatic awareness. There was a strong positive relation between individual cortisol response and symptomatic awareness i.e. those subjects with a low symptom score rating ("hypoglycemic unaware") also had a reduced cortisol response during Day 1 HYPO. During the day 2 exercise trial, plasma glucose concentrations were maintained (>4.5 mmol/L), and isotope-determined hepatic glucose output, RER, insulin, cortisol, and IL-6 responses were comparable between

antecedent HYPO and EU exposure. Individual assessment of the data revealed that 7 (of 10) subjects' average sprint times and 6 (of 10) subjects' TT performance were improved after antecedent HYPO vs. EU exposure, however, these differences were not statistically significant.

These results indicate that prior exposure to hypoglycemia (and stimulation of the gluco-counterregulatory response) in healthy, well-trained men has no effect on metabolic and neuroendocrine responses to a subsequent bout of hypoglycemia, nor to exercise performed within 24 h, and that the ability to regulate blood glucose homeostasis is maintained. Furthermore, antecedent exposure to hypoglycemia has no impact on next-day exercise performance, RPE or cognitive performance.

A common finding in studies in which cognitive function is impaired due to fatigue or alcohol intoxication is that slowing of responses on motor and attention tasks is accompanied by significant increases in the variability of responses. It has been demonstrated that changes in mean estimates of response speed between groups or between assessments arise as a consequence of an increased proportion of slow responses (or increased 'trial-to-trial variability') rather than any change in the maximum speed of responses. The contribution of alterations in response variability to the commonly observed slowing of motor and attentional speed during hypoglycemia is yet to be investigated. Hence, by using the cognitive data collected in the previous study, we sought to determine whether cognitive slowing commonly observed during hypoglycemia is associated with an increase in the proportion of slow responses in the reaction time distribution (i.e. increased skewness) (presented under **Addendum B**). The data indicated that the response slowing commonly observed on choice reaction time tasks in hypoglycemic individuals is not associated with increased skewness in the reaction time distribution. Rather, such dysfunction appears to result from a general slowing of responses.

In summary, in accordance with our original hypothesis this thesis demonstrated that when commencing exercise with normal glycogen stores combined with CHO ingestion during exercise, the altered metabolic (glucose, FFA) and hormonal (insulin) milieu elicited by preexercise CHO meals with differing GI is rapidly negated and has no impact on exercise metabolism and performance. However, it was demonstrated that there is a large variability in postprandial glucose responses between and within individuals. This variability may explain the inconsistent findings noted in the literature on the metabolic and performance effects of preexercise CHO intake, and questions the validity and clinical "usefulness" of the GI, particularly in sports nutrition education.

It was also demonstrated that there is an individual range in sensitivity to declining blood glucose concentrations, which in turn differentially affects fatigue development. In addition, though this thesis supports evidence on the ergogenic effect of CHO supplementation, it indicates that the

ergogenic potential is highly variable between subjects, specifically when exercising in a CHO-depleted state. In contrast to the original hypothesis, CHO oxidation was similar in placebo and glucose infusion trial, and maintenance of glucose availability with glucose infusion did not enable the majority of well-trained subjects to complete 2.5 h of exercise in a glycogen depleted state. Hence, in contrast to what is commonly been suggested in the literature, these results indicate that ergogenic effect of CHO cannot simply be explained by the maintenance of euglycemia and CHO oxidation at a time when muscle glycogen concentrations are low. These data are in support of a different mechanism whereby glycogen content *per se* may affect fatigue development.

Finally, it was demonstrated that exposure to acute bouts of hypoglycemia in well-trained athletes at rest results in a significant neuroendocrine and IL-6 response, and that individual symptomatic responsiveness and aspects of cognitive performance were significantly correlated to the cortisol response. Furthermore, prior exposure to hypoglycemia in well-trained athletes did not alter their ability to mount gluco-regulatory metabolic and neuroendocrine responses needed for effective maintenance of glucose homeostasis during subsequent (next-day) prolonged exercise, neither did it alter perceived exertion and exercise performance during or cognitive function immediately after prolonged exercise.

THESIS FORMAT

This thesis is presented in the form of a literature review (CHAPTER 1), followed by the presentation of the findings from 5 primary studies, presented as CHAPTER 2, 3, 4, 5, 6 and Addendums A and B. CHAPTER 7 contains the overall conclusions of the thesis, and CHAPTER 8 contains the list of references used throughout the thesis.

The two experimental chapters presented as Addendum A and B play an important role in the evolution of this thesis. Addendum A highlights the important methodological implications of the variability in postprandial glycemic responses, and further the interpretation of the findings from studies involving preexercise CHO ingestion. Addendum B furthers our understanding of short-term repeated bouts of hypoglycemia on cognitive function. These two chapters are placed as addendums at the end so that the flow of the dissertation and central hypotheses are not impeded.

Each of the study chapters and addendums are presented in the form of a scientific journal publication with added detail, and in the format of an introduction (brief literature overview and stating the aims of the investigation), followed by a Methods, Results and Discussion section.

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LIST OF ABBREVIATIONS

% - percentage	SA – specific activity
* - significant difference between treatments	SD – standard deviation
# - significant increase compared to baseline	sec – seconds
ACTH – adrenocorticotrophic hormone	SNS – sympathetic nervous system
AMPK – 5'AMP-activated protein kinase	SR – sarcoplasmic reticulum
ANOVA – analysis of variance	SRT – simple reaction time
ATP – adenosine triphosphate	TT – time-trial
BCAA – branched-chain amino acid	VCO ₂ – carbon dioxide production
BM – body mass	VO ₂ – oxygen consumption
BMI – body mass index	VO _{2max} – maximal oxygen consumption
Ca ²⁺ - calcium	wk – week
CaCl – calcium chloride	wks - weeks
CHO – carbohydrate	W _{peak} – maximal sustained power output in Watts
ChRT – choice reaction time	w.w. – wet weight
CI – euglycemic glucose infusion trial (chapter 4)	
CON – control trial (chapter 2)	
CNS – central nervous system	
CV – coefficient of variation	
dpm – disintegrations per minute	
EU – euglycemic clamp trial (chapters 5 & 6)	
FFA – free fatty acid	
Fig. - figure	
G-6-P - glucose 6-phosphate	
GLUT-4 – glucose transporter 4	
GI – glycemic index	
h – hour	
hrs - hours	
HCl – hydrochloric acid	
HClO ₄ – perchloric acid	
HCO ₃ - bicarbonate	
HGI – high glycemic index trial (chapter 2)	
HPLC – high performance liquid chromatography	
HYPO – hypoglycemic clamp trial (chapters 5 & 6)	
IL-6 – interleukin 6	
K ₂ CO ₃ – potassium carbonate	
LGI – low glycemic index trial (chapter 2)	
L - liter	
min – minutes	
NaOH – sodium hydroxide	
NS – Not significant	
p – level of significance	
PDH – pyruvate dehydrogenase	
P _i – inorganic phosphate	
PI – placebo (saline) infusion trial (chapter 4)	
R _a – rate of appearance	
R _d – rate of disappearance	
RER – respiratory exchange ratio	
RPE – rating of perceived exertion	
rpm – revolutions per minute	
RQ – respiratory quotient	
RT – reaction time	

CHAPTER 1

LITERATURE REVIEW

University of Cape Town

1.1 INTRODUCTION

Maintenance of blood glucose concentrations is essential to ensure an adequate supply of substrate to the glucose-dependent brain, thereby maintaining cerebral metabolism and cognitive function³³⁴. Contracting skeletal muscle is able to use carbohydrate (CHO) and fat-derived substrates to sustain prolonged exercise. With an increase in exercise intensity, there is a linear increase in contraction-induced muscle glycogenolysis and increased sympathetic nervous system activity, resulting in a shift in the balance of substrate utilization from fat to CHO⁴⁷. Hence, muscle glycogen and blood-borne glucose are the most heavily metabolized fuels during exercise performed at moderate to high intensities (>60 $VO_{2\text{ max}}$), accounting for at least 60 to 70% of the total energy requirement^{89,360,365,366}. However, relative to fat, the CHO stores in the body are limited²⁰ and depletion of these CHO reserves may ultimately result in a reduction in blood glucose concentration⁶⁷ and glucose supply to the working muscle as well as the glucose-dependent brain, which will challenge a series of glucoregulatory systems to restore euglycemia³²⁵. The full extent and interplay of these systems are not yet fully understood, especially as they operate during prolonged exercise; how it is influenced by short- and long-term nutrition intervention (known to alter metabolic and hormonal responses), and ultimately how exercise performance may be affected.

Decades of research in the field of carbohydrate (CHO) metabolism during prolonged exercise have clearly demonstrated that CHO supplementation during prolonged (>90 min), moderate to high-intensity ($>60\%$ $VO_{2\text{ max}}$) exercise can delay the development of fatigue^{19,67,73,74,89,91}. The ergogenic effect of CHO has mainly been ascribed to the prevention of hypoglycemia and the maintenance of high rates of CHO oxidation throughout exercise^{73,91}. These findings have stimulated a wave of research investigating nutritional strategies to optimize bodily CHO fuel stores as well as formulating effective nutritional strategies for CHO supplementation during prolonged exercise to optimize exercise performance (for reviews see^{76,220}). However, the exact mechanisms whereby CHO metabolism is regulated, the interplay between fuel substrates, and the manner in which CHO affects endurance exercise capacity, still remain to be fully elucidated.

Closer inspection of the literature reveals that CHO supplementation does not merely enhance performance via maintenance of euglycemia and high rates of CHO oxidation throughout exercise, as several studies have reported similar levels of glycemia and CHO oxidation between CHO supplemented and placebo trials (e.g. refs.^{35,38,440,441}). Indeed, in a very recent study, simply rinsing the mouth with a CHO solution then spitting it out appeared to have an effect on attenuating fatigue development during a 1-h cycle time-trial performance⁶¹. In addition, there are studies in which the development^{87,146,228,230,312}, prevention¹⁴⁶ or reversal⁷³ of hypoglycemia had no significant effect on exercise performance. The ergogenic effect of CHO

supplementation between individuals also does not seem to be uniform as a range of metabolic responses and variable effects on performance within a study population are often seen. This seems to hold true even in apparently homogenous study populations (i.e. controlled for training status, body mass, age).

This literature review will aim to characterize and discuss factors known to be involved in the regulation of CHO metabolism and glucose homeostasis during prolonged exercise, and specifically relate changes in CHO availability and the metabolic sequelae during exercise with resistance to fatigue.

1.2 FACTORS AFFECTING GLUCOSE HOMEOSTASIS

Glucose homeostasis is essential in order to ensure an adequate supply of substrate to the glucose-dependent brain. Therefore, blood glucose concentrations are tightly regulated with often redundant mechanisms of control, ostensibly to prevent hypoglycemia and associated sequelae.

Section 1.2 will focus on factors affecting glucose homeostasis predominantly under resting conditions, and section 1.4 will focus on the key factors involved in the regulation of glucose homeostasis during prolonged exercise.

1.2.1 *The glucose counterregulatory response*

In both fed and fasted states, insulin is one of the key factors that regulates glucose production and use. Insulin reduces endogenous glucose production, mainly by reducing hepatic glucose output via an increase in glycogen synthase and decrease in glycogenolysis³⁸². On the other hand, glucoregulatory hormones including glucagon, epinephrine, cortisol and growth hormone, all have biologic effects that can oppose those of insulin²⁷.

When blood glucose concentrations decline, a series of counterregulatory responses are elicited in hierarchical fashion in order to restore euglycemia, mainly in aid of preserving brain function³⁰⁹. With an initial 0.6-0.8 mmol/L decline in blood glucose (below postabsorptive levels) to ~4.0-4.2 mmol/L, the first counterregulatory response involves suppression of endogenous insulin and an increase in glucagon production in aid of stimulating endogenous glucose production¹¹⁰. Thus, hypoglycemia may be defined as any decrease in plasma glucose concentration below 4.0 mmol/L²⁷. The release of other counterregulatory hormones occurs after an additional similar decrease in plasma glucose to approximately 3.7 mmol/L^{138,309,371}.

There is evidence to suggest that the reduction in insulin secretion may be via a local response within the pancreatic islets, which responds directly to the reduction in blood glucose and also to central neurogenic signals⁴⁰⁵. The control of glucagon secretion is less clear and remains controversial. There is evidence to suggest that local factors within the pancreas may be responsible for glucagon secretion by the alpha cells. It has been suggested that the pancreatic alpha cell may respond directly to the prevailing glucose concentration¹⁰⁶, and that the glucagon-secreting pancreatic alpha cells may respond to the insulin secretory response of the beta cells, however, evidence to support this is currently lacking (for review see³⁸⁵). In addition, there is also evidence for central involvement in the glucagon response via activation of the sympathetic¹⁶⁸ and parasympathetic²⁴ nervous systems. In further support of central control of glucagon secretion, neuroglycopenia has been shown to result in elevations in epinephrine and glucagon concentrations in aid of increasing systemic glucose production and glucose supply to the brain⁴⁰⁵. Furthermore, studies in dogs in which cerebral normoglycemia was maintained whilst inducing systemic hypoglycemia have shown a large attenuation in the glucagon-epinephrine counterregulatory hormone response^{17,157}. Glucagon and epinephrine are referred to as “rapid-acting” hormones critical for glucose counterregulation in the early phase of hypoglycemia²⁷.

When the initial insulin-glucagon response fails to restore blood glucose and concentrations decline further to ~ 3.7 mmol/L, sympathetic activation and epinephrine secretion (both under neurological control) result in peripheral and hepatic responses to augment glucose concentrations via stimulation of glycogenolysis and gluconeogenesis²⁷. In addition, lipolysis is stimulated, resulting in enhanced free fatty acid release, thereby providing substrate for gluconeogenesis, whilst peripheral glucose uptake is reduced¹⁵⁷. The release of growth hormone and adrenocorticotrophin hormone contribute to endogenous glucose production and reduce peripheral glucose uptake^{111,112}. Their secretion and peripheral effects to augment glucose concentrations typically show a delay in response (also referred to as “slow-acting” hormones), but their effects are longer-lasting and important in the long-term maintenance of blood glucose concentrations²⁷. As part of the counterregulatory response, a complex of hypoglycemic symptoms are elicited (for review see^{27,385}). Failure of these responses to correct hypoglycemia may reduce glucose supply to the brain, which may alter cerebral metabolism and impair cognitive function³³⁴.

1.2.2 Glucose sensing

Based on the involvement of the sympathetic centers and hypothalamic-pituitary-adrenal (HPA) axis in aid of correcting declining blood glucose concentrations, it seems clear that the series of responses initiated are largely centrally mediated³⁸⁵. Furthermore, seeing that glucose supply to

the brain is so critical for sustaining normal cerebral function, it has been suggested that the brain would be the main organ for sensing reductions in blood glucose concentrations³⁸⁵.

1.2.2.1 *The role of the brain as primary "glucose-sensor"*

Numerous studies indicate that the brain is the prominent organ or center for the sensing of hypoglycemia (for review see²⁷). Dogs in which insulin-induced hypoglycemia was induced peripherally whilst euglycemia was maintained in the brain, displayed near complete attenuation of counterregulatory responses compared to responses in dogs with brain neuroglycopenia^{17,157}. These findings have lent support to the presence of a glucose sensor in the region of the cerebral and/or vertebral arteries. In contrast, other similar studies concluded that the response to moderate⁵⁷ or deep⁵⁸ hypoglycemia did not involve putative hypothalamic glucoreceptors nor receptors in the carotid perfused tissue. These studies^{57,58} did, however, show some reduction in the epinephrine response to hypoglycemia.

Recently, much effort has been invested in determining which specific area(s) of the brain may be involved in glucose-sensing. Livingston et al.²⁸¹ found that the glucose transporter isoform, GLUT-4, is expressed in the hypothalamus. The authors suggested that this brain region, which is outside the blood-brain barrier and therefore sensitive to circulating insulin concentrations, may experience stimulation of glucose uptake in response to insulin. They further proposed that this may allow regions of the hypothalamus to respond directly to elevated blood glucose, constituting a form of metabolic regulation by allowing circulating glucose (and therefore insulin) in concert with other mechanisms to maintain blood glucose homeostasis²⁸¹. Hence, this area of the brain may be a sensing mechanism which responds directly to blood glucose and/or insulin concentrations, which may, in turn, alter neurohormonal responses^{281,385}. Through a series of studies in rats, Borg and co-workers³¹⁻³³ demonstrated that the ventromedial hypothalamus triggers counterregulation during hypoglycemia, suggesting that, at least in rats, this area of the brain is one of the key sites acting as a glucose sensor. However, results from one study suggest that various regions of the brain may be involved in glucose sensing and inducing the glucagon and epinephrine hormonal response to acute central nervous system fuel deprivation¹⁵⁷.

Frizzell et al.¹⁵⁷ demonstrated that the humoral responses (cortisol, glucagon, catecholamines, and pancreatic polypeptide) to systemic hypoglycemia were minimally attenuated when euglycemia was maintained in the carotid or vertebrobasilar arteries in dogs, whilst significant counterregulation occurred when hypoglycemia was induced in either of these circulations. These observations are in support of the hypothesis that more than one center is important in hypoglycemic counterregulation in the dog and that they are located in brain regions supplied by the carotid and vertebrobasilar arteries¹⁵⁷. These authors suggested that counterregulation

during hypoglycemia is probably directed by widespread brain regions that contain glucose-sensitive neurons such that the sensing sites may be redundant ¹⁵⁷.

1.2.2.1.1 *Glucose-sensing neurones*

It has also now been shown that there are populations of neurons that uniquely use glucose as a signaling molecule that may alter firing rate in response to changes in local glucose supply, rather than using it as metabolic substrate ³⁸⁸. These neurons comprise of subpopulations that are either glucose-sensitive i.e. respond to decreasing blood glucose concentrations, or glucose-responsive i.e. responds to increasing blood glucose concentrations ³⁸⁵. Furthermore, changes in blood glucose concentration have been shown to alter neuronal release of neurotransmitters such as dopamine and gamma amino butyric acid in rats ^{126,278} and central nervous system (CNS) hormones involved in food intake and appetite regulation such as neuropeptide Y ³⁰⁴. Hypothalamic dopaminergic neurons are stimulated by reduced glucose supply and can regulate secretion of several glucoregulatory hormones ⁴⁵.

Evidence for similar involvement of these pathways in hypoglycemia counterregulation in humans is still lacking. However, results from human studies showing delayed onset and reduced magnitude of counterregulatory responses to hypoglycemia by provision of non-glucose substrates (especially ketones and lactate), provide support for locating the hypoglycemia sensor in the brain (for review see ³⁸⁵).

In addition, neural feedback from the working muscles may play a role in regulating hormonal and metabolic responses to exercise ²⁵⁷.

1.2.2.2 *Glucose sensing by the liver*

Though evidence suggest that the brain is the primary glucose sensing organ, studies performed on dogs ^{121,122,181} suggest the existence of hepatic gluco-sensors involved in activating a sympathoadrenal response to hypoglycemia. A study on adrenalectomized, somatostatin-infused dogs (i.e. absence of counterregulatory hormone response) found that the liver stimulated hepatic glucose production during marked hypoglycemia (2 mmol/L). Maintenance of brain euglycemia did not prevent the increase in hepatic glucose production, thus demonstrating autoregulation of hepatic glucose production and evidence that hypoglycemia must therefore be sensed within the liver as well ¹⁸¹.

1.2.2.3 Hepatic portal glucose sensing

The portal vein, draining the gastrointestinal tract to the liver, has also been suggested as a potential site for monitoring ("sensing") glucose entry from the gastrointestinal tract into the circulation. Animal studies have indicated that cells in the region of the portal vein contain beta-cell GLUT 2 transporters^{50,51}, and have demonstrated a reduction in hormonal response to systemic hypoglycemia when glucose supply to the portal vein was maintained²⁰⁸. Portal vein glucose sensing have not only been implicated in the hormonal responses to hypoglycemia, but also in the control of hepatic glucose uptake (perhaps via a portal-arterial glucose concentration gradient acting as a signal for an insulin-dependent net glucose uptake as measured in perfused rat liver)¹⁶⁵; and tissue glucose utilization⁵¹, though the exact mechanism(s) remain unclear.

Data from human studies are at present limited. Smith et al.³⁸⁶ recently reported results in favor of a glucose sensor in the liver or hepatic portal vein in humans. Their data also suggested that portal vein glucose sensors in humans are the primary hypoglycemia sensors, which may, interact with and modulate the centrally mediated adrenergic responses. The portal signal may be conveyed to the central sympathetic pathways via nerves³⁸⁶.

From the available literature it seems clear that glucose homeostasis is critical, primarily to ensure constant fuel supply to the glucose-dependent brain. The full extent and integration of the control and responses of the glucoregulatory systems in humans are not yet fully clear as in many of these experimental models one system has been controlled or blocked thereby influence the response of another system. However, several key components have been identified as playing a role in the maintenance of glucose homeostasis at rest including a) sensing of the prevailing blood glucose concentration and a component of central regulation; and b) a component of integration of a series of hormonal and neural responses in the periphery in aid of restoring euglycemia. In addition, there seems to be a component of redundancy in the glucoregulatory mechanisms. The remainder of this literature review will explore how these gluco-regulatory components operate during exercise, how it is affected by certain dietary manipulations and ultimately aim to explore its impact on endurance exercise capacity or performance.

1.3 BRIEF OVERVIEW OF FUEL SUBSTRATE SELECTION DURING PROLONGED EXERCISE

As previously noted, maintenance of glucose homeostasis is necessary to ensure constant fuel supply to brain. In addition, glucose is also an important fuel source to contracting skeletal muscle, and optimal glucose homeostasis and fuel supply has been linked to improved

endurance capacity (for review see ⁷⁶). However, blood glucose is not the only fuel source utilized by the muscle. Muscle glycogen, blood-borne glucose (derived from liver glycogenolysis, gluconeogenesis and/or exogenous supply), and fat (intramuscular triglycerides (IMTG) and plasma free fatty acids (FFA)) have been identified as the major substrates utilized for energy production during prolonged exercise ^{220,233,360,393}. The various types of substrates and their relative contributions during exercise have been extensively researched and reviewed (for reviews see ^{220,233,414}).

The relative contribution of these substrates to energy production during exercise is largely dependent on the intensity and duration of exercise ^{220,360,393}, substrate availability ¹⁷⁵, and training status ¹⁵.

1.3.1 Effect of exercise intensity on substrate selection

A classic study by Romijn et al. ³⁶⁰ on fuel utilization during various exercise intensities demonstrated that during exercise performed below 40% $VO_{2\text{ max}}$, plasma FFA were the major source of fuel for muscle contraction. CHO oxidation remained low at this low intensity and the liver was capable of supporting this relatively low rate of blood glucose utilization via gluconeogenesis. At higher exercise intensities (>60% $VO_{2\text{ max}}$) the contribution of fat to the overall energy demand decreased. Utilization of IMTG also decreased with increasing exercise duration ³⁶⁰. Though a progressive increase in FFA mobilization and oxidation was observed with increasing exercise duration, CHO remained the major source of fuel for muscle contraction when exercise was performed at higher intensities ³⁶⁰.

CHO oxidation during exercise is influenced primarily by the exercise intensity, duration and CHO availability. It has been well established that with an increase in exercise intensity, there is a linear increase in muscle glycogenolysis and muscle glucose uptake (for example see refs. ^{220,414}). Muscle glycogen and blood-borne glucose, as derived from endogenous (mainly liver glycogenolysis and to a lesser degree gluconeogenesis) and exogenous sources, are the most heavily metabolized fuel during exercise performed at moderate to high intensities (above 60 to 75% $VO_{2\text{ max}}$), accounting for at least 60 to 70% of the total energy requirement ^{89,360,365,366}. This is largely due to contraction-induced increases in muscle glycogenolysis and glycolysis ²¹⁷ as well as increased sympathetic nervous system activity ¹²⁰.

However, relative to fat, the CHO stores in the body are limited. Skeletal muscle can only provide 300 to 500 g and the liver 60 to 100 g of glycogen (dependent on body size and CHO content of the diet) ²⁰. Depletion of these CHO reserves is often associated with impaired exercise performance and/or the development of fatigue ^{52,67,76}.

1.3.2 Effect of CHO availability on fat oxidation

The general view is that in exercising muscle, the state of the endogenous CHO stores and CHO availability affects FFA uptake and fat oxidation, whilst increased FFA availability enhances fat oxidation and inhibits CHO metabolism³⁹³.

Interestingly, in a recent study by Kjaer et al.²⁵³ fatty acid kinetics and CHO metabolism during involuntary, electrically-induced leg cycling in spinal cord-injured individuals were compared to voluntary cycling exercise in healthy control subjects. They demonstrated a reduced fat metabolism in the absence of intact neural mechanisms during exercise, which underlines the importance of somatic afferent and efferent neural activity for regulation of lipolysis during exercise. Blood-borne mechanisms alone, including direct feedback on lipolysis by decreasing plasma FFA levels are not sufficient to elicit a normal increase in FFA mobilization during exercise²⁵³. In addition, their results were in support of the view that in exercising muscle, FFA delivery enhances FFA uptake and inhibits CHO metabolism whilst CHO oxidation inhibits FFA uptake. Their study illustrated that substrate availability is an important determinant of fuel selection during exercise, however, the exact mechanisms regulating this interplay still remain to be clarified²⁵³.

CHO (glucose) availability and the insulin response associated with preexercise CHO ingestion has been shown to regulate fat metabolism during exercise^{93,211,212}. Raised insulin concentrations (10-30 $\mu\text{U/ml}$) reduce FFA appearance mainly by inhibiting adipose tissue lipolysis^{28,56}. However, it appears that a reduction in FFA availability *per se* is not the sole factor responsible for the reduced fat oxidation observed with increased CHO availability and raised insulin concentrations. In the study of Sidossis and Wolfe³⁷⁹, CHO oxidation was accelerated by means of a hyperglycemic, hyperinsulinemic clamp whilst FFA concentrations were held constant with lipid-heparin infusion. Despite the maintenance of FFA availability, fat oxidation was inhibited likely due to a direct effect of elevated glucose or insulin concentrations³⁷⁹.

In contrast to the above, some studies have shown that despite a significant suppression of FFA and glycerol concentrations, CHO ingestion during exercise had no effect on fat oxidation during the first 120 min of exercise at moderate- to high-intensity (65 to 75% $\text{VO}_{2\text{max}}$)^{89,91}. This may be due to the effect of higher-intensity exercise on suppressing the insulin response⁸⁹. The suppression of the insulin response at higher exercise intensities may also explain the results of studies where CHO ingestion had no effect on CHO oxidation and muscle glycogenolysis^{36,38,89,91,440}. In support of these findings, the study of Horowitz et al.²¹² demonstrated that though CHO ingestion during 2 h of exercise at low intensity (25% $\text{VO}_{2\text{max}}$) resulted in a 3-fold increase in insulin and a >50% reduction in lipolytic rate and plasma FFA concentration throughout the

final 1 h exercise, fat oxidation was not reduced until 80-90 min of exercise. CHO ingestion during 2 h of exercise at moderate- to high-intensity (68% $\text{VO}_{2\text{max}}$) resulted in a small elevation ($\sim 3 \mu\text{U/ml}$) in insulin response in the second hour and a reduction in lipolysis and plasma FFA concentration by $\sim 20\text{-}25\%$ during this time, however, fat oxidation remained unaffected throughout the 2-h exercise bout. Despite suppression of lipolysis with CHO ingestion, plasma FFA availability was in excess of oxidation during both exercise intensities, indicating that reduced lipolysis and FFA availability were not solely responsible for the reduction in fat oxidation in the low-intensity exercise trial. Other additional mechanisms whereby increased glucose availability and insulin may suppress fat oxidation may be related to the effects of insulin on increasing glucose uptake into the muscle²⁴⁸, increasing glycolysis and glycolytic flux³⁷⁷, and by controlling the rate of fatty acid oxidation by controlling the rate of long-chain fatty acid entrance into the mitochondria³⁷⁸. It should be noted though, that the other gluco-regulatory hormones (glucagon, catecholamines, cortisol and growth hormone) are also important in the regulation of substrate utilization, mainly through their action on glucose homeostasis (for review see⁴³⁴). The effects of these hormones are discussed in more detail under various subsequent sections of this literature review.

Fat oxidation during exercise is also largely regulated by the status of the endogenous CHO stores^{23,35,162,175,440,441,445}. The studies by Weltan et al.^{440,441} demonstrated an increase in fat oxidation when performing exercise in a glycogen-depleted state. Surprisingly, fat oxidation during exercise in the glycogen-depleted state remained elevated despite euglycemic⁴⁴⁰ or hyperglycemic ($\sim 10 \text{ mmol/L}$)⁴⁴¹ glucose infusion. In contrast, when exercise was commenced and performed with normal^{23,175,440,441} or supercompensated^{35,162,175,445} glycogen stores, fat oxidation was reduced and muscle glycogen oxidation enhanced. These studies have also indicated that reduced muscle glycogen content does not alter the rate of plasma glucose oxidation⁴⁴¹, but rather results in an increase in fat oxidation to meet the energy demands.^{35,440,441} It was hypothesized⁴⁴⁰ that the shift towards fat oxidation during exercise with low muscle glycogen stores may be mediated by a muscle afferent pathway that ultimately results in an alteration in norepinephrine and insulin concentrations.

1.3.3 Effect of training status

Regular exercise training can alter the interplay in fuel substrate selection. Training results in, amongst other things, an increase in insulin sensitivity (e.g. ref.²⁰⁰), an increased activity of oxidative enzymes, a higher proportion of type I skeletal muscle fibers, greater capillary density, higher skeletal muscle GLUT-4 protein expression (e.g. refs.^{130,184}), which enables enhanced glucose uptake by the contracting muscle²⁶⁵ as well as an increase in the ability to oxidize fat as fuel with potential "sparing" of the limited CHO fuel stores^{15,179,199,202,218}.

When assessing the effects of training on CHO (glucose) metabolism, it has been noted that training reduces glucose flux at the same absolute workload (compared to pre-training), but not the same relative workload¹⁵⁴. In one of the very few longitudinal studies of the effect of training status on whole-body glucose kinetics during exercise¹⁵⁴, it was shown that training does not affect glucose kinetics (glucose appearance or disappearance) when expressed at a given relative power output. This is in contrast to results from cross-sectional designs where results of highly trained and untrained populations were compared⁷⁸. However, results of highly trained athletic populations may be confounded by specific genetic characteristics related to substrate utilization, such as an increased ability to oxidize fat¹⁵⁴. However, irrespective of study population and/or design, it has been shown that even after training, endogenous CHO energy sources are the predominant fuel for muscle contraction when exercising at intensities $>50\% \text{VO}_{2\text{max}}$ (for review see⁴⁷). Furthermore, even when the ability to oxidize fat is increased with training, there is still a limitation in the overall rate of fat oxidation³⁶¹.

In this regard, Bergman et al.¹⁴ investigated the effect of endurance training on IMTG oxidation by studying leg FFA and glycerol exchange during 1 h of cycling exercise at two intensities (45% and $65\% \text{VO}_{2\text{max}}$) before training, and after training at 65% of pretraining $\text{VO}_{2\text{max}}$ (i.e. the same absolute workload), and 65% of posttraining $\text{VO}_{2\text{max}}$ (i.e. the same relative workload). Testing was conducted before and after 9 wks of cycling exercise, 5 times per wk at $75\% \text{VO}_{2\text{max}}$. The power output that elicited $\sim 65\% \text{VO}_{2\text{max}}$ before training, elicited $\sim 54\% \text{VO}_{2\text{max}}$ after training due to a $\sim 15\%$ increase in $\text{VO}_{2\text{max}}$. Training significantly decreased pulmonary (whole-body) RER values at the same absolute intensity, but was the similar at the same relative intensity. After training, leg respiratory quotient (RQ) was not significantly different at either the same absolute or relative intensity. Measurements of leg RQ, net FFA uptake, and glycerol release by working legs indicated no change in leg FFA oxidation, FFA uptake, or IMTG lipolysis during cycling exercise that elicits 65% pre- and 54% posttraining $\text{VO}_{2\text{max}}$. The authors concluded that training increases working muscle FFA uptake at $65\% \text{VO}_{2\text{max}}$, but high RER and RQ values at all work intensities indicate that FFA and IMTG are of secondary importance as fuels in moderate and higher-intensity exercise¹⁴.

The limitation in fat oxidation during exercise at higher intensities may be due, partly, to reduced availability of blood-borne FFA (due to reduced adipose tissue blood flow), and partly due to other regulating mechanisms involved in fat oxidation such as inhibition of β -oxidation by malonyl-CoA at higher exercising intensities^{235,237}. These mechanisms are not fully understood and discussion of this aspect is beyond the scope of this review (for detailed reviews on the topic see refs.^{235-237,392}).

1.3.4 The “crossover concept”

The above observations have contributed to the development of the “crossover concept” by Brooks ^{47,48} that explains the interplay between CHO and fat metabolism and oxidation during exercise in humans.

According to this concept ⁴⁸, endurance training results in biochemical adaptations that enhance fat oxidation during low to moderate-intensity exercise as well as decrease the sympathetic nervous system (SNS) response to given submaximal exercise stresses. In contrast, an increase in exercise intensity is associated with an contraction-induced increase in muscle glycogenolysis, altered fiber-type recruitment patterns, and increased SNS activity, resulting in a shift (“crossover”) in the balance of substrate utilization from fat to CHO, regardless of genotypic or phenotypic adaptations ⁴⁷. Hence, it is proposed that the individual pattern of substrate utilization at any point in time depends, amongst other things, on the crossover between the exercise intensity-induced responses (such as SNS-related responses to increase CHO utilization) and the endurance training-induced responses (which promote fat oxidation) ⁴⁸. The crossover point is defined as the workload (power output) at which oxidation of endogenous CHO-based fuels (liver and muscle glycogen, blood glucose, and liver, muscle and blood lactate) predominates over that derived from fat ⁴⁸. As exercise intensity (power) increases, the relative contribution of energy derived from CHO-based substrates will increase and the relative contribution of energy derived from fat-based substrates will further decrease ⁴⁸. In order to support the increased energy substrate flux rate needed to sustain muscle contraction during high-intensity exercise, SNS-mediated responses along with other hormonal responses (e.g. insulin and glucagon), blood lactate concentrations, nutritional strategies and training history will contribute to coarse and fine settings of substrate utilization patterns during exercise of differing intensities ⁴⁸.

As evident from the literature, there is an extensive interplay between the various endogenous oxidative substrates capable of contributing to the energy demand by the working muscle. However, from the various endogenous fuel stores, CHO oxidation seems paramount to sustain muscle contraction during prolonged exercise at moderate- to high-intensities. This setting, combined with limited endogenous CHO stores may ultimately result in a reduction in blood glucose concentration and glucose supply to the working muscle as well as the glucose-dependent brain, which will challenge glucoregulatory systems to restore euglycemia.

1.4 KEY FACTORS INVOLVED IN THE REGULATION OF GLUCOSE HOMEOSTASIS DURING PROLONGED EXERCISE

During exercise, glucose homeostasis is mainly comprised of activation of liver and muscle glycogenolysis as well as up-regulation of insulin-dependent and insulin-independent glucose transport pathways. Several hormonal and humoral factors have been identified as key regulators of glucose homeostasis during exercise (discussed in detail below). In brief, with increased muscle contraction there is increased uptake of glucose, likely mediated via increased insulin signaling, as well as insulin-independent, contraction-stimulated increases in 5'AMP-activated protein kinase (AMPK), which both result in increased translocation of the GLUT-4 transporter protein and subsequent glucose uptake (for review see ^{250,447}). With increased glucose uptake by the contracting muscles, hepatic glycogenolysis and hepatic glucose output are activated, and the subsequent hormonal responses involve changes in the gluco-regulatory hormones such as a reduction in insulin, and an increase in glucagon, cortisol, growth hormone and catecholamines ⁴⁵⁷. Prolonged exercise and progressive glycogen depletion will result in a gradual decline in blood glucose concentrations and further changes in glucose counterregulatory hormones ⁴⁰⁴, whilst CHO supplementation during such exercise has been shown to attenuate these hormonal responses ^{44,180,275,277}.

1.4.1 *Contribution of the liver to the maintenance of glucose homeostasis during exercise*

The liver is the predominant source of endogenous glucose production during exercise (via glycogenolysis and gluconeogenesis) and therefore plays an essential role in the maintenance of glucose homeostasis during exercise (for review see ²⁵²).

Though several studies have aimed to investigate the effect of exercise intensity on the rates of hepatic glycogenolysis and gluconeogenesis, findings are contradictory and unclear (documented in Trimmer et al. ⁴¹²). It seems that one of the main reasons for the discrepancy in results is related to the methodological limitation in the use of carbon tracers and correction factors for estimations of rates of gluconeogenesis *in vivo* ⁴¹². However, a more sophisticated measurement technique has been developed ^{206,315} called the mass isotopomer distribution analysis (MIDA) technique, which enables a more true estimation of precursor pool enrichment without the need of correction factors ⁴¹².

In a recent study, Trimmer et al. ⁴¹² employed the MIDA and isotope-dilution techniques to assess the relative contribution of gluconeogenesis and hepatic glycogenolysis to glucose production, and its ability to match the rate of peripheral glucose utilization during prolonged

exercise at moderate and high-intensity. In a randomized order (at least 5 days apart), 8 overnight-fasted, endurance-trained men cycled for 90 min at either 45% or 65% $\text{VO}_2 \text{ max}$. In agreement with previous findings^{14,154,155,411}, glucose production increased in an intensity-dependent manner (rest, 2.0 ± 0.1 ; 45%, 4.0 ± 0.4 ; 65% $\text{VO}_2 \text{ max}$, 5.8 ± 0.6 mg/kg/min, $p < 0.05$), however, the rate of peripheral glucose utilization exceeded rate of appearance during the last 30 min of exercise at 65% $\text{VO}_2 \text{ max}$. During high-intensity exercise, the fraction from gluconeogenesis decreased significantly to account for only 21% of glucose production, and the highest sustained rate of gluconeogenesis observed was 1.2 ± 0.3 mg/kg/min in lieu of a glucose utilization rate of > 6.0 mg/kg/min. The use of the MIDA technique yielded results that are ~30-45% higher than that obtained with similar study designs but with the use of the carbon tracer dilution technique. Hence, this study demonstrated that after an overnight fast, though the absolute rate of gluconeogenesis increases during exercise compared to rest, the relative contribution of gluconeogenesis (as a fraction of glucose production) remains the same at moderate-intensity exercise, and decreases with higher-intensity exercise (constituting ~20-25% of glucose production). Hepatic glycogenolysis is more important in the maintenance of glucose homeostasis during exercise. However, in overnight-fasted state (i.e. compromised hepatic glycogen content), neither increased glycogenolysis or gluconeogenesis (nor in combination) were sufficient to prevent a decline in plasma glucose concentration during 90 min of high-intensity exercise (65% $\text{VO}_2 \text{ max}$), despite an elevation in precursor (lactate and glycerol) supply⁴¹².

Hence, with prolonged exercise performed in a postabsorptive (fasted) state, especially at intensities $> 65\% \text{VO}_2 \text{ max}$, maintenance of glucose homeostasis may be compromised⁴¹². This will inevitably elicit a complex of gluco-regulatory systems, such as alterations in hormonal responses²⁷¹ in attempt to restore euglycemia. The key components of these regulatory systems, as they operate during exercise will be discussed below.

1.4.2 Regulation of hepatic glucose output

Hepatic glucose production at rest has been shown to be regulated primarily by feedback from the extracellular glucose concentration, which has a direct glucoregulatory effect on hepatic glucose production⁴²⁹. The extracellular glucose concentration has been shown to modulate the neural discharge from glucoreceptors in the liver, the hypothalamus and hind-brain, the intestine and pancreas (for review see ref.⁴²⁹). However, during exercise, hepatic glucose production has been shown to be regulated by more complex interaction between hormonal and neural mechanisms, that often display a component of redundancy, but are as of yet not completely understood²⁵².

Initially it was thought that the well-established humoral feedback mechanism that regulates glucose homeostasis at rest, also operates during exercise⁴²⁹. This thought was strengthened by studies in dogs demonstrating an initial (temporary) decline in blood glucose concentrations at the onset of moderate-intensity exercise, which may provide a feedback stimulus to increase hepatic glucose production to restore glucose homeostasis (for review see⁴²⁹). Further support for a humoral feedback mechanism operating during exercise has come from studies during low to moderate intensity exercise in humans^{13,224} and animals⁴³⁰ demonstrating that the increase in hepatic glucose output during exercise can be prevented by an intravenous glucose infusion, or with glucose ingestion in trained men²⁹⁷. These findings indicated that hepatic glucose output is subject to feedback regulation by the prevailing glucose concentration, causing alterations in glucagon and insulin concentrations^{209,453}. However, during high-intensity exercise, changes in glucagon and insulin are insufficient to account for the marked increase in hepatic glucose output^{77,380}. Furthermore, glucose infusion during high-intensity exercise in rats reduced but did not completely suppress hepatic glucose output^{446,448}, indicating that metabolic (humoral) feedback mechanisms (arising from the prevailing blood glucose concentration) cannot entirely prevent the exercise-induced increase in hepatic glucose production during such exercise. The rapid increase in glucoregulatory hormonal responses and glucose production seen during exercise at higher intensities is unlikely to be due entirely to humoral feedback mechanisms^{303,422}.

During high-intensity exercise, there is evidence for feed-forward activation as shown by a greater increase in the rate of glucose production than glucose uptake, and a subsequent increase in plasma glucose concentrations (particularly early in exercise)^{192,213,258}. Hence, during high-intensity exercise, hepatic glucose output may rather be regulated by neural feed-forward mechanisms whereby motor centers in the brain may activate neuroendocrine responses that increase glucose production^{258,430}. The neural feed-forward mechanism has the benefit of a more rapid supply of glucose to meet the large increase in substrate need by the working muscle, as opposed to a slower rise in glucose from a humoral feedback mechanism⁴²⁹.

Recent studies have attempted to investigate the role of central neural control of metabolism in exercise. In this regard, Kjaer et al.²⁵⁸ have shown that neuromuscular blockade in humans induced higher perceived exertion and a higher glucose production compared to controls exercising at the same absolute workload, which provided evidence of central command regulation of glucose mobilization in exercise. After conducting a series of experiments in cats and humans, Vissing⁴²⁹ presented compelling evidence that mobilization of glucose and secretion of glucoregulatory hormones are regulated primarily by neural mechanisms. It was suggested that this neural mechanism involves an efferent (central) component "...associated with a simultaneous drive of somatomotor and autonomic circuitry from cerebral motor centers, that in parallel stimulate locomotion, neuroendocrine activity and circulation" and hence stimulate

glucose mobilization; as well as “an afferent (peripheral) mechanism associated with reflex stimulation from working muscle”⁴²⁹. Further, the author proposed that “the muscle reflex mechanism likely has a fast component caused by mechanoreceptor stimulation at the onset of exercise and a slower component related to muscle metaboreceptor stimulation during continued exercise”⁴²⁹. Vissing suggested that these two components are likely integrated in the central nervous system. The potential chemical mediator responsible for eliciting the muscle reflex is still unclear, but there seems to be no direct correlation between sympathetic activation and the levels of acidity, lactate, inorganic phosphate and glycogen during exercise⁴²⁹. Levels of nitric oxide and potassium are potential mediators in the muscle reflex mechanism (for review see⁴²⁹). However, the results from this series of experiments by Vissing⁴²⁹ should be interpreted and extrapolated with caution as it was conducted under non-physiological conditions in animals and diseased humans. Though it provides significant insights into potential mechanisms of hepatic glucose production and regulation of glucose homeostasis during exercise, further studies on healthy humans in a more physiological milieu should be conducted to reaffirm the results.

In summary, though the humoral feedback mechanisms are efficient to adjust for small fluctuations in glucose homeostasis at rest, it is unlikely that they account for the dramatic increase in fuel (glucose) demand of the working muscle, especially during moderate to high intensity exercise. Rather, evidence suggests that direct neural control of neuroendocrine activity and subsequent glucose mobilization is responsible for a rapid delivery of glucose to match the increased metabolic demand of the working muscle.

1.4.2.1 Role of glucose availability per se in the regulation of hepatic glucose output in humans exercising at moderate to high intensities

A study by Howlett et al.²¹³ attempted to elucidate the relative importance of feedback and feed-forward mechanisms for regulation of hepatic glucose output during high-intensity exercise (40 min cycling at ~77% $\text{VO}_{2\text{max}}$) in trained men. Glucose was infused during the exercise trial at a rate equal to the average hepatic glucose output as was measured during a control trial (without glucose infusion). Glucose concentrations in the control trial gradually increased from 5 to 6 mmol/L, and from ~5-7 mmol/L with glucose infusion ($p < 0.05$). Glucose infusion and an increase in glucose availability completely inhibited the rise in hepatic glucose output during exercise. Insulin, glucagon, epinephrine and norepinephrine concentrations were lower with glucose infusion, though not statistically significant. It was suggested that the lack of significance might have been attributed to the small sample size ($n=5$) and inter-individual variability. The insulin to glucagon ratio was significantly higher throughout exercise with glucose infusion. The authors concluded that metabolic feedback signals (arising from the prevailing blood glucose concentration) can override feed-forward activation of hepatic glucose production during

strenuous exercise²¹³. Similar results were found in trained males exercising for 2 h at 70% $\dot{V}O_2$ _{max} when plasma glucose was increased to a level of 10 mmol/L, however, hepatic glucose output was reduced but not completely abolished during euglycemia¹⁹⁴.

Glucose supplementation and increased glucose concentrations *per se* have been shown to directly inhibit hepatic glycogenolysis by decreasing glycogen phosphorylase and increasing glucokinase activity with a subsequent reduction in hepatic glucose output³⁶². However, in the studies of Howlett et al.²¹³, and Hawley et al.¹⁹⁴ alterations in the glucoregulatory hormone concentrations may also have played a role in altering hepatic glucose output¹⁶².

1.4.2.2 Role of pancreatic hormones on hepatic glucose output: Insulin and glucagon

Glucose infusion results in alterations in the key glucoregulatory hormone concentrations¹⁶². It has been shown in dogs and humans that hepatic glucose output during low- to moderate-intensity exercise is mainly mediated by alterations in pancreatic hormones, insulin and glucagon, playing an essential role in the maintenance of glucose homeostasis (for review see⁴³⁴). The decline in insulin is responsible for the increase in glycogenolysis, whereas the rise in glucagon is responsible for the increase in gluconeogenesis when performing exercise at low-to moderate intensities^{209,434}.

Recently, Coker et al.⁷⁹ investigated the importance of basal glucagon to the stimulation of net splanchnic glucose output during exercise by means of a pancreatic islet clamp in healthy men. In one group (BG), glucagon was replaced at basal levels and insulin was adjusted to achieve euglycemia, and in another group (GD), only insulin was replaced at the identical rate used in BG, and basal glucagon was not replaced. In their study, exogenous glucose infusion was necessary to maintain euglycemia during exercise in BG. Arterial glucagon was at least twofold greater in BG than in GD throughout the pancreatic islet cell clamp. Splanchnic glucose output was greater in BG than in GD at 10 min of moderate exercise, most likely due to the residual effect of basal glucagon replacement. However, splanchnic glucose output increased slightly and remained similar throughout the remainder of moderate and heavy exercise in BG and GD. The authors concluded that a mechanism independent of changes in pancreatic hormones and/or the level of glycemia contributes toward modest stimulation of glucose production during moderate and heavy exercise⁷⁹.

Insulin and glucagon seem to be of lesser importance in humans exercising at higher intensities, where the alterations in glucagon and insulin are insufficient to account for the marked increase in hepatic glucose output^{72,214,287,380}. However, it has been noted that small changes in peripheral concentrations of these hormones might not reflect significant changes at the level of

the portal vein, which could account for the alterations in hepatic glucose output ²¹⁴. It does seem though that with exercise at higher intensities, catecholamines become more important in the regulation of glucose homeostasis than the pancreatic hormones ^{287,380}.

1.4.2.3 Role of catecholamines on hepatic glucose output: Epinephrine and norepinephrine

With an increase in exercise intensity and duration, there is an exponential increase in plasma catecholamines concentrations and sympathetic neural activity ³⁷². It has been shown that catecholamines (particularly epinephrine) become important to maintain hepatic glucose output and prevent hypoglycemia when alterations in insulin and glucagon do not occur ²⁸⁶, suggesting that there is redundancy in the counterregulatory responses.

The role of epinephrine in regulation of hepatic glucose output is not clearly defined. With high-intensity exercise, a causal relationship between plasma epinephrine and hepatic glucose output has been suggested, but not established, by those studies where an increase in plasma epinephrine was found to occur simultaneously with an increase in hepatic glucose output ^{55,256,287,342,360,380}.

Kjaer et al. ²⁵⁴ infused a high physiological dose of epinephrine in human subjects during intense exercise and observed an increase in hepatic glucose output. However, interpretation of these results is complicated not only because a non-physiological dose of epinephrine was used, but subjects had also undergone anesthetic blockade of the celiac ganglion, which impairs sympathetic activity to the liver, pancreas and adrenal medulla, and these results can therefore not be extrapolated to normal physiological conditions.

Howlett et al. ²¹⁴ investigated the effect of epinephrine infusion on hepatic glucose output in humans during exercise under more physiological conditions. In the control trial, six trained men cycled for 20 min at 40% $\text{VO}_{2 \text{ max}}$ followed by 20 min at 80% $\text{VO}_{2 \text{ max}}$. In a second trial the subjects cycled for 40 min at 40% $\text{VO}_{2 \text{ max}}$, with epinephrine infusion during the latter 20 min, which resulted in plasma concentrations similar to those measured during intense exercise in the control trial. Epinephrine infusion increased hepatic glucose output at 40 min of exercise, though to a significantly lesser extent than that observed during the high-intensity exercise period in the control trial ²¹⁴. These results indicate that though epinephrine can increase hepatic glucose output during exercise in trained men, the epinephrine concentrations seen with high-intensity exercise (80% $\text{VO}_{2 \text{ max}}$) cannot fully account for the rise in hepatic glucose output. The results of this study ²¹⁴ are in contrast to studies proposing that epinephrine is the main mediator of the rise in hepatic glucose output during high-intensity (>80 $\text{VO}_{2 \text{ max}}$) exercise ^{256,287,342,380}.

Howlett et al.²¹⁴ noted that other gluco-regulatory factors must contribute to the increase in hepatic glucose output during intense exercise. Sympathetic neural innervation of the liver has been proposed to play a role in regulating hepatic glucose output during exercise in human subjects^{77,287,342,380}. However, it seems unlikely that direct sympathetic neural innervation plays a major role in the regulation of hepatic glucose output during exercise, given that liver transplant patients display a normal exercise-induced increase in hepatic glucose output²⁵⁵, and that hepatic glucose output is not reduced during intense exercise in normal subjects when sympathetic activity was impaired by anesthetic blockade of the celiac ganglion²⁵⁴. However, extrapolating findings from liver transplant patients to healthy individuals should be done with caution³⁸⁰. The study of Howlett et al.²¹³ suggests reduced sympathetic activity that may have contributed, at least in part, to the decrease in hepatic glucose production. Hepatic neural activation may play a more important role during exercise performed at higher absolute or relative intensities⁷⁷, than those used in the above-mentioned studies. However, this aspect remains equivocal and warrants further investigation.

In summary: At present it is yet unclear at which glucose concentration feedback inhibition can override feed-forward activation during exercise. It seems likely that hepatic glucose production during strenuous exercise (>70% $\text{VO}_2 \text{max}$) contains both a neural feed-forward and a humoral feedback component, and that whichever component dominates is determined by the interaction between exercise intensity (which also determines motor center activity) and prevailing plasma glucose and insulin concentrations.

1.4.2.4 Role of hepatic glucose sensors in glucose homeostasis during exercise

Seeing that hepatic glucose production is essential for the maintenance of glucose homeostasis during exercise, and since there is evidence that hepatic afferents (via the vagus nerve) monitor the availability of liver glycogen and glucose metabolites that play a role in appetite regulation, it has been hypothesized that the liver may play a similar sensory role during exercise to elicit metabolic adjustments to sustain the high energy demand during exercise (for review see²⁷¹).

It is postulated²⁷¹ that the sensory role of the liver during exercise would be similar to its role in controlling food intake in that a decrease in liver glycogen (or a related metabolic intermediate) is sensed by the liver, that sends a signal to the central nervous system most likely via the afferent activity of the hepatic vagus nerve, where it contributes to metabolic and hormonal responses to exercise in order to alter substrate mobilization and/or utilization (for review see²⁷¹). For example, recent animal data points to the existence of a link between a decrease in liver glycogen concentration and an increase in FFA mobilization from adipose tissue during exercise^{88,271}. There is currently no evidence that an afferent pathway from the liver directly influences

glucose or glycogen utilization by contracting skeletal muscle ²⁷¹. However, there is evidence that hepatic denervation results in peripheral insulin resistance in animals ²⁶⁹. In this regard, it has been postulated that postprandial insulin release activates a hepatic parasympathetic reflex resulting in the release of a humoral factor by the liver termed hepatic insulin-sensitizing substance (HISS) that sensitizes skeletal muscle to insulin, or has a direct insulin-like action ^{270,271}. In a recent review of the data (predominantly animal research), Luttich ²⁷⁰ suggests that the glucose disposal effect of insulin after a meal is accounted for in equal measure by the direct action of insulin and the action of HISS on skeletal muscle to stimulate glucose storage. However, the existence of the HISS hypothesis has not yet been confirmed in humans.

Further evidence for the existence of an afferent hepatic glucose sensor comes from studies where intraportal glucose infusion resulted in a twofold increase in insulin and an attenuated norepinephrine response during exercise compared with an equivalent peripheral vein glucose infusion ²⁶³. In addition, the normal responses in insulin, glucagon and norepinephrine during exercise were attenuated in hepatic-vagotomized compared with sham-operated rats ²⁷².

Though there is evidence to suggest the existence of hepatic sensors that may respond to decreasing liver glycogen or glucose intermediates and ultimately influence, likely through an afferent pathway, the hormonal responses and ultimately substrate mobilization / utilization during exercise, it has not been confirmed in human studies. The manner in which the human liver responds to acute exercise and regular training warrants further investigation.

1.4.3 Potential role of interleukin 6 (IL-6) in glucose homeostasis

Cytokines, such as IL-6, have traditionally been researched in respect to its involvement in the immune response. More recently, IL-6 concentrations have been shown to markedly increase during exercise, and skeletal muscle has been identified as the major source of the circulating IL-6 (for reviews see ^{143,337}). The brain ³²⁷, peritendon ²⁶⁸ and adipose tissue ⁴²⁴ have also been identified as sites of IL-6 production in response to exercise. IL-6 has been shown to act on peripheral and central organs and have been suggested to act in a hormone-like manner to mobilize extracellular substrates and/or augment substrate delivery during exercise ³³⁷. Specifically, IL-6 seems to play an important role in signaling between the muscles and other organs in order to maintain energy supply. IL-6 can enhance lipolysis and fatty acid mobilization ^{244,328,396,424} and might play a role in glucose homeostasis ^{140,396,400} via increased glycogenolysis ^{86,357} during exercise.

Studies have shown that IL-6 can contribute to the hepatic glucose output necessary to maintain blood glucose homeostasis during exercise ^{140,394,400}. IL-6 is a potent activator of the HPA-axis

^{291,335}, and may mediate blood glucose concentrations through its effect on raising glucose-regulating hormones such as cortisol ³⁹⁸ and epinephrine ³¹⁷. IL-6 and its receptor are co-expressed at similar sites in the human adrenal gland, which also seems to be an important source of IL-6 production ³³⁵. Path et al. ³³⁵ suggested that under conditions of prolonged stress when corticotrophin-releasing hormone and adrenocorticotrophin hormone (ACTH) release are suppressed by feedback inhibition due to raised circulating glucocorticoids, IL-6 may directly stimulate adrenocortical steroidogenesis via autocrine/paracrine mechanisms to raise cortisol concentrations. This may explain the high systemic cortisol levels in the absence of adequate plasma concentrations of ACTH observed in patients after long-term treatment with IL-6 ³³⁵.

The liver is also capable of producing IL-6, which seems to have a regulatory function by directly acting on hepatocytes to increase hepatic glucose release ³⁵⁷, thereby contributing further to glucose homeostasis. Thus, the liver may produce IL-6 to stimulate hepatic glucose output if blood glucose concentrations are compromised. In contrast, CHO ingestion during exercise has been shown to reduce or augment IL-6 production by the contracting skeletal muscle ^{144,394}.

However, the results of CHO ingestion during exercise on skeletal muscle IL-6 gene expression remain inconsistent. One study showed that CHO ingestion during prolonged exercise tended ($p < 0.07$) to decrease skeletal muscle IL-6 mRNA levels ³¹⁸, whereas three other studies ^{144,317,394} found that CHO ingestion had no effect on skeletal muscle mRNA levels. Whilst the effect of CHO ingestion *per se* on muscle IL-6 mRNA is unclear, low muscle glycogen has been shown to increase muscle IL-6 mRNA concentrations up to 100-fold in response to exercise ^{201,245,397}. This suggests that IL-6 release from the working muscle may be regulated by substrate availability such as circulating blood glucose and muscle glycogen concentrations ¹⁷⁰.

IL-6 release from the contracting skeletal muscle has also been shown to enhance the IL-6 production in adipose tissue ^{143,424}. Adipocytes express the IL-6 receptor ³³⁵, and binding of the IL-6 released from skeletal muscle may trigger adipose IL-6 gene induction, thereby enhancing IL-6 production in adipose tissue. Febbraio et al. ¹⁴⁴ demonstrated that though CHO ingestion did not influence IL-6 gene expression in skeletal muscle, the release of IL-6 from the contracting skeletal muscle was attenuated. This may explain the decreased IL-6 gene expression in adipose tissue seen with CHO ingestion during exercise. Furthermore, it has been shown that IL-6 induces an increase in appearance and disappearance of free fatty acids (FFA) ²⁴⁴. The increased IL-6 production in adipose tissue late in exercise may provide a link from the contracting skeletal muscle to enhance fat metabolism, thereby contributing FFA oxidation to the energy demand (at a time when glycogen stores are reduced).

In summary, these results indicate that IL-6 may play an important role in glucose homeostasis during exercise, mainly by mediating hepatic glucose production, but may also play a role in enhancing FFA release and fat oxidation late in exercise. In this regard, evidence suggest that IL-6 release from the working muscle may be regulated by substrate availability such as circulating blood glucose and muscle glycogen concentrations ¹⁷⁰. Though recent studies are continually demonstrating a role for IL-6 beyond that played in immune system function, the full physiological range of these functions and their relative importance, as well as the mechanisms underlying these responses, especially during exercise, are not yet fully known.

(The cerebral IL-6 response and potential involvement of IL-6 in fatigue development during prolonged exercise will be discussed under section 1.5.2.2.6.).

1.4.4 Regulation of muscle glycogenolysis during exercise

The primary regulators of muscle glycogenolysis during exercise include muscle glycogen availability and exercise intensity.

1.4.4.1 Muscle glycogen availability

Muscle glycogen concentration at the onset of exercise is an important determinant of muscle glycogenolysis during exercise. It is now well-known that reduced muscle glycogen availability results in a lower rate of glycogenolysis during submaximal exercise ^{173,190}, whilst higher concentrations of muscle glycogen at the onset of exercise increase muscle glycogenolysis during exercise ^{35,190,375}. During the initial stages of exercise, the rate of muscle glycogenolysis is most rapid and contributes ~85% to CHO oxidation ^{35,89}, but as exercise duration increases and muscle glycogen is depleted, the rate of muscle glycogenolysis gradually declines ^{174,431} whilst plasma glucose oxidation increases ³⁶⁰.

1.4.4.2 Exercise intensity

As exercise intensity increases linearly, the rate of muscle glycogenolysis increases exponentially ^{366,425}. An increase in exercise intensity results in greater sympathetic nervous system activation ¹²⁰ and greater contraction-induced muscle glycogenolysis and glycolysis ²¹⁷, which consequently results in greater reliance on CHO fuel stores.

In the study of Romijn et al. ³⁶⁰ 5 endurance-trained men cycled on three separate occasions in random order for 120 min at 25 and 65% $\text{VO}_{2 \text{ max}}$ and 30 min at 85% $\text{VO}_{2 \text{ max}}$. Stable isotope tracers and indirect calorimetry were used to determine the rate of endogenous CHO and fat

oxidation. The results indicated that muscle glycogen was not used at the lowest intensity, but contributed ~80-83% of the CHO oxidized when exercising at 65 and 85% $VO_{2\max}$ ³⁶⁰.

1.4.4.3 Training status

An increase in glucose uptake during strenuous exercise in the glycogen-depleted state has been reported after 3 weeks of endurance training²⁶⁵. This result was attributed to a training-induced increase in GLUT-4 protein content. These findings are consistent with studies showing a greater plasma glucose uptake in well-trained subjects when commencing moderate-intensity exercise with low compared to high muscle glycogen content^{391,450}. In contrast, similar rates of glucose uptake were reported when moderately-trained subjects commenced moderate-intensity exercise with either low or high muscle glycogen content^{35,190,440}. The inconsistent findings from these studies may be related to the differences in training status of subject populations between studies.

Recently Arkinstall et al.¹⁰ employed a study design to assess the relative effects of exercise intensity and preexercise muscle glycogen content on rate of whole-body fuel oxidation in the same subjects (i.e. controlling for training status). Moderately-trained subjects performed, in random order, four 60 min rides, two at 45% and two at 70% of $VO_{2\max}$ after exercise-diet intervention to manipulate muscle glycogen content at the start of exercise. All trials were performed after a 10-12 h over-night fast. During low-intensity exercise (45% $VO_{2\max}$), high preexercise muscle glycogen content resulted in greater rates of whole body CHO oxidation (glycogen and blood glucose oxidation) than exercising at the a higher intensity (70% $VO_{2\max}$) with low muscle glycogen concentration. The rate of plasma glucose uptake was similar when muscle glycogen content was elevated in both the 45 and 70% $VO_{2\max}$ trials, as also shown in other studies with moderately-trained individuals. Rather, the additional energy required to perform the exercise was derived from the oxidation of fat¹⁰. Thus, this study¹⁰ indicated that high preexercise muscle glycogen content increases CHO oxidation to a greater extent than an increase in exercise intensity from 46 to 70% $VO_{2\max}$ and that glycogen availability does not influence the rates of plasma glucose disposal during exercise in moderately-trained individuals, in support of previous findings^{35,190,440}.

1.4.4.4 Role of epinephrine on muscle glycogen utilization

Muscle glycogenolysis is catalyzed by glycogen phosphorylase, existing in an active 'a' and a less active 'b' form. During exercise, conversion of phosphorylase b to the active a form is regulated by increases in cytosolic Ca^{2+} (released during muscle contraction) and an epinephrine-mediated increase in cAMP²¹⁶. Additional factors linked to the energy state of the

cell (such as AMP and IMP) or substrates (such as glycogen and inorganic phosphate) then make further adjustments to the flux rate based upon the metabolic demands (for review see ²³⁹).

The majority of studies have demonstrated that increased epinephrine results in increased muscle glycogen utilization during exercise in humans ^{142,222,353,356,437}, however, others have not shown such an effect ^{63,442}. The discrepancy in results may be related to the infusion of epinephrine in doses above physiological levels ²²², and/or the differences in exercise intensities employed by these studies ^{63,142,437} or differences in the training status of the study population ^{63,442}.

Epinephrine infusion at physiological concentrations during moderate (65% $VO_{2\max}$) or intense exercise (80% $VO_{2\max}$) had no effect on the rate of muscle glycogenolysis when the study populations were untrained ⁶³. In contrast, epinephrine infusion (~2 vs. 1 nmol/L, epinephrine vs. control trial) in trained men exercising for 40 min at 70% $VO_{2\max}$ resulted in increased muscle glycogen utilization, glycogenolysis and overall rate of CHO oxidation ¹⁴². Furthermore, when exercising at high intensities (>80% $VO_{2\max}$) glycogenolysis may already be maximally activated, irrespective of further increases in epinephrine concentrations ⁴³⁷.

The exact mechanism(s) whereby epinephrine exerts its action on CHO metabolism have not been fully established. In contrast to the potential stimulatory effect of epinephrine on muscle glycogenolysis and CHO oxidation ¹⁴², some evidence suggest that epinephrine inhibits skeletal muscle glucose uptake in exercising humans ^{215,222} whilst another study demonstrated an increase in glucose uptake during moderate-intensity exercise ²⁶⁴.

In a more recent study, Watt et al. ⁴³⁷ examined the combined effects of elevated plasma epinephrine and 20 min of exercise at ~60% $VO_{2\max}$ on skeletal muscle glycogenolysis, glucose uptake and pyruvate dehydrogenase (PDH) activation in moderately trained men. With increased glycolytic flux, an increase in pyruvate flux must also occur to facilitate a higher CHO oxidation. The entry of CHO into the oxidative pathways is regulated by PDH, and PDH activation is linked to exercise intensity, substrate availability and intrinsic regulators related to energy state of the cell (discussed in detail in ref. ⁴³⁷). The study of Watt et al. ⁴³⁷ comprised of either saline or epinephrine infusion 5 min prior to and throughout exercise. Glucose kinetics were determined by the use of a 6,6-[2 H]glucose tracer and muscle samples were obtained prior to and at 1 and 20 min of exercise. Epinephrine infusion resulted an increase in total CHO oxidation by 18%, mainly due to greater skeletal muscle glycogenolysis ($p < 0.05$) and PDH activation ($p < 0.05$). Glucose rate of appearance was not different between trials, but the epinephrine infusion significantly decreased muscle glucose uptake, and elevated muscle glucose 6-phosphate (G-6-P) and lactate concentrations compared to saline infusion. No

changes were observed for pyruvate, creatine, phosphocreatine, ATP and the calculated free concentrations of ADP and AMP. It was concluded that elevated plasma epinephrine concentrations during moderate exercise in untrained men increase skeletal muscle glycogenolysis and PDH activation, which results in greater CHO oxidation. The greater muscle glycogenolysis appears to be due to increased glycogen phosphorylase transformation whilst the increased PDH activity cannot be readily explained. Finally, the decreased glucose uptake observed during exercise with epinephrine infusion is likely to be due to the increased intracellular G-6-P and a subsequent decrease in glucose phosphorylation ⁴³⁷.

In a subsequent study, Watt et al. ⁴³⁶ examined the effect of epinephrine on glucose disposal during 40 min of exercise at 60% $VO_{2\max}$ when glycogenolytic flux was limited by low preexercise skeletal muscle glycogen availability in moderately trained men. Subjects cycled for 40 min at ~60% $VO_{2\max}$ either with saline or epinephrine infusion starting after 20 min of exercise. On the day before each experimental trial, subjects completed fatiguing exercise and then maintained a low CHO diet to lower muscle glycogen content. Muscle samples were obtained after 20 and 40 min of exercise, and glucose kinetics were measured using a 6,6-[2 H]glucose tracer. Exercise increased plasma epinephrine above resting concentrations in both trials, and plasma epinephrine was significantly higher during the final 20 min with epinephrine infusion compared to saline infusion. Muscle glycogen concentrations were low after 20 min of exercise (28 ± 6 vs. 29 ± 5 mmol/kg w.w. for saline vs. epinephrine infusion, respectively). Under these conditions, epinephrine infusion over the next 20 min of exercise had no effect on net muscle glycogen breakdown or on muscle G-6-P concentrations. Plasma glucose increased with epinephrine infusion (i.e. from 20-40 min), due to a decrease in glucose disposal (Rd) (40 min: 34 ± 3 vs. 21 ± 5 $\mu\text{mol/kg/min}$ for saline vs. epinephrine infusion, respectively, $p < 0.05$) as the exercise-induced rise in rate of glucose appearance was similar between trials. These results show that glucose Rd during exercise is reduced by elevated plasma epinephrine, even when muscle glycogen availability and utilization are low. In contrast to the previous findings ⁴³⁷, these results suggest that the effect of epinephrine does not appear to be mediated by increased G-6-P, secondary to enhanced muscle glycogenolysis, but may be linked to a direct effect of epinephrine on sarcolemmal glucose transport ⁴³⁶.

Collectively taken, the bulk of the evidence suggests that epinephrine may increase glycogenolysis and increase CHO oxidation, whilst decreasing muscle glucose uptake during moderate-intensity exercise in trained athletes. However, other factors involved in muscle glycogenolysis such as exercise intensity and energy status and demand of the muscle may at times dictate the rate of muscle glycogenolysis irrespective of epinephrine concentration. Although the underlying mechanism remains unclear, the results ⁴³⁶ suggest that, in contrast to situations in which preexercise glycogen content is normal ^{142,437}, epinephrine may not enhance

muscle glycogenolysis when commencing moderate-intensity exercise with low glycogen content.

1.4.4.5 *Effects of elevated FFA concentrations on CHO oxidation, and in particular, muscle glycogen utilization*

Studies in which intralipid and heparin were co-infused during exercise in order to increase FFA availability (to 1 to 2 mmol/L), have demonstrated an increase in fat oxidation and a concomitant decrease in CHO oxidation, predominantly through reduced muscle glycogen utilization (for example refs. ^{87,128,329,361}).

Costill et al. ⁸⁷ elevated plasma FFA concentrations to ~1.0 mmol/L with heparin infusion in 7 endurance-trained men during 30 min of treadmill exercise (~70% $VO_{2\text{ max}}$) and demonstrated a 40% reduction in muscle glycogenolysis compared to the control trial. The elevation of FFA in the study of Costill et al. ⁸⁷ was also associated with an increase in blood glucose concentration, which the authors ascribed to a possible inhibitory effect of accumulated glucose 6-phosphate on hexokinase, thereby limiting the entry of glucose into the cell ^{166,433}.

In a study by Romijn et al. ³⁶¹, intralipid and heparin infusion during 20 to 30 min of exercise at 85% $VO_{2\text{ max}}$ resulted in increased FFA availability and a 27% increase in fat oxidation with a concomitant 15% reduction in the rate of muscle glycogen utilization. This effect has been attributed to the inhibitory effect of citrate on phosphofructokinase ¹⁶⁶. Increased FFA oxidation produces an accumulation of citrate in rat heart and skeletal muscle, thereby limiting the rate of glycolysis ^{167,354}. However, this effect has not consistently been demonstrated in humans ¹²⁹. Hargreaves et al. ¹⁸⁹ found that lipid-heparin infusion during 1 h of knee extension exercise (80% of work capacity) increased FFA concentrations to 1.1 mmol/L, but had no effect on muscle glycogen utilization nor were muscle citrate release or G-6-P concentrations altered. They did demonstrate a 33% reduction in muscle glucose uptake.

In a study by Dyck et al. ¹²⁹ lipid-heparin infusion raised FFA concentrations to ~1.4 mmol/L during short-term (15 min) high-intensity exercise (85% $VO_{2\text{ max}}$) and this resulted in significant muscle glycogen sparing, but with no differences in acetyl-CoA or citrate concentrations, or PDH activity. The authors suggested that the glycogen sparing effect may result from an attenuation at the level of glycogen phosphorylase. To investigate this mechanism, Dyck et al. ¹²⁸ repeated the previous study and found that 7 of the 11 subjects demonstrated glycogen sparing, however glycogen phosphorylase transformation into the active *a* form was unaltered whilst the calculated free ADP, AMP and Pi contents were lower. AMP is an allosteric activator of glycogen phosphorylase *a*, and Pi a substrate of glycogen phosphorylase ¹²⁸. In support of these findings,

Odland et al.³²⁹ also demonstrated a 23% reduction in muscle glycogen utilization and reduced free AMP accumulation with lipid-heparin infusion during 60 min of moderate-intensity exercise (65% $\text{VO}_2 \text{max}$). In their study glycogen phosphorylase was not measured, but the glycogen sparing was associated with higher muscle citrate concentrations and a reduced muscle PDH activity in the more active form¹²⁸.

Though adaptations to long-term high fat diets are beyond the scope of this thesis, a series of studies by Helge et al.^{199,202,203} on the interaction between diet and training on substrate metabolism have yielded very interesting results. It was shown that adaptation to a high fat diet and exercise training for 7 wk, followed by 1 wk of high CHO intake in healthy men resulted in an increase in fat utilization and reduced muscle glycogenolysis compared to 8 wk on a high CHO training diet. It was demonstrated that even when muscle glycogen concentrations were elevated with the high CHO intake following prolonged high fat diet adaptation, muscle glycogenolysis during exercise appeared to be impaired, and that subjects were unable to fully make use of the muscle glycogen stores in that exhaustion was reached with large amounts of glycogen left-over in the muscle¹⁹⁹. The molecular mechanism behind the impaired muscle glycogenolysis observed after prolonged adaptation to a high fat diet followed by a high CHO diet for 1 wk remains to be clarified. Additionally, in a subsequent study following a similar research design, it was demonstrated that leg glucose uptake during 60 min of exercise at 70% $\text{VO}_2 \text{max}$ was reduced after prolonged adaptation to a high-fat diet followed by a shift to a high CHO diet for 1 wk²⁰³.

From the bulk of available data it is evident that increased FFA availability, specifically when FFA concentrations in the blood are >1 mmol/L, can increase fat oxidation and reduce CHO oxidation, mainly through reducing the rate of muscle glycogen utilization and perhaps also reduced glucose uptake by the muscle. However, the regulatory factors involved in this effect remains to be fully elucidated.

1.4.4.6 Effects of exogenous CHO supplementation during exercise on muscle glycogen utilization

CHO intake before exercise and a concomitant increase in insulin concentration have been shown to increase CHO oxidation and thus increase reliance on muscle glycogen for energy⁸⁷. The effect of pre-exercise CHO ingestion will be discussed in more detail in section 5.1, whereas this section will focus specifically on the effects of CHO ingestion during exercise on muscle glycogen utilization.

The intake of CHO during prolonged exercise has been shown to enhance CHO availability and to improve endurance exercise capacity when exercising at a constant speed or workload, and to improve performance during a high-intensity exercise test (time-trial) performed immediately after a prolonged intermittent or continuous, steady-state exercise bout (for review see ⁴¹⁴). The exact mechanism(s) whereby CHO ingestion may delay fatigue has not been established, but the two major effects regarded as being responsible for the ergogenic effect of CHO supplementation, has been the maintenance of euglycemia and CHO oxidation throughout exercise, and/or a decreased rate of muscle glycogen utilization (discussed in detail under section 1.5.2).

Whilst CHO ingestion during prolonged exercise has been shown to decrease the rate of muscle glycogen utilization in some studies ^{38,132,185,415,418,455,456}, others demonstrated similar rates of muscle glycogenolysis with CHO or placebo ingestion ^{89,149,193,306}. The discrepancy in results may be related to the ingestion of differing amounts of CHO, different exercise protocols (e.g. exercise intensity as discussed under section 4.3.1, or intermittent vs. steady-state exercise).

The bulk of the studies have employed cycling as the mode of exercise ⁴¹⁴. It appears that exogenous CHO supplementation (~25 to 100 g/h) during steady-state cycling protocols (~70% $VO_{2\text{ max}}$) in trained, non-CHO loaded subjects has minimal effect on the rate of muscle glycogen oxidation ^{89,193,306}. However, these results are not clear-cut as results from the studies of Coyle et al. ⁸⁹, Erickson et al. ¹³² and Bosch et al. ³⁸, performed under similar conditions, suggest the opposite. For example, in the study of Coyle et al. ⁸⁹ trained, non-CHO loaded male subjects cycled to exhaustion at 70% $VO_{2\text{ max}}$ firstly without CHO supplementation, and, on another occasion, with CHO supplementation (2 g CHO/kg at 20 min, followed by 0.4 g CHO/kg every 20 min thereafter). CHO ingestion maintained plasma glucose concentration and enabled subjects to exercise for 4 h compared to 3 h in the placebo trial. The pattern of muscle glycogen utilization was similar between trials over the first 3 h of exercise and muscle glycogen concentrations were equal at the end of 3 h of exercise (~100 mmol/kg w.w.). However, despite having a considerable amount of muscle glycogen left in the muscle, no further muscle glycogen was used during the additional hour of exercise in the CHO ingestion trial, whilst CHO oxidation was maintained. These data suggest that CHO ingestion may have resulted in muscle glycogen sparing within the last hour of exercise. Similarly, in the study of Bosch et al. ³⁸ trained, non-CHO loaded male subjects ingested either 50 g/h of CHO or placebo whilst cycling for 180 min at 70% $VO_{2\text{ max}}$. Total CHO oxidation was similar in the CHO-ingestion and placebo trials and declined similarly during exercise. Muscle glycogen disappearance was identical during the first 2 h of exercise in both groups and continued at the same rate in the placebo ingestion trial, however, no net muscle glycogen disappearance occurred during the final hour in the CHO-ingestion trial. It was concluded that the ingestion of 500 ml/h of a 10% CHO solution during

prolonged exercise in non-CHO loaded subjects has a marked liver glycogen-sparing effect, maintains plasma glucose concentration and has a muscle glycogen-sparing effect. Erickson et al.¹³² demonstrated a reduction in the amount of muscle glycogen used over a somewhat shorter exercise period as that of Coyle et al.⁸⁹ and Bosch et al.³⁸. During 90 min of steady-state exercise at 65-70% $\text{VO}_{2\text{ max}}$ the amount of muscle glycogen used was significantly lower after glucose ingestion (1 g/kg) compared to placebo ingestion (62 vs. 91 mmol/kg w.w. for glucose vs. placebo ingestion, respectively).

Evidence of reduced muscle glycogenolysis with CHO supplementation during exercise has frequently been observed in studies where a variable-intensity or self-paced cycling^{185,445,456}, or 60-100 min constant or intermittent running protocols were employed (reviewed in⁴¹⁴).

A detailed revision of the literature on CHO ingestion and muscle glycogen utilization by Tsintzas and Williams⁴¹⁴ suggest that the magnitude of the insulin response in addition to an increase in blood glucose concentrations may determine whether glycogen sparing will take place or not. The magnitude of the insulin response may largely be influenced by the exercise intensity, as the greater catecholamines stimulation at higher exercise intensities result in greater suppression of insulin secretion compared with lower intensities⁴¹⁴.

It is clear from the above evidence that the rate of muscle glycogen utilization is controlled and affected by a complex interplay of factors, of which exercise intensity and muscle glycogen availability at the onset of exercise perhaps are the main determinants of muscle glycogen use during exercise, and will to a large extent determine to what extent the other factors (such as CHO ingestion) will affect the ultimate rate of glycogen utilization. Though the ergogenic effect of CHO supplementation may be explained by a reduction in the rate of muscle glycogen utilization during exercise, it cannot explain the ergogenic effect in the bulk of the studies where the rate of muscle glycogen utilization did not differ between CHO compared to placebo supplementation.

Further research on the effect of CHO supplementation on muscle glycogen utilization is clearly warranted, specifically to investigate the effect of hormonal responses as elicited at varying exercise intensities and/or with varying nutritional strategies. Specifically, there is limited literature available on the effects of CHO supplementation on muscle glycogen utilization in humans commencing exercise in the CHO-depleted state.

1.4.5 Regulation of muscle glucose uptake during exercise

It is believed that glucose transport during exercise is mainly regulated by the contraction-stimulated AMPK pathway and this effect lasts 3-6 h post-exercise^{250,355}. AMPK increases

GLUT-4 translocation from the intrasarcolemmal vesicles to the membrane to enable glucose uptake by the muscle cell^{250,355,447}. AMPK has been referred to as an intracellular “masterswitch” which activates fat oxidation and glucose uptake in relation to the increased rate of ATP utilization during muscle contraction⁴⁰¹. However, this suggestion is not universally accepted as changes in muscle AMPK or components of this signaling pathway have not consistently shown to track changes in the substrate (fat) oxidation¹.

Factors such as exercise intensity (with increasing exercise intensity, the rate of plasma glucose uptake and oxidation increases^{89,220,360,365,393}), glucose availability⁹², prevailing epinephrine concentration, membrane transport and intracellular metabolism (as discussed in section 1.4.4.4) have also been identified as playing a regulating role in muscle glucose uptake during exercise.

The bulk of the scientific literature indicate that the combination of insulin and exercise act synergistically (albeit via different mechanisms) to increase membrane GLUT-4 and glucose uptake by the muscle⁴⁵⁷. In addition, studies using the isolated perfused rat hind-limb model have shown that increased muscle blood flow and increased glucose delivery may add to the effect of muscle contraction to increase glucose uptake⁴⁵⁷.

There is evidence to suggest that the rate of muscle glucose uptake is at least partially dependent on the prevailing plasma glucose concentrations. This has been shown in men cycling at 73% $VO_{2\text{ max}}$ when hyperglycemic (10-12 mmol/L)⁹² and with CHO ingestion and subsequent increase in plasma glucose concentration during low-intensity⁴ and prolonged strenuous exercise²⁹⁹.

1.4.6 Interaction between muscle glycogen availability and blood glucose uptake during exercise

Hargreaves et al.¹⁹¹ studied six men during 40 min of cycling (65-70% $VO_{2\text{ max}}$) to examine the relationship between leg glucose uptake and muscle glycogen concentration. Like others^{118,173}, they observed a significant inverse relationship between muscle glycogen concentration and glucose uptake during exercise, and suggested a possible regulatory influence of muscle glycogen on glucose uptake during the early stages of exercise.

However, the effect of preexercise glycogen concentration on glucose uptake has yielded inconsistent results. Some studies found that tracer-determined rates of glucose uptake were unaffected by preexercise muscle glycogen availability during a similar 40 min cycling protocol¹⁹⁰ than that used by the study of Hargreaves et al.¹⁹¹ mentioned above, as well as during a more prolonged exercise protocols (180 min, 70% $VO_{2\text{ max}}$)^{35,440,441}. However, with this experimental

approach where glycogen content is often manipulated after ingestion of either a low CHO (high fat) vs. high CHO diet, substrate availability and the hormonal milieu is typically altered^{162,440,441}, which may influence glucose uptake by the contracting muscles independent of glycogen availability. For example, a high vs. low dietary CHO intake results in altered epinephrine secretion¹⁶², which has been demonstrated to influence glucose uptake⁴³⁷.

Another experimental approach used to determine the effect of glycogen content on glucose uptake during exercise is to deplete one leg of glycogen by doing single-limb exercise 24 to 48 h prior to performing two-legged exercise. Studies employing this approach have demonstrated a higher glucose uptake in the leg that commenced exercise with lower glycogen content (due to prior exercise) compared to the previously rested leg that started exercise with normal glycogen content^{23,173}. This approach has the advantage of ensuring a similar substrate availability and hormonal milieu during the subsequent two-legged exercise trial. However, GLUT-4 transporter protein will most likely be increased in the limb that did prior exercise⁴⁴⁹, which may in turn be responsible for the increase in glucose uptake during subsequent exercise, and not the glycogen content *per se*. GLUT-4 gene and protein expression were not measured during these studies^{23,173}.

Steensberg et al.³⁹⁹ aimed to clarify the effect of glycogen content on glucose uptake by performing two series of experiments. In the first series, 7 healthy subjects performed 4 h of two-legged knee extensor exercise, 16 h after performing 60 min of single-leg cycling in order to deplete muscle glycogen content of one leg (series 1). In the second series, 6 subjects performed two trials of 3 h of two-legged knee extensor exercise: on the one occasion after performing 60 min of two-legged cycling 16 h prior to the trial followed by a low CHO diet, and on the other occasion with similar exercise followed by a high CHO diet (series 2). Glucose uptake, glycogen content and GLUT-4 gene and protein expression were measured. Muscle glycogen was decreased by 40% when comparing the pre-exercised leg with the control (previously rested) leg prior to exercise in series 1. In addition, muscle glycogen was decreased by the same magnitude when comparing the effects of the low vs. high CHO diet interventions in series 2. In series 1, glucose uptake was 3-fold higher in the pre-exercised leg (series 1) in the first 60 min of exercise, in the presence of unchanged pre-exercise GLUT-4 protein compared to that in the control (previously rested) leg. These data suggest that it is the lower glycogen and not the exercise the day before that might have provided the stimulus for increased glucose uptake. Despite the same magnitude of difference in pre-exercise glycogen concentration when comparing series 1 with series 2, neither direct- nor isotope-determined rate of glucose uptake were altered when comparing the low vs. high CHO diet intervention in series 2. However, arterial concentrations of insulin and glucose were lower, while free fatty acids and epinephrine concentrations were higher in low CHO compared with high CHO diet intervention. These data

suggest that pre-exercise glycogen content may influence glucose uptake during subsequent exercise. However, this is only the case when delivery of substrates and hormones remains constant. When delivery of substrates and hormones is altered, the potential effect of glycogen on glucose uptake is negated³⁹⁹.

From the results of Steensberg et al.³⁹⁹ it seems likely that the metabolic and hormonal alterations elicited by a low CHO diet may bring about alterations in other parts of the gluco-regulatory system (such as hepatic glucose output, glycolysis, glycogenolysis), as well as alterations in the availability of alternative oxidative substrates (e.g. FFA) which may alter the fuel selection and relative contribution of substrate oxidation within the muscle.

In review of this section it is clear that glucose (derived from endogenous production or exogenous supplementation) is an important fuel source (for brain and skeletal muscle) during prolonged exercise. We know that during such exercise, muscle and subsequently liver glycogen stores become depleted^{78,91}, which poses a challenge for the maintenance of glucose homeostasis. A complex of neuro-hormonal factors have been identified, mainly in animal models, with some being confirmed in humans, as playing a role in the regulation of glucose and overall substrate metabolism at rest and during exercise, however, several of these factors and the interaction between them are not fully understood. It is clear that factors such as the status of the endogenous CHO stores, substrate availability and exercise intensity and duration play an important role in altering the neuro-humoral milieu, substrate selection and oxidation and ultimately glucose homeostasis. The full extent of the interplay of these factors is not yet fully understood.

The following section of this literature review will consider the impact of interventions resulting in alterations in endogenous CHO stores, hormonal milieu and substrate availability prior to and during prolonged exercise and how it affects gluco-regulatory responses, exercise metabolism and ultimately exercise performance.

1.5 EFFECT OF ANTECEDENT NUTRITIONAL INTERVENTION ON GLUCOSE HOMEOSTASIS AND RELATED NEURO-HORMONAL RESPONSES AND FATIGUE DEVELOPMENT DURING PROLONGED EXERCISE

1.5.1 *Acute alterations in hormonal milieu: Effect of type and timing of CHO intake immediately prior to prolonged exercise on hormonal responses, substrate utilization and exercise performance*

The depletion of muscle glycogen and development of hypoglycemia have been associated with fatigue development, and as a result CHO supplementation immediately before and during prolonged exercise have been identified as strategies that can contribute to substrate availability with potential performance-enhancing benefits (for review see ^{52,220}). However, whilst the ergogenic potential of CHO supplementation during prolonged exercise is well established, the effect of preexercise CHO ingestion on exercise metabolism and performance is less clear.

Though preexercise CHO ingestion can help to optimize liver and muscle glycogen, particularly where an overnight fast or previous exercise has reduced these bodily CHO stores ¹⁹⁵, this strategy has raised some concerns, particularly related to CHO consumed during the hour prior to exercise as it may result in hyperglycemia and hyperinsulinemia, and a potential fall in glucose (rebound hypoglycemia) during exercise ^{3,67,152,260,288}. The rebound hypoglycemia at the onset of exercise is in consequence to the combined effects of relative hyperinsulinemia and muscle contraction on increasing glucose uptake and inhibition of the exercise-induced increase in hepatic glucose output ^{145,266}. Other potential consequences of relative hyperinsulinemia include an increase in CHO oxidation and a reduction in the rate of fat oxidation (as discussed in detail under section 1.3.2), which in turn may lead to increased muscle glycogen oxidation ⁹⁰.

The development of hypoglycemia following preexercise CHO ingestion has been observed in some studies ^{67,152,167,260,266,305,426}, however, this finding is not universally consistent ^{69,114,147,171,166,279,286,294,346,374}. Furthermore, whilst two studies on preexercise CHO (sugar) intake within the hour prior to exercise reported a large decline in blood glucose concentrations (<3.5 mmol/L) and an increase in muscle glycogen use during exercise ^{67,187}, several others have found no effect on the rate of muscle glycogenolysis ^{147,171,166,259,279}. One possible explanation for this discrepancy may be that the hypoglycemia in the latter studies ^{147,171,186,259,279} was not as pronounced compared to that found in the studies of Costill et al. ⁶⁷ and Hargreaves et al. ¹⁸⁷.

Though the metabolic and hormonal sequelae of preexercise CHO intake in the studies mentioned above have traditionally been associated with premature fatigue development during endurance exercise, when examining the effect of preexercise CHO ingestion on exercise

performance, whilst one study found an ergolytic effect ¹⁵², the bulk of subsequent studies reported either no effect ^{69,141,145,186,228,230,312,389,389,439} or an improvement in performance during endurance exercise ^{171,251,330,376,408}. Based on these results, ingesting CHO within the hour prior to exercise is a strategy that should not be discouraged as evidence for its potential ergogenic effect far outweighs evidence for a potential ergolytic effect.

Whilst the discrepancies in the results may be related to differences in research design, a recent series of systematic experiments by the same laboratory have indicated that when methodological factors are controlled for, the amount of CHO ingestion (25; 75 or 200 g CHO ²²⁸); timing of CHO feeding (15, 45 or 75 min prior to exercise ³¹²); type of CHO intake (glucose, trehalose or galactose, ²³⁰); and intensity of exercise (40, 65 or 80% maximum work rate ²) have no effect on exercise performance. Furthermore, when the average group data were examined, none of the above factors resulted in rebound hypoglycemia (plasma glucose <3.5 mmol/L). However, when examining the individual data in these studies, rebound hypoglycemia was observed in some of the subjects. In particular, n=3 to 4 (of n=8) developed hypoglycemia when exercise intensity was varied between 40 to 80% of maximal work rate; n=2 vs. 3 vs. 5 (of n=8) developed hypoglycemia when CHO ingestion was delayed from 15 to 45 to 75 min before exercise, respectively; prevalence of hypoglycemia increased when high glycemic index (glucose, n=4 out of n=8) was ingested compared to trehalose (n=1) or galactose (n=0); and 4 to 6 subjects (of n=9) when the amount of CHO ingested varied from 25 to 200 g. Kuipers et al. ²⁶⁶ observed that hypoglycemia was more likely to occur when moderate-intensity exercise was commenced after ingesting small amounts of CHO (40-60 g). When 70 and 80 g of CHO was ingested preexercise, no hypoglycemia (plasma glucose <3.0 mmol/L) was observed ²⁶⁶. However, this is clearly not a given seeing that ~50% of subjects demonstrated rebound hypoglycemia in the study of Jentjens et al. ²²⁸ when up to 200 g of CHO was ingested preexercise.

It is not yet known why some individuals are predisposed to the development of rebound hypoglycemia following preexercise CHO ingestion and others not. It has been suggested that an increased insulin sensitivity (e.g. as a consequence of exercise training ^{34,200}) may lead to the development of rebound hypoglycemia. Recently, this theory was discounted when it was shown that subjects, varying in training status, who developed rebound hypoglycemia did not have a higher insulin sensitivity than those who did not develop rebound hypoglycemia ²²⁹. In fact, there was a similar range in insulin sensitivity index (ISI) between the group who developed hypoglycemia (2.1 to 6.0) and those who did not (2.6 to 7.4) ²²⁹.

Kuipers et al. ²⁶⁶ proposed that rebound hypoglycemia may be more likely in those individuals in whom sympathetic-induced counterregulation is less activated. This effect may be related to

increased parasympathetic activity in trained athletes⁶². In the study of Kuipers et al.²⁶⁶ 50 g of glucose was ingested after a 4-h fast and 30 min prior to 40 min of exercise at 60% maximal power output. Six subjects (of n=19) developed transient rebound hypoglycemia (hypo group, plasma glucose <3 mmol/L; n=10 with plasma glucose <3.5 mmol/L) during the first ~30 min of exercise (and a return to basal levels towards the end of exercise), and displayed reduced norepinephrine concentrations throughout most of the exercise bout compared to the group of subjects who did not develop hypoglycemia (non-hypo group). Insulin concentrations were similar between the two groups. This study indicated that rebound hypoglycemia may not only occur after an over-night fast, but also when consuming CHO after a 4-h fast. Furthermore, though epinephrine concentrations were similar between groups, a large inter-individual variation was noted. Norepinephrine concentrations, on the other hand, were lower in the hypo-group compared to the non-hypo group, which reflects a lower activation of the sympathetic-mediated counterregulation. However, exercise intensity *per se* may activate the sympathetic system (separate from declining blood glucose concentrations) and improve blood glucose maintenance, as was shown when two of the subjects from the hypo-group repeated the protocol, but exercised at 75% of maximal power output without developing hypoglycemia. In contrast, the study by Achten and Jeukendrup² showed that occurrence of rebound hypoglycemia in susceptible individuals was unrelated to exercise intensity.

Further research is needed to elucidate the extent of inter-individual variability in sympathetic activation and counterregulatory responses and the degree to which this may affect postprandial plasma glucose concentrations at rest and during exercise.

In summary, it appears that in the majority of cases, any perturbations in glucose and insulin levels due to preexercise CHO intake are transient and overridden by the metabolic response to exercise (for review see¹⁹⁵). However, a small percentage of athletes may be sensitive to exaggerated hormonal and metabolic responses to preexercise feedings of CHO. It is possible that these responses may be attenuated by choosing CHO sources which produce a minimal glycemic-insulinemic response¹⁹⁵. Such CHO sources include fructose¹⁸⁶ and CHO-rich foods with a low glycemic index (GI). Ingestion of low-GI CHO foods produce less metabolic disturbances (reduced glycemic and insulinemic response) postprandially, compared with the intake of the same quantity of high-GI CHO²²⁶. A further benefit may be a more sustained glucose release during prolonged endurance events (e.g. swimming) or strenuous work environments (fire-fighting) where frequent CHO supplementation to maintain blood glucose concentrations may not be possible.

1.5.1.1 Type of CHO ingested preexercise: Role of Glycemic Index (GI)

The GI is determined by the rate at which an ingested CHO is made available to intestinal enzymes for digestion and absorption²²⁶. The GI is calculated as a percentage value based on the area under the blood glucose curve following the ingestion of a food containing 50 g of available CHO, divided by the area of the blood glucose response to 50 g CHO in a reference food, multiplied by 100²²⁶. It has been suggested that the manipulation of the GI of meals may have application in the area of sports nutrition to manipulate metabolic and hormonal perturbations and to optimize CHO availability for exercise, particularly prolonged moderate intensity exercise¹⁹⁵.

Thomas et al.⁴⁰⁸ were the first to study the role of the GI of different CHO-containing foods and their effects on metabolism and exercise performance. They reported that the ingestion of a low GI CHO-rich meal (lentils), 1 h prior to exercise increased the time to exhaustion during the cycling trial at 67% $\text{VO}_{2\text{max}}$, compared with the ingestion of an equal amount of CHO eaten in the form of a high GI CHO-rich food (potatoes). Time to exhaustion after ingestion of the low GI food was increased compared to preexercise glucose feeding, or a water trial. These findings were attributed to lower glycemic and insulinemic responses to the low GI trial compared with the other CHO feedings. This promoted a more stable blood glucose response during exercise, increased FFA concentrations and also reduced exercise respiratory exchange ratio (RER) values. Although muscle glycogen was not measured, the authors suggested that glycogen sparing may have occurred with the low GI CHO trial⁴⁰⁸.

This study supports the view regarding the metabolic perturbations caused by CHO feedings in the hour before exercise, and suggests an alternative way to increase CHO availability during exercise while decreasing the likelihood of rebound hypoglycemia in response to preexercise CHO feedings. These findings have been widely publicized and are largely responsible for the advice of some sports nutrition experts that athletes should choose preexercise meals based on low GI CHO-rich foods and drinks.

In contrast, a subsequent study by Febbraio and Stewart¹⁴⁵ reported no differences in the performance of a cycle time-trial conducted after 2 h of cycling at 70% $\text{VO}_{2\text{max}}$, when preexercise meals eaten 45 min before the bout consisted of either a low GI CHO (lentils), high GI CHO (potatoes) or placebo (diet jelly). They reported no difference in total CHO oxidation between the two CHO treatments, and similar muscle glycogen utilization in all three trials. These findings are more consistent with the view that glycemic differences at the onset of exercise are short-lived and of little practical significance¹⁹⁵.

Other more recent studies with similar research designs have also failed to support the findings of the first investigation by Thomas et al.⁴⁰⁸. Ingestion of a low GI compared to a high GI preexercise meal has been shown to reduce CHO oxidation during exercise in some^{389,409,439}, but not all studies^{145,210,251}. Furthermore, in the few studies where muscle glycogen content was measured, a high versus a low GI preexercise meal either did not have any effect^{145,251}; or showed a tendency ($p=0.07$) towards augmenting the rate of muscle glycogenolysis¹⁴¹. Interestingly, a recent study by Li et al.²⁸⁰ demonstrated that a low GI (vs. high GI) meal ingested 3 h prior to a 90 min treadmill run (70% $VO_{2\text{ max}}$) resulted in significantly higher plasma glucose concentrations between 15 to 45 min of exercise, but a reduced neutrophil/lymphocyte ratio, whilst cortisol, growth hormone and IL-6 concentrations were similar between trials.

Febbraio et al.¹⁴¹, by performing multiple biopsies, aimed to investigate if muscle glycogenolysis may be affected in a biphasic manner, i.e. altered rates of glycogenolysis early compared to late in exercise. It was hypothesized that a high GI meal will result in a more perturbed glycemic and insulinemic response, which would result in an enhanced rate of plasma glucose uptake and oxidation and reduce glycogenolysis early in exercise, but a reduced rate of glucose oxidation and an augmented rate of glycogenolysis later in exercise. A meal consisting of a high or low GI (1 g CHO/kg body mass (BM), isocaloric) or placebo was ingested 30 min prior to 120 min of steady-state exercise (70% $VO_{2\text{ max}}$) followed by a 30-min time trial. Glucose and insulin concentrations were significantly higher and FFA concentrations more suppressed after the high GI preexercise meal, particularly in the 30 min postprandial period and over the initial ~30 min of exercise, where after responses became more comparable between trials. Blood glucose concentrations during the high GI trial remained above ~4.3 mmol/L. CHO oxidation was higher and fat oxidation lower after ingestion of the high GI compared to the low GI meal, however, exercise performance was unaffected. The increased rate of CHO oxidation in the high GI trial was most likely due to the increased rate of plasma glucose oxidation, as muscle glycogen utilization tended to be augmented, but not significantly so. In addition, the high GI meal made no difference to the rate of muscle glycogenolysis early in exercise. Their data also indicated that even when glucose and insulin concentrations returned to similar levels than in the low GI trial, the rate of glucose oxidation remained elevated and FFA's depressed, suggesting the persistent effect of hyperinsulinemia after insulin returns to normal levels during exercise¹⁴¹.

In contrast, in a similar study performed by the same laboratory¹⁴⁵, similar rates of muscle glycogenolysis and FFA concentrations were reported after ingestion of a low vs. a high GI meal. An explanation for the discrepancy in results posed by Febbraio et al.¹⁴¹ is the possible variation in insulin sensitivity between study populations used in these studies. Where no difference in FFA concentrations were observed between the high and low GI trials, subjects may have been more sensitive to even just a small rise in insulin associated with the low GI trial, but enough to

blunt lipolysis to the same extent as the large rise in insulin associated with the high GI trial ¹⁴¹. However, direct measures of insulin sensitivity were not made and this suggestion therefore remains speculative.

Lastly, when assessing the effect of a low compared to high GI preexercise meal on exercise performance, three studies reported an improvement in exercise performance (time to exhaustion) following a low GI ^{117,408} or moderate GI meal ²⁴⁹, whilst others found no effect on exercise performance when employing time to complete a set amount of work to assess endurance cycling ^{141,145,388,409} or running performance ⁴³⁹. It should be noted though that time to complete a set amount of work (or time-trial) as measure of performance is more representative of real-life racing conditions (where there is a defined end-point) as well as being a more reliable and reproducible measure of endurance exercise performance ^{232,301}.

Collectively taken, the results from the various studies discussed above suggests a wide inter-individual range of metabolic responses to preexercise CHO ingestion, even when preexercise CHO ingestion is quantified in terms of glycemic index, the hormonal and metabolic responses remains complex and largely individual. However, despite potential alterations in the metabolic and hormonal response, exercise performance as measured by the time to complete a set amount of work, seems to be unaffected.

1.5.2 Longer-term alteration in hormonal milieu: The effect of altering preexercise glycogen availability via a low-CHO diet

Muscle glycogen stores can be manipulated by alterations in dietary macronutrient intake and exercise. A reduction in glycogen stores, for example by following a low CHO diet, has been shown to elicit an altered hormonal and metabolic milieu ⁴⁴⁰ such as an increase in glucagon, ACTH and cortisol concentrations, providing a stimulus for enhanced hepatic glycogenolysis and gluconeogenesis ^{223,440,441} and a shift towards fat oxidation ³⁰⁷. Furthermore, it seems that this altered metabolic response persists during exercise even when fuel availability is maintained through exogenous CHO supplementation. In fact, Weltan et al. ⁴⁴¹, demonstrated that, only under conditions of insulin infusion or hyperglycemia in subjects starting exercise in a glycogen-depleted state, were the metabolic effects of a low CHO diet and low glycogen counteracted.

CHO-loading diets have traditionally been associated with improved endurance capacity or performance, ascribed mainly to increased endogenous CHO availability and maintenance of a high rate of CHO oxidation throughout exercise. Low CHO diets, on the other hand, have been associated with impaired endurance exercise capacity, mainly ascribed to the reduction of muscle glycogen availability and declining blood glucose concentration (development of

hypoglycemia) and a concomitant decrease in the rate of total CHO oxidation^{73,74,89,162,190,223,234,440}. However, the exact mechanism(s) whereby reduced muscle glycogen and/or hypoglycemia affect endurance exercise capacity is not fully clear.

1.5.2.1 Potential mechanisms for the purported association between muscle glycogen availability and fatigue development during exercise

A strong relationship between muscle glycogen depletion and the onset of fatigue have been documented in the literature¹⁴⁸. Furthermore, an interesting observation from the literature is that the typical level of muscle glycogen below which fatigue usually sets in, is ~40 mmol/kg w.w. It has long been believed that the main association between fatigue and glycogen depletion is the reduction in available fuel to sustain muscle contraction. Yet, no association between ATP (fuel) depletion at the point of fatigue have ever been demonstrated, in fact, minimal ATP depletion in the light of substantially depleted muscle glycogen and creatine phosphate stores have been reported⁴³².

In studies employing steady-state cycling protocols performed to exhaustion at moderate-high exercise intensities (~70% $\dot{V}O_{2\text{ max}}$), subjects commonly develop fatigue at muscle glycogen concentrations of ~40 mmol/kg w.w.^{35,37,73,89}. This observation might be explained by recent evidence suggesting that muscle glycogen may act as a feed-forward metabolic signal that informs the individual at what point they should slow down or stop exercise to prevent the development of absolute substrate depletion³⁵⁰. It has been suggested³²⁸ that peripheral inhibitory input is conveyed from metabo- and chemo-receptors that are sensitive to chemical changes within the muscle, which may, via group III and IV afferents, affect supraspinal parts of the central nervous system which may then reduce central motor drive. Some evidence in support of this suggestion comes from studies demonstrating, for example, that group III and IV muscle afferents invoke circulatory and ventilatory reflexes during exercise³⁰⁸ and provide feedback from metabolic disturbances such as lactic acid production³⁸³, pain⁸⁵ and mechanoreception^{308,340}.

Other mechanisms that have been explored to explain the relationship between muscle glycogen depletion and fatigue development suggest that apart from a metabolic role, muscle glycogen availability may also have a structural role in that it may be critical for excitation-contraction coupling^{64,241,403}. Low glycogen content has been associated with reduced calcium (Ca^{2+}) uptake and release in animals^{64,241,403} and humans²⁹. In this regard, reduced glycogen concentrations may relate to fatigue via its association with reduced Ca^{2+} release from the sarcoplasmic reticulum (SR), with a subsequent reduction in force production^{29,64,241}. The link between reduced glycogen (and creatine phosphate) concentrations and fatigue may be explained by the

concomitant increase in metabolites such as inorganic phosphate (P_i), which is known to have an inhibitory effect on both Ca^{2+} release from the SR^{159,241} as well as the contractile apparatus¹⁴⁸.

It has also been hypothesized that the glycogen concentration *per se* exerts a protective action on excitation-contraction coupling^{12,403}. Barnes et al.¹² recently demonstrated that skinned rat muscle fibers from muscles with a higher glycogen content reached 50% rundown after a larger number of depolarizations and displayed consistently larger average response capacity values. It was concluded that skinned fibers originating from muscles with a higher glycogen content have an increased ability to respond to T-system depolarization when the effect of metabolite accumulation is minimized and the function of glycogen acting as an energy source is by-passed. These data provided direct support to the hypothesis that glycogen has a protective role in maintaining fiber excitability¹². However, this hypothesis remains to be tested and confirmed *in vivo* in human skeletal muscle and with intact metabolic and neuro-humoral systems.

Thus, the impact of altered glycogen concentrations on endurance exercise capacity and the mechanism(s) involved remain unclear. Furthermore, the effect of low starting muscle glycogen concentration on glucose uptake, substrate metabolism and fatigue development remains unclear.

1.5.2.2 Potential mechanisms for the purported association between development of hypoglycemia and fatigue during exercise

Blood glucose concentrations may decline during prolonged exercise as muscle and subsequently liver glycogen gets depleted⁹¹ and hepatic glucose production fails to keep up with glucose utilization by the working muscles¹⁴⁸.

1.5.2.2.1 Reduction in the rate of total CHO oxidation

A decline in blood glucose concentrations during prolonged exercise has traditionally been associated with a reduction in exercise capacity, mainly ascribed to a concomitant decline in CHO oxidation^{73,75,76}. However, other more recent studies do not support this finding^{36,100,298,416}, indicating that the frequently-quoted decline in CHO oxidation when glycogen stores are compromised compared to when CHO are in plentiful supply, are not always observed.

Alternative explanations for the ergogenic effect of CHO supplementation have come from McConell et al.²⁹⁸, suggesting that CHO supplementation (compared to placebo) may enhance endurance exercise performance by lowering muscle inosine monophosphate levels (a marker of the rate of ATP degradation vs. resynthesis), thereby improving muscle energy balance. Similarly, though still controversial, it has also been suggested that CHO supplementation may

enhance endurance exercise performance in some subjects via alternations in central nervous system function^{100,326,394}.

1.5.2.2.2 Effect of hypoglycemia on the central nervous system, and its potential association with fatigue development during exercise

With declining blood glucose concentrations during prolonged exercise (due to progressive muscle and liver glycogen depletion⁹¹ and hepatic glucose production failing to keep up with glucose utilization by the working muscles¹⁴⁶), the development of fatigue (loss of force-generating capacity) may be related, in part, to central factors¹⁶³. In human studies it is often difficult to differentiate between the contribution of peripheral vs. central factors in fatigue development during exercise, and observations are often based on indirect measures and/or temporal associations. Ratings of perceived exertion (RPE) is often used as a subjective rating of the effort perception / sensation of fatigue, however, it cannot fully differentiate between aspects of peripheral and central fatigue. Nevertheless, it has been shown to be highly reproducible and are often employed as an assessment of central drive / central fatigue during exercise. Effort perception may primarily be a feedforward system that results from the interpretation of afferent sensations around the body. It is unclear how the brain interprets all this feedback, but it has been suggested that an integration of these sensory cues may indirectly and unconsciously influence effort perception during exercise, and thereby fatigue^{182,331}.

Several studies have shown an association between the development of hypoglycemia and increased RPE, conversely, a lower RPE has been associated with a higher plasma glucose concentrations, higher CHO oxidation, and lower plasma cortisol during prolonged exercise after CHO compared to placebo supplementation despite no differences in muscle glycogen content⁴²⁰. These findings support a physiological link between RPE and CHO substrate availability as well as selected hormonal regulation during prolonged exercise. In contrast, during an ultra-marathon race a progressive increase in RPE was noted without an accompanying increase in heart rate nor a decrease in blood glucose⁴²¹. The authors concluded that during competitive self-paced exercise the perceptual responses may be mediated through other neurological and physiological mechanisms.

Hence, central fatigue is essentially difficult to measure in humans and therefore difficult to fully elucidate. Only with the development of more sophisticated research and measurement techniques will central fatigue and its impact of central / neural drive and exercise performance be better understood and clarified.

1.5.2.2.3 *Effect of hypoglycemia on central nervous system (CNS) activation*

Nybo ³²⁵ compared the effects of CHO supplementation vs. placebo on CNS activation of the skeletal muscles during prolonged exercise. In 8 endurance-trained males voluntary force production and central activation ratios, assessed by the twitch interpolation technique, were determined during a 2-min sustained maximal knee extension in a baseline condition and immediately after 3 h of constant-load cycling, either with or without CHO (glucose) supplementation. In the placebo trial, plasma glucose concentration declined from 4.5 ± 0.2 to 3.0 ± 0.2 mmol/L, whereas blood glucose homeostasis was maintained during the glucose-supplemented trial. The development of hypoglycemia during prolonged exercise was associated with a significantly lower force production during the sustained maximal voluntary muscle contraction (MVC), and accompanied by a reduced level of CNS activation ($p < 0.05$).

Interestingly, the same force was developed at the onset of the isometric contraction, suggesting that hypoglycemia impairs the ability to sustain a high neural drive to the muscle rather than affecting the ability to mobilize maximal force for a limited period of time ³²⁵. Furthermore, the author noted that the lower force production after the prolonged exercise bout also involved peripheral fatigue, as the average force in the glucose-supplemented trial was lower compared to baseline whilst the level of CNS activation was similar between these two conditions. It was concluded that exercise-induced hypoglycemia attenuates CNS activation during a sustained maximal muscle contraction, resulting in a reduced force development, and that central fatigue appears to be effectively counteracted when hypoglycemia is prevented with CHO supplementation ³²⁵. Hence, this study is in support of the idea that the ergogenic effect of CHO supplementation during prolonged exercise is related to the prevention of hypoglycemia, and that this effect is, at least in part, due to the counteraction of central fatigue.

1.5.2.2.4 *Reduction in cerebral glucose availability and fatigue*

Hypoglycemia may affect central fatigue by reducing substrate (glucose) availability to the brain ³²⁸ and/or altering the levels of certain neurotransmitters (discussed under section 1.5.2.2.5). It has been estimated that at least 50% of whole-body glucose uptake (at rest) is a result of noninsulin-dependent uptake of glucose by the brain ²⁷. Animal research indicate that exercise results in the activation of several major brain regions such as the motor cortex, cardiorespiratory, vestibular and visual regions of the brain regulation ¹¹⁶. In healthy humans, rates of cerebral glucose uptake and cerebral metabolism may become impaired at a systemic glucose concentration of ≤ 3.6 mmol/L ⁴⁰. Though glucose is the major energy source for the brain ³³⁴, the cerebral tissue also has the enzymatic capacity to metabolize other fuel sources such as lactate and ketones ²⁸⁵. It has been shown that during maximal exercise, cerebral uptake of lactate is increased ³⁹⁹. This supports the notion that cerebral metabolic function may

be maintained during exercise even in the presence of hypoglycemia, seeing that the lactate released from the exercising muscle substitutes for glucose as cerebral metabolic fuel source^{146,427}. However, during prolonged exercise blood glucose concentrations might decrease to very low levels¹⁴⁶, and it is not yet known whether the rate of utilization of alternate fuel sources (lactate and ketones) is sufficient to compensate for the reduced blood glucose availability.

Nybo et al.³²⁶ investigated whether hypoglycemia-induced fatigue during 3 h of cycling at 60% $\text{VO}_{2\text{max}}$ could be related to alterations in cerebral metabolism. Blood glucose was maintained during the one trial, whilst it decreased from 5.2 to 2.9 mmol/L after 3 h in the placebo trial. With the development of hypoglycemia, cerebral glucose uptake decreased (from 0.34 ± 0.05 to 0.28 ± 0.04 $\mu\text{mol/g/min}$) whilst β -hydroxy (OH) butyrate uptake was increased. However, according to the reduced cerebral oxygen consumption and reduced carbon dioxide production, the uptake of ketone bodies or other substrates was too small to compensate for the reduced blood glucose availability and cerebral uptake. The temporal association between increased RPE and reduced cerebral glucose uptake indicated that fatigue was related to inadequate substrate availability for the brain³²⁶.

1.5.2.2.5 Alterations in neurotransmitter release and fatigue

Exercise-induced changes in cerebral serotonin concentrations has been associated with central fatigue³²⁸. Serotonergic neurons has been shown to play a role in, amongst other things, activation of the HPA-axis, arousal, and locomotion³²⁸. In addition, the ingestion of CHO during prolonged exercise has been proposed to postpone central fatigue by attenuating the release of serotonin in the brain²². The "serotonin-fatigue hypothesis" has been reviewed in detail in refs. 22,328 and is beyond the scope of this review and will therefore only be referred to in brief.

The proposed mechanism involves the availability of free tryptophane which has the ability to cross over the blood-brain-barrier (in competition with branched-chain amino acid (BCAA) concentrations) into the brain, where it acts as a precursor for serotonin synthesis. In the systemic circulation, tryptophane may be bound to albumin (unable to cross the blood-brain-barrier) or as free tryptophane (able to be transported into the brain). During prolonged exercise, BCAA concentration may decline, resulting in a higher free tryptophane:BCAA ratio and a situation where more free tryptophane can cross into the brain. A further consequence of prolonged exercise (particularly in the absence of CHO supplementation), is an increase in FFA concentrations, which also binds to albumin as a carrier protein, and may displace some of the albumin-bound tryptophane thereby also increasing the free tryptophane concentrations. CHO supplementation during prolonged exercise can attenuate the rise in FFA's, thereby allowing

more albumin to be bound to tryptophane, reducing the concentrations of free tryptophane and uptake into the brain and hence reducing serotonin synthesis (for reviews see ^{22,328}).

The review of the literature by Nybo and Secher ³²⁸ indicated that the though animal evidence are in support of the serotonin-fatigue hypothesis, the results from human studies are unconvincing. In humans, direct measures of tryptophane kinetics and cerebral serotonin concentrations cannot be assessed (for ethical reasons). However, tryptophane administration, resulting in 7-10 fold increase in systemic levels, failed to affect exercise time to exhaustion when cycling at 70% maximal power output ⁴²³, whilst it either had no effect ⁴⁰² or improved endurance performance ³⁷³ during high-intensity running. Furthermore, pharmacological manipulation of cerebral serotonin levels (i.e. with serotonin inhibitors) also had inconsistent results of exercise performance (for review see ³²⁸).

After detailed assessment of the available data by Nybo and Secher ³²⁸, it appears that the "serotonin-fatigue hypothesis" in humans does not become relevant unless the exercise is associated with marked elevations in FFA's and free tryptophane and perhaps only with very prolonged endurance exercise. Nybo and Secher ³²⁸ suggested that the central effect of CHO (glucose) supplementation during exercise may primarily be related to a direct effect of increased glucose availability for the glucose-dependent brain (as discussed under sections 1.5.2.2.3 and 1.5.2.2.4).

1.5.2.2.6 Effect of prolonged exercise on IL-6 release and its potential role in fatigue development

In section 1.4.3 the potential role of IL-6 in glucose homeostasis and fatty acid mobilization during exercise were highlighted. In addition to the large increase in circulating IL-6 concentrations produced by skeletal muscle during prolonged exercise, the brain has also been shown to produce small amounts of IL-6 during exercise. Interestingly, whereas systemic IL-6 release is increased under glycogen-depleted conditions ^{201,245,397}, cerebral IL-6 release is abolished with the development of hypoglycemia during exercise (e.g. exercising without CHO supplementation) ³²⁷. The reason for this response is not clear, but it has been suggested that cerebral IL-6 response may be attenuated as a consequence of cerebral dysfunction due to hypoglycemia, or that the response may be blunted because the systemic IL-6 response during exercise without CHO supplementation may be increased to an extent where it overrides the cerebral response ³²⁶.

Spath-Schwalbe et al. ³⁹⁰ demonstrated that when low doses of rh-IL-6 were administered to healthy individuals at rest, the elevated IL-6 concentrations were associated with increased

cortisol and ACTH concentrations and an increased heart rate. In addition, subjects reported an increased sensation of fatigue, altered ability to concentrate and disrupted sleep patterns. Furthermore, when an IL-6 antagonist (humanized anti-IL-6 receptor antibody) was administered to patients diagnosed with IL-6 related immune-inflammatory diseases (Castleman's disease and rheumatoid arthritis), subjects reported an immediate attenuation of fatigue symptoms³²⁰. Changes associated with elevated levels of plasma IL-6 include malaise, fatigue, elevated levels of adrenocorticotrophic hormone and cortisol, and increases in heart rate and core temperature^{217,351,417}. It has therefore, at least in part, been suggested that IL-6 may play a role in the development or perception of fatigue.

Gleeson¹⁷⁰ suggested that the large release of IL-6 from exercising skeletal muscle could act as a feedback mechanism contributing to the development of central fatigue. To investigate the role of IL-6 in fatigue development during exercise, Nybo et al.³²⁷ measured cerebral IL-6 balance during exercise with and without hyperthermia. It was hypothesized that the exercise-induced increase in IL-6 would result in cerebral uptake thereof, especially during exercise with hyperthermia, which is associated with central fatigue³²⁷. At rest there was a net balance of IL-6 across the brain, however, during exercise IL-6 was released rather than taken up by the brain, and in the face of systemic IL-6 concentrations that increased over 10-fold during the same 2 h exercise period. Cerebral release of IL-6 appeared to be more a function of exercise duration and not an increase in body temperature, as arterial and cerebral IL-6 responses were similar during hyper- and normothermia³²⁷.

Recent data obtained from an exercise performance-related study have indicated that IL-6 may play a role in affecting the sensation of fatigue during running in trained male athletes³⁵⁹. In this study, recombinant human IL-6 was administered subcutaneously prior to a 10 km running time-trial in amounts equivalent to those produced during 2 h of strenuous running (9 ± 2 pg/ml vs. baseline concentrations of 1 ± 0.5 pg/ml)³⁹⁴. With IL-6 infusion (compared to placebo), subjects reported an increased sensation of fatigue, which ultimately resulted in a decrement in running performance. This has led to the conclusion that IL-6 may possibly act as a circulating "fatiguogen" during exercise. In recent years, the role of the central nervous system (CNS) in the development during exercise has received increasing attention⁵⁹. It is suggested that afferent signals relating the physiological and metabolic status of the body are relayed back to the CNS. Based on these signals, efferent signals from the CNS / brain are related back to the appropriate bodily structures, and in respect to exercising skeletal muscle, result in a de-recruitment of skeletal muscle (reduced exercise capacity) in order to maintain a homeostatic environment¹⁸³. Exercise induced changes in various brain neurotransmitters (serotonin, dopamine and acetylcholine) and neuromodulators (ammonia and various cytokines) have been suggested to be possible mediators in the onset of exercise related fatigue⁵⁹. Further studies are needed to

assess the range of physiological functions of IL-6 during exercise, especially pertaining to its potential effect on the development of central fatigue.

1.5.2.2.7 Putative CHO receptors in the mouth and its relationship with central drive and exercise performance

Further evidence for an alternate mechanism for the ergogenic effect of CHO, unrelated to mere maintenance of euglycemia / prevention of hypoglycemia and high CHO oxidation rates, comes from studies where CHO ingestion immediately before or during relatively short (~1 h), high-intensity (>75% $\text{VO}_2 \text{max}$) exercise resulted in improved performance^{9,11,60,61,131,231,316}. Though again, this effect has not been universal amongst all studies^{70,119,262,299}.

One suggestion for the improvement in performance observed under these exercise conditions have been the maintenance of a high rate of CHO oxidation, however, Jeukendrup et al.²³¹ demonstrated that this effect is unlikely seeing that only 5-15 g of exogenous CHO were oxidized in the first hour of exercise and too small to affect CHO oxidation and performance. In fact, there does not seem to be a clear metabolic explanation for this effect^{60,61}. When glucose was infused at a rate of 1 g/min compared with saline during a 40-km (~1-h) simulated cycling time-trial (TT), there was a small but significant increase in CHO oxidation rate, however, 40-km TT performance was unaffected (Carter et al., unpublished observations 2004, as reported in⁶¹).

An intriguing finding from a very recent study demonstrated an improvement in 1-h cycling TT performance by simply rinsing the mouth with a CHO-containing solution⁶¹. Power output was higher in the CHO compared to placebo trial, but RPE remained similar. The fact that the CHO solution was not swallowed makes it unlikely that the improvement in performance was related to a metabolic effect of CHO. Rather, the authors suggested the involvement of an alternate mechanism, such as the involvement of a central pathways, increasing central motor drive or motivation rather than having any metabolic cause⁶¹. This effect may be mediated by receptors in the mouth that may be triggered by CHO and may result in the stimulation of the reward and/or pleasure centers in the brain⁶¹. Though there is some animal evidence for the potential existence of CHO receptors in the mouth, the nature and role of these receptors remains to be fully explored and substantiated.

1.5.2.3 Variability in response to the development of hypoglycemia during exercise and its effect of exercise performance

A number of studies have found that exogenous CHO supplementation, especially when endogenous glycogen concentrations are low, can maintain blood glucose and total CHO oxidation and delay the onset of fatigue and thereby, in part, negate or 'over-ride' the potential

ergolytic effects of low endogenous glycogen stores^{35,60,73,74,89}. Furthermore, CHO supplementation during exercise appear to minimize the performance differences in studies in which subjects ingested different preexercise diets and therefore began exercise with different muscle and liver glycogen concentrations^{53,445}.

These findings contributed to the popular premise that the improved endurance capacity associated with CHO-loading or CHO supplementation during exercise, or both, may result from the prevention of hypoglycemia^{53,73,242,445}. Indeed, some studies have demonstrated that CHO supplementation improved endurance exercise capacity only in the individuals who became hypoglycemic during the placebo treatment^{19,91,92}. For example, Coyle et al.⁹¹ reported that CHO ingestion during prolonged exercise at 74% VO_2 max increased endurance time by ~17%, but only in those subjects who developed hypoglycemia during the placebo trial.

However, closer evaluation of the literature suggest that the ergogenic potential of maintaining CHO availability during exercise is not simply due to the prevention of hypoglycemia and a decline in CHO oxidation (as discussed previously). There are studies that demonstrated that CHO supplementation during exercise lowered RPE and improved endurance performance even in individuals who did not become hypoglycemic during the placebo trials^{60,293,313}, nor did the CHO supplementation alter the rate of CHO oxidation during exercise^{36,60,100,132,298,313,416}.

Perhaps the most intriguing findings are from those studies that found that the development of hypoglycemia^{87,146,228,230,312} or reversal of hypoglycemia¹⁶² (plasma glucose <3.5 mmol/L) during exercise had little effect on exercise performance, nor were RPE any different between CHO and placebo supplementation trial. Similarly, as discussed in detail under section 1.5.1, a series of studies investigating the effect of type, amount and timing of preexercise CHO intake have indicated that some subjects are prone to the development of rebound hypoglycemia during subsequent exercise (for reasons yet unclear), yet the development of hypoglycemia had no effect on their exercise performance^{228,230,312}.

Collectively taken, the results from the available literature suggest that the fatiguing effects of declining blood glucose concentrations differ between individuals. The nature of this difference is not clear, but may relate to differences in cerebral sensitivity to reductions in blood glucose concentrations⁷, and/or phenotypic differences in metabolism during exercise^{2,172}. The phenotypic differences seen might be related to genetic differences, or to differences in habitual dietary and training regimes^{102,172}.

It has become apparent that (a) the ergogenic effect of improved endogenous and exogenous CHO availability on exercise capacity is not simply due to the prevention of hypoglycemia and

the maintenance of a high rate of CHO oxidation *per se*, and (b) the development of hypoglycemia does not necessarily have a negative impact on exercise performance; (c) that there is individual variability in the metabolic, hormonal and performance response to altered glucose availability, and finally (d) that the mechanisms by which improved or reduced CHO availability may alter exercise performance still need to be fully elucidated. Clarity on these aspects may assist with development of specific dietary advice and strategies to enhance athletic performance, especially in strenuous training or competition situations where recovery time is limited and endogenous CHO stores may be compromised.

1.5.3 Longer-term alteration in hormonal milieu: Metabolic and neuroendocrine responses to antecedent exposure to hypoglycemia and/or exercise

1.5.3.1 Exercise-associated adaptation of the HPA-axis

Exercise has been identified as a potent activator of the HPA-axis^{275,276}. An interesting observation in the literature is that endurance-trained men might develop an adaptation of the HPA-axis to repeated and prolonged exercise-induced increases in cortisol secretion (or repeated stimulations of the HPA-axis)¹²⁵. These adaptive mechanisms may include decreased sensitivity to cortisol to protect muscle and other glucocorticoid-sensitive tissues against the increased cortisol secretion commonly observed during and up to 2 h after exercise^{124,125}. The potential impact of such an adaptation in HPA-axis activity on glucoregulatory hormonal responses and maintenance of glucose homeostasis have not been fully explored.

Though some researchers consider this blunting of the HPA-axis and cortisol response as a maladaptation or pathology in athletes²⁷⁶, Viru et al.⁴²⁸ examined the possibility that fatigue development *per se* may modify the hormone responsiveness to subsequent exercise. Twelve endurance-trained men ran for 2 h (blood lactate ~2 mmol/L) in order to induce fatigue. A 10-min exercise bout at 70% $\text{VO}_{2\text{max}}$ was performed before (1st test) and after (2nd test) the 2 h run to assess hormone responsiveness, the 10-min exercise test was followed by 10 min of rest, followed by a 1-min anaerobic power test to assess muscle power. The 1st test resulted in a significant increase in cortisol and growth hormone, a decrease in insulin and no change in testosterone concentrations. The 2 h run caused decreases of insulin, increases of growth hormone concentration and variable responses in the concentrations of cortisol and testosterone. The 2nd test decreased insulin concentration further, but responses of the concentrations of testosterone, growth hormone and cortisol were variable. In 6 subjects (group A) cortisol displayed an increase from baseline concentrations, while in the other 6 subjects (group B) a decrease or no change was seen. Growth hormone concentration was substantially higher in group A than group B following the 2nd test. In group A anaerobic muscle power was significantly higher, while in group B it was lower after the 2 h run than before the 2 h run⁴²⁸.

The findings suggest that fatigue from prolonged endurance activity may introduce a resetting in the pituitary-adrenocortical component of the endocrine system, expressed either by intensified endocrine functions in some individuals or by suppressed endocrine functions in others.

1.5.3.2 Hypoglycemia-associated counterregulatory failure, hypoglycemia "unawareness" syndrome and the sensation of fatigue

As discussed previously, with the development of hypoglycemia, a point may be reached where glucose transport from the circulation is no longer sufficient to meet cerebral metabolic demands, and a series of central and peripheral counterregulatory responses are elicited, as well as the development of warning symptoms which makes the person aware of hypoglycemia, all in aid of restoring euglycemia. It has been shown that the glycemic threshold for these counterregulatory (metabolic, hormonal and symptomatic) responses can be shifted to lower glucose concentrations following a single episode of hypoglycemia. A series of studies have now demonstrated that antecedent exposure to hypoglycemia can blunt subsequent counterregulatory responses to hypoglycemia (or related physiological stresses such as exercise¹⁶⁰) in diabetic (for review see refs. ^{27,385} and healthy individuals ^{102,160,205}. For example, in healthy subjects, rates of brain glucose uptake were initially impaired at a systemic glucose concentration of 3.6 mmol/L, however, after 56 h of intermittent hypoglycemia (3.0 mmol/L) brain uptake was preserved at normal rates even when blood glucose was as low as 2.5 mmol/L⁴⁰. It was demonstrated that counterregulatory hormone and symptomatic responses were also triggered at lower glucose concentrations following recurrent hypoglycemia⁴⁰.

As hypoglycemia and exercise trigger similar autonomic nervous system (ANS) and neuro-endocrine responses, it has been suggested that exercise may also attenuate counterregulatory responses to other related physiologic stresses in non-diabetic populations, including exercise. Additionally, both hypoglycemia and exercise lead to elevated glucocorticoid concentrations, and it has been demonstrated that prior elevations of cortisol (or challenge to the HPA-axis) produce blunted responses to subsequent hypoglycemia^{102,106,107,160,205}.

Davis et al.¹⁰² reported that antecedent hypoglycemia resulted in significant blunting of neuro-endocrine (glucagon, insulin, catecholamines) and metabolic (endogenous glucose production, lipolysis, ketogenesis) responses during a subsequent exercise bout (the following day) in healthy subjects. The group exposed to antecedent hypoglycemia required a 10 fold higher rate of glucose infusion in order to maintain euglycemia during the exercise bout, compared to the antecedent euglycemic group¹⁰². These results makes it difficult to elucidate whether the neuro-endocrine and metabolic responses were caused by, or resulted in the high rate of glucose infusion required to maintain euglycemia. It is yet unclear how the neuro-endocrine and

metabolic responses would be affected when healthy individuals are left to regulate their blood glucose concentration (without exogenous glucose infusion intervention) during exercise after antecedent hypoglycemic exposure. Specifically, these responses have not been systematically investigated in a population of well-trained individuals.

In a subsequent study, Galassetti et al.¹⁶⁰ demonstrated that antecedent prolonged exercise with euglycemic glucose infusion in healthy individuals blunted neuro-endocrine and metabolic counterregulatory responses to subsequent hypoglycemia the following day. Again, blood glucose concentrations during exercise were manipulated with glucose infusion, which complicates interpretation of the subsequent glucose counterregulatory responses.

Factors that have been identified as independent determinants of the magnitude of subsequent counterregulatory failure include depth of antecedent hypoglycemia¹⁰⁸, number of prior episodes of hypoglycemia^{103,108,205,444}, and gender¹⁰⁵. Though the factors and mechanisms responsible for the phenomenon of counterregulatory failure and hypoglycemia unawareness have received much focus in recent years, it still remains to be fully elucidated.

1.5.3.2.1 Factors affecting the magnitude of counterregulatory failure

1.5.3.2.1.1 Magnitude (depth) of antecedent hypoglycemia

In men, neuroendocrine and SNS responses were sensitive to prior exposure of even modest (3.9 mmol/L) hypoglycemia¹⁰⁸. Antecedent exposure to hypoglycemia of 3.9 mmol/L for 2 h resulted in significantly blunted epinephrine, muscle sympathetic nerve activity, and glucagon responses during next-day hypoglycemia (2.9 mmol/L), whereas antecedent exposure to hypoglycemia of 3.3 mmol/L resulted in additional significant blunting of pancreatic polypeptide, norepinephrine, growth hormone, endogenous glucose production, and lipolytic responses. Deeper antecedent hypoglycemia of 2.9 mmol/L produced similar day 2 counterregulatory failure as day 1 antecedent hypoglycemia of 3.3 mmol/L. In summary, this study indicated that in healthy overnight-fasted men, mild antecedent hypoglycemia of 3.9 mmol/L significantly blunted sympathoadrenal and glucagon, but not other forms of neuroendocrine counterregulatory responses to subsequent hypoglycemia. Antecedent hypoglycemia of 3.3 mmol/L resulted in additional significant blunting of all major neuroendocrine and metabolic responses to subsequent hypoglycemia. The authors concluded that in normal humans, there is a hierarchy of blunted counterregulatory responses that are determined by the depth of antecedent hypoglycemia¹⁰⁸.

1.5.3.2.1.2 Duration and magnitude of antecedent hypoglycemia

Significant blunting of counterregulatory responses during subsequent (next-day) exposure to hypoglycemia has been demonstrated after antecedent hypoglycemic exposure consisting of 2 h in the morning followed by 2 h in the afternoon¹⁰⁸, and with 30 min hypoglycemic exposure in the morning followed by a 2 h exposure in the afternoon²⁰⁵. However, one single episode of antecedent hypoglycemia (3.0 mmol/L) of 2 h duration has been shown to produce a generalized reduction of the neuroendocrine and symptomatic responses to subsequent hypoglycemia (2.8 mmol/L) in healthy men²⁰⁵.

Peters et al.³³⁸ investigated whether subsequent neuroendocrine and symptomatic responses are attenuated after short-term hypoglycemic episodes of less than 1-h duration in healthy men. They studied hypoglycemia on 4 consecutive days and after an 8-day pause. Plasma glucose was reduced to < 2.8 mmol/L on study days 1, 2, 3, 4, and 12 after intra venous insulin boluses (0.04 U/kg). Counterregulatory hormone concentrations increased similarly during the hypoglycemic episodes in all instances, and maximal concentrations on study day 4 were not attenuated. On each study day, symptoms of hypoglycemia were produced after induction of hypoglycemia, and there was no decrease in the degree of symptom responses on subsequent days. The multivariate analysis of variance showed no day-to-day differences in plasma glucose, counterregulatory hormones, or hypoglycemic symptoms. It was concluded that with short-term (<1 h) hypoglycemic episodes, the neuroendocrine and symptomatic responses remain completely intact in healthy individuals³³⁸.

Davis et al.¹⁰³ investigated the effect of two identical episodes of short-duration, intermediate-duration and prolonged hypoglycemia each performed during a 2 h morning and afternoon experiment in healthy humans. In all the hypoglycemic experiments, blood glucose was initially reduced from baseline to 2.9 mmol/L over a 30 min period. Thereafter, in the short-duration hypoglycemia experiment blood glucose was maintained at 2.9 mmol/L for 5 min then rapidly restored to euglycemia for the remainder of the 2 h. With intermediate hypoglycemic exposure, blood glucose was maintained at 2.9 mmol/L for 30 min, and with prolonged hypoglycemia it was maintained at 2.9 mmol/L for 90 min and rapidly restored to euglycemia for the remaining 2 h. The same procedure was repeated in the afternoon. Euglycemia was maintained for 2 h between the morning and afternoon experiments. The following day involved a single 2 h hypoglycemic (2.9 mmol/L) clamp experiment. Epinephrine, norepinephrine, glucagon, growth hormone and muscle sympathetic nerve activity responses were similarly significantly blunted by all differing-duration hypoglycemic experiments (compared to euglycemia). Plasma cortisol responses were reduced in a step-wise manner from ~745 nmol/L after antecedent euglycemia to ~580, ~611 and ~497 nmol/L in short-, medium and prolonged-duration antecedent hypoglycemia, respectively. Endogenous glucose production was similarly significantly blunted

and glucose infusion rates (to maintain day 2 hypoglycemia) increased after all differing-duration hypoglycemia experiments. Hypoglycemic symptom scores (principally autonomic symptoms) were significantly reduced after intermediate- and prolonged-duration hypoglycemia, but were unaffected by prior short-duration hypoglycemia ¹⁰³.

The study by Davis et al. ¹⁰³ ruled out the possibility that short-duration antecedent hypoglycemia could selectively blunt some but not all subsequent neuroendocrine responses. Furthermore, muscle sympathetic nerve activity was similarly significantly blunted even after short-duration antecedent hypoglycemia, which indicates the sensitivity of the sympathetic nervous system to the deleterious effects of even minimal antecedent hypoglycemia.

1.5.3.2.1.3 Effect of gender

Davis et al. ¹⁰⁵ demonstrated that whilst prolonged (90-min) antecedent hypoglycemia exposure at 3.3 or 3.9 mmol/L significantly blunted counterregulatory responses in healthy men, it had virtually no effect in healthy women. Antecedent hypoglycemia of 2.9 mmol/L in women was needed to produce significant blunting of subsequent counterregulatory responses ¹⁰⁵. In support of these findings, Galassetti et al. ¹⁶¹ also demonstrated a sexual dimorphism in the counterregulatory response to antecedent hypoglycemia, with a significantly greater attenuation of response in men, when compared to women.

Davis et al. ¹⁰⁴ demonstrated that differential glycemic thresholds are not the cause of the sexual dimorphism present in counterregulatory responses to hypoglycemia, but that it is rather a reduced CNS efferent input that underlies the mechanism responsible for lowered neuroendocrine responses to hypoglycemia in women. They also demonstrated that the physiological counterregulatory responses (neuroendocrine, cardiovascular, and autonomic nervous system) are reduced across a broad range of hypoglycemia in healthy women compared with healthy men ¹⁰⁴.

1.5.3.2.1.4 Role of cortisol in the pathogenesis of reduced counterregulatory responses

The exact mechanism(s) responsible for the reduced neuroendocrine and autonomic-adrenomedullary responses observed after repeated exposure to hypoglycemia (or related physiological stresses) in healthy ^{103,103,109,205} and insulin-treated diabetic patients ^{25,96,101,385}, are not fully known.

One possibility might be related to the role that glucocorticoids play in protecting the body against its own defense mechanisms, which might cause damage if allowed to proceed unregulated (for example reducing the inflammatory response). Glucocorticoids have been shown to reduce the

autonomic-adrenomedullary response to a variety of differing stress in animals and humans^{281,287}. Tappy et al.⁴⁰⁶ demonstrated that dexamethasone administration resulted in a reduced norepinephrine response during hyperinsulinemic-euglycemia in humans. Komesaroff and Funder²⁸¹ reported that prior dexamethasone administration resulted in reduced autonomic-adrenomedullary responses to hypoglycemia in sheep. Furthermore, bilateral adrenalectomy with resultant glucocorticoid deficiency has been shown to result in exaggerated autonomic responses to stress exposures such as surgery in nonhuman primates⁴¹⁹ and cold exposure in rats³⁴¹.

These studies gave rise to the study of Davis et al.¹⁰⁶ who aimed to investigate if hypoglycemia-associated autonomic failure is caused by antecedent increases of plasma cortisol. Healthy subjects underwent two separate, 2-day experiments. On day 1 subjects underwent either two 2-h bouts of hyperinsulinemic hypoglycemia (2.9 mmol/L) separated by 2 h of euglycemia (~5 mmol/L), or 2 bouts of hyperinsulinemic euglycemia followed by a 2 h bout of hypoglycemia (2.9 mmol/L) on day 2. In another group of subjects day one consisted of similar 2-h bouts of euglycemia with cortisol infusion to simulate concentrations reached during clamped hypoglycemia, followed by similar day 2 hypoglycemia. Their results indicated that antecedent physiologic increases of plasma cortisol resulted in significantly reduced autonomic nervous system (ANS) responses (epinephrine, norepinephrine, glucagon, and muscle sympathetic nerve activity) to subsequent hypoglycemia. The magnitude of reduction in ANS responses was similar to that observed with antecedent hypoglycemia. It was concluded that increased plasma cortisol is the suggested mechanism underlying the effect of antecedent hypoglycemia on subsequent hypoglycemia-associated autonomic failure¹⁰⁶. Similar findings and conclusions were reported in a more recent study by McGregor et al.³⁰⁰.

In the study of Davis et al.¹⁰⁶, the magnitude of reduction in the glucagon response was greater after antecedent hypoglycemia compared to antecedent cortisol infusion. As discussed previously, glucagon secretion may be controlled by the sympathetic¹⁶⁸ and parasympathetic²⁴ nervous systems. The results of Davis et al.¹⁰⁶ are in support of the ANS as well as alpha-cell glucose sensing in regulating glucagon release seeing that hypoglycemia would impair both ANS input into as well as direct glucose sensing by the alpha cell, resulting in a greater suppression of glucagon secretion. Whereas antecedent cortisol would only impair ANS input into the pancreas whilst leaving direct glucose sensing by the alpha cell intact and hence less suppression of glucagon secretion.

Along similar reasoning, the different magnitude of the glucagon responses could explain why antecedent hypoglycemia resulted in greater suppression of hepatic glucose production compared to antecedent cortisol infusion¹⁰⁶.

In a subsequent study, Davis et al.¹⁰⁷ assessed whether prevention of the plasma cortisol response during antecedent hypoglycemia preserves autonomic nervous system counterregulatory responses during subsequent hypoglycemia in healthy humans. They demonstrated that the prevention of an increase in cortisol during antecedent hypoglycemia preserved many critical ANS counterregulatory responses to subsequent hypoglycemia. It was concluded that hypoglycemia-induced increases in plasma cortisol levels are a major mechanism responsible for causing subsequent hypoglycemic counterregulatory failure, and that other mechanisms, apart from cortisol, do not play a major role in causing hypoglycemia-associated autonomic failure¹⁰⁷.

In contrast to these findings, Raju et al.³⁴⁵ more recently demonstrated that elevations in cortisol (for 2.5 h in the morning and afternoon during euglycemia) to levels that occur during hypoglycemia did not reduce sympathoadrenal or neurogenic symptom responses to subsequent hypoglycemia in healthy humans. The study population and duration of the elevation in cortisol concentrations were comparable between studies, hence the reason for the discrepancy in results are not readily apparent.

Interestingly, De Galalan et al.¹¹³ tested whether antecedent elevations in epinephrine concentrations (for 1 h) plays a role in hypoglycemia-associated autonomic failure during subsequent hypoglycemia (induced 3 h later) in healthy humans. The subsequent bout of hypoglycemia induced similar counterregulatory hormonal responses as well as similar autonomic, neuroglycopenic and cognitive function responses compared to prior saline infusion¹¹³. Their results indicated that prior elevation in epinephrine plays a limited role in the concept of hypoglycemia-associated autonomic failure.

Collectively taken, there is literature to suggest that prior elevations of cortisol can exert a restraining effect of ANS activity with reductions in neuroendocrine responses and hepatic glucose production and hence impaired maintenance of glucose homeostasis during subsequent exposure to a variety of stress that may increase cortisol concentrations^{106,107}. However, there are also research to suggest that neither prior elevations of cortisol³⁴⁵ nor epinephrine¹¹³ are responsible for causing hypoglycemia-associated autonomic failure. Hence, the mechanism behind the concept of hypoglycemia-associated autonomic failure remains to be fully elucidated.

1.5.3.2.2 *Dissociation between the degree of counterregulatory hormone failure and symptomatic response and cognitive function*

1.5.3.2.2.1 *Symptomatic response*

The recognition or symptomatic response to hypoglycemia is one of the key responses elicited in the defense against the development of even more severe hypoglycemia. Symptomatic awareness of hypoglycemia is key to stimulate food intake and hence restore euglycemia and ultimately, prevent neuroglucopenia³⁸⁵. There seems to be a distinct hierarchy of responses to decrements in plasma glucose, such that the threshold for activation of counterregulatory hormone secretion occurs at higher plasma glucose levels than that for initiation of autonomic warning symptoms, which in turn occurs at higher plasma glucose levels than that for onset of neuroglycopenic symptoms and deterioration in cerebral function³⁰⁹.

Though there is good agreement in the literature that neuroendocrine responses are blunted by prior hypoglycemia of differing durations in normal healthy humans (e.g. refs.^{102,103,105,108,205,444}), the effect on the symptomatic response and cognitive function seem less clear.

In this regard, studies in humans with insulin dependent diabetes mellitus (IDDM) demonstrated that neuroendocrine failure is not always associated with symptomatic response failure and cognitive impairment. Studies have reported that prior hypoglycemia had either no effect on thresholds for hypoglycemic symptoms^{71,443} or significantly reduced the glucose level at which symptoms were provoked^{95,443}. Furthermore, Dagogo-Jack et al.⁹⁷ reported that rigorous avoidance (3-4 wks) of hypoglycemia in type 1 diabetics resulted in a return of hypoglycemic symptoms but not neuroendocrine responses to subsequent hypoglycemia. Additionally, several studies have shown a blunting of neuroendocrine responses, yet cognitive function was preserved in IDDM⁹⁸ and healthy humans²¹⁹.

In the study of Davis et al.¹⁰³ 2 episodes (1 in the morning and 1 in the afternoon) of short-duration hypoglycemia (5 min at 2.9 mmol/L and 20 min of lowering and raising between 2.9 and 3.9 mmol/L) in healthy humans resulted in similar magnitude of neuroendocrine and muscle sympathetic nerve activity blunting than 2 prior episodes of intermediate (30 min) and prolonged (90 min) hypoglycemia. However, hypoglycemic symptoms (particularly autonomic symptoms) were blunted by intermediate and prolonged antecedent hypoglycemia, but unaffected by prior short-duration hypoglycemia¹⁰³. It therefore appears that duration of antecedent hypoglycemia can produce a hierarchy of blunted physiological responses during subsequent hypoglycemia, with hypoglycemic symptom awareness less vulnerable than neuroendocrine responses^{103,333,385}. Furthermore, cardiovascular responses were unaffected by prior hypoglycemia (of any duration)¹⁰³. It therefore seems that prior hypoglycemia may blunt some but not all autonomic nervous

system responses during subsequent hypoglycemia, as has also been observed by Paramore et al.³³³

1.5.3.2.2.2 Cognitive function

Though neuroendocrine changes after induction of hypoglycemia, in patients with diabetes and healthy persons, are thoroughly investigated, cognitive adaptation processes are still not fully understood²⁸². It has generally been shown that cognitive functioning becomes impaired when blood glucose falls below 3.1 mmol/L^{156,158}, and that altered cognitive functioning is often related to development of neuroglycopenic symptoms¹⁵⁶. However, it has been demonstrated that there are regional differences in the susceptibility to neuroglycopenia within the brain, with the cerebral cortex being most sensitive while deeper structures are more resistant^{156,282}. Furthermore, the degree of dysfunction differs in various areas of the brain and a battery of psychometric tests is usually required to assess impairment of cognitive function during hypoglycemia. Complex, attention-demanding and speed-dependent responses are most impaired with accuracy often preserved at the expense of speed (reviewed in detail by Frier¹⁵⁶).

Evans et al.¹³⁵ examined the time course for the onset of, and recovery from, acute hypoglycemia in healthy subjects in 8 healthy males after plasma glucose was allowed to fall rapidly to ~2.65 mmol/L and maintained for 90 min before euglycemia was rapidly restored (vs. euglycemia throughout). Cognitive function assessed by a battery of sensitive tests (4-choice reaction time (RT), Stroop word, and color-word test) became significantly impaired immediately at onset of hypoglycemia compared to the euglycemic experiment. Counterregulatory hormone responses (epinephrine, norepinephrine, glucagon, cortisol, and growth hormone) and symptomatic awareness of hypoglycemia (assessed by a questionnaire) were relatively delayed, being detected 20 min after the onset of hypoglycemia. There was no diminution (adaptation) of any responses, cognitive, humoral, or symptomatic during the 90 min of sustained hypoglycemia. During recovery, the 4-choice RT continued to be abnormal even after symptomatic awareness was restored. It was concluded that cognitive performance during hypoglycemia may become impaired before symptomatic awareness. Furthermore, during recovery from hypoglycemia, recovery of cognitive function lags behind the restoration of glucose concentrations symptomatic awareness^{135,156,282}.

In a study on healthy men where cerebral use of alternate fuel sources (FFA's) during hypoglycemia was assessed¹³⁴ by means of intralipid-heparin infusion, the raised FFA, glycerol, and beta-hydroxybutyrate concentrations significantly reduced epinephrine and growth hormone responses. These hormonal responses were also delayed from a blood glucose threshold of 3 mmol/L to 2.8 mmol/L, with a trend toward reduced cortisol responses. Similarly, hypoglycemic

symptom scores were significantly diminished during intralipid infusion. However, there was no significant effect on the deterioration in four-choice RT, one measure of cognitive deterioration. With intralipid-heparin infusion during euglycemic clamping, there was no rise in hormones, four-choice RT, or symptoms other than hunger and tiredness. Hence, raised concentrations of FFA and glycerol were able to reduce neurohumoral responses to hypoglycemia, but could not protect cognitive function. This suggests that regional differences exist in human brain metabolism between glucose-sensing and cognitive areas of the brain, which may be important in the understanding of the mechanisms of glucose sensing and in the genesis of hypoglycemia unawareness ¹³⁴.

Similar to the reduction in neuroendocrine and symptomatic responses that may follow exposure to a single, recent bout of hypoglycemia, a single episode of mild antecedent hypoglycemia (3.1 mmol/L) has also been shown to attenuate several aspects of cognitive dysfunction during subsequent hypoglycemia 18-24 h later ¹⁵⁸. Fruehwald-Shultes et al. ¹⁵⁸ reported that cognitive performance, assessed by auditory-evoked brain potentials (AEBPs) and reaction time during a vigilance task and short-term memory recall, deteriorated during stepwise hypoglycemia. However, after subsequent exposure to hypoglycemia 18 – 24 h later, the hypoglycemia-induced decrease in the amplitude of the P3 of the AEBP was distinctly reduced, and short-term memory and reaction time performance was less impaired in the prior-hypo compared to the control group ¹⁵⁸. These results may be explained by data indicating that cerebral adaptations may occur that allow for normal brain glucose uptake and cerebral function to be maintained during recurrent systemic hypoglycemia ⁴².

To test whether the glycemic thresholds for hypoglycemic cognitive dysfunction, like those for neuroendocrine responses and symptoms of hypoglycemia, shift to lower plasma glucose concentrations after recent antecedent hypoglycemia, Hvidberg et al. ²¹⁹ exposed 16 healthy subjects (7 women and 9 men) to 2 h of hypoglycemia (2.6 mmol/L) vs. euglycemia on day 1, followed by exposure to stepped hypoglycemia (4.7, 4.2, 3.6, 3.0, 2.8, 2.5, and 2.2 mmol/L) the following morning (day 2) whilst neuroendocrine, symptomatic, and cognitive responses were assessed. Cognitive function tests included measures of information processing (Serial Addition), attention (Stroop Arrow Word), pattern recognition and memory (Delayed Non-Match to Sample), and declarative memory (Paragraph Recall). As expected, plasma glucagon, epinephrine, and pancreatic polypeptide responses to stepped hypoglycemia were significantly reduced, and symptomatic responses tended to be reduced after day 1 hypoglycemia compared to euglycemia. Performance on the cognitive function tests deteriorated significantly during day 2 stepped hypoglycemic clamps, but there were no significant overall effects of antecedent hypoglycemia on hypoglycemic cognitive dysfunction. Although deterioration was reduced from the 2.8 mmol/L to the 2.5 mmol/L steps on the Serial Addition and Delayed Non-Match to Sample

tasks after day 1 hypoglycemia, comparable differences were not found on the Stroop Arrow Word or Paragraph Recall tasks. These data indicated that glycemic thresholds for hypoglycemic cognitive dysfunction, unlike those for neuroendocrine responses to and symptoms of hypoglycemia, do not seem to shift to substantially lower plasma glucose concentrations after recent antecedent hypoglycemia in healthy humans ²¹⁹.

The exact site(s) and mechanism(s) responsible for hypoglycemia-associated autonomic failure are still not clear. In particular, it is unclear whether the mechanism(s) of counterregulatory failure act at multiple sites or one co-coordinating center within the brain, and what impact it has on symptomatic response and the magnitude of cognitive impairment. It is also yet unknown how these potential alterations in neuroendocrine, metabolic, symptomatic and cognitive responses may affect the sensation of fatigue and exercise performance in a setting where exercise is performed after antecedent exposure to hypoglycemia and/or performed without exogenous CHO supplementation. In the studies where either antecedent hypoglycemia resulted in reduced neuroendocrine and metabolic responses to subsequent, next-day endurance (90 min) exercise ¹⁰², or where antecedent exercise produced a blunting of these responses to next-day hypoglycemia, measurements of perceived exertion, exercise performance and cognitive function were not reported.

1.5.4 Potential effects of prolonged exercise on cognitive functioning

Measurement of cognitive function has been shown to be impaired during exposure to hypoglycemia ¹³⁵, but a single episode of mild antecedent hypoglycemia (3.1 mmol/L) in healthy men can attenuate several aspects of cognitive dysfunction during subsequent hypoglycemia 18-24 h later ¹⁵⁸.

An improvement in cognitive function (such as simple and choice reaction time tasks) have been reported when performed during or immediately after exercise lasting >20 min up to an hour ^{66,80,81}. It has been suggested that the effect of exercise (including duration and intensity) on cognitive function follows an inverted U-shaped curve (termed the Inverted U-Hypothesis) with optimal arousal corresponding to an intermediate arousal level ⁴⁶, which is as of yet not clearly defined. When arousal exceeds this "optimal" level, cognitive performance may decline.

The improvement in cognitive functioning is suggested to be the result of an increase in metabolic load which induces an increase in arousal level that would improve cognitive functioning ^{46,177}. Other suggested mechanisms for the improvement in cognitive performance is an increase cerebral blood flow, or neurotransmitter (catecholamines / endorphin) release,

although, the exact mechanism(s) supporting a functional link between these factors are still unknown⁴⁶.

Results on the effect of prolonged (>1 h in duration) exercise on cognitive functioning are limited. During such exercise, cognitive functioning may be confounded by the development of fatigue symptoms, hypoglycemia and individual motivation (for review see⁴⁶). During prolonged exercise, apart from marked increases in metabolic load, symptoms of central (e.g. reduced neural drive) and peripheral (e.g. decreased muscle excitability) fatigue are also commonly reported¹⁷⁷, and it is yet unclear what relationship exists between cognitive function, metabolic and hormonal responses, perceived exertion and endurance exercise performance. Muscle glycogen depletion and hypoglycemia have been related to the development of peripheral and central fatigue^{52,76} and CHO ingestion has been shown to delay the onset of fatigue^{52,76} and attenuate the perception of effort towards the latter stages of prolonged exercise^{46,420}. Except for one study²²¹, other studies have demonstrated that CHO ingestion during ~2 h of cycling³⁵² and running⁸¹ performed at 60-75% $\text{VO}_{2\text{ max}}$ may benefit cognitive functioning. Hence, CHO ingestion can minimize the negative effect of central fatigue induced by prolonged exercise⁴⁶, perhaps via and attenuation of free tryptophane concentrations and ultimately reduced serotonin release in the brain and reduced central fatigue¹⁰⁰, however, more research is needed to investigate the role of other central factors on cognitive performance⁴⁶.

Grego et al.¹⁷⁷ recently demonstrated that P300 components were affected by prolonged exercise (3 h at ~66% $\text{VO}_{2\text{ max}}$) in trained male cyclists. P300, a component of event-related brain potentials, has been related to neural activity underlying basic aspects of cognition¹⁷⁷. P300 amplitude has been related to the amount of memory and attentional resources employed in the processing capacity of a given task, whereas P300 latency relates to the speed of cognitive processing¹⁷⁷. In this study¹⁷⁷, exercise resulted in a progressive increase in FFA, heart rate and RPE (after 108 min), and glycerol, epinephrine and cortisol (after 144 min) and a significant decrease in blood glucose (after 108 min) and insulin (144 min). However, blood glucose remained above 4 mmol/L. The results showed a temporary increase in P300 amplitude between the 1st and the 2nd hour (an indication of an improvement in cognitive function) and an increase in latency after the 2 h of exercise concomitant with some hormonal changes, including an increase in cortisol and epinephrine and a decrease in blood glucose (an indication of an alteration of information processing speed with fatigue). These findings suggest a combined effect of arousal and central fatigue on electrocortical indices of cognitive function during acute physical exercise¹⁷⁷.

Most recently, Grego et al.¹⁷⁸ examined the influence of 3 h of cycling at 60% $\text{VO}_{2\text{ max}}$ on simple and complex cognitive performance in 8 well-trained male subjects before, during, and

immediately after the exercise task. A significant improvement in speed of response and a decrease in error number during the map recognition task were recorded between 80 min and 120 min when compared with the first 20 min of exercise. After 120 min the number of recorded errors was significantly greater indicating a shift in the accuracy-speed trade-off. These results provide some evidence for exercise-induced facilitation of cognitive function. However this positive effect disappears during prolonged exercise - as evidenced by an increase in errors during the complex task and an alteration in perceptual response (i.e. the appearance of symptoms of central fatigue) ¹⁷⁸.

During prolonged exercise, apart from marked increases in metabolic load, symptoms of central (e.g. reduced neural drive) and peripheral (e.g. decreased muscle excitability) fatigue are also reported ¹⁷⁸, and it yet unclear what relationship exists between cognitive function, metabolic and hormonal responses, perceived exertion and endurance exercise performance. In addition, it is not yet clear how cognitive function may be affected at the end of prolonged exercise and after prior recent exposure to hypoglycemia (which may alter neuro-endocrine and metabolic responses) in well-trained men.

1.6 IN CONCLUSION

When blood glucose concentrations decline, a series of counterregulatory responses are elicited in hierarchical fashion in order to restore euglycemia, mainly to preserve brain function ³⁰⁹. Based on the involvement of the sympathetic centers and HPA-axis in aid of correcting declining blood glucose concentrations, it seems clear that the series of responses initiated are largely centrally mediated ³⁸⁵. There seems to be a component of redundancy in the gluco-regulatory mechanisms which may account for the discrepancy in results obtained from different studies where one component of the gluco-regulatory system was altered or blocked, causing a variable response in the other systems. In addition, the central and peripheral integration of these neural, metabolic and hormonal counterregulatory responses are not yet fully understood.

Muscle glycogen, blood-borne glucose (derived from endogenous and/or exogenous supply), and lipids (IMTG and FFA's) have been identified as the major substrates utilized for energy production during prolonged exercise ³⁸⁰. CHO oxidation during exercise is influenced primarily by the exercise intensity, duration and CHO availability. An increase in exercise intensity results in a shift in the balance of substrate utilization from fat to CHO, regardless of genotypic or phenotypic adaptations ⁴⁷. This is largely due to contraction-induced increases in muscle glycogenolysis and glycolysis ²¹⁷ as well as increased sympathetic nervous system activity ¹²⁰. Hence, sufficient CHO supply is paramount for sustaining muscle contraction during prolonged exercise at moderate- to high-intensities (>60% $VO_{2\max}$).

With increased glucose uptake by the contracting muscles, hepatic glycogenolysis and hepatic glucose output are activated, and the subsequent hormonal responses involve changes in the gluco-regulatory hormones such as a reduction in insulin, and an increase in glucagon, cortisol, growth hormone and catecholamines⁴⁵⁷. More recently, IL-6 has also been identified as playing a role in glucose homeostasis¹⁴⁰ via its potential effects on increasing hepatic glucose production⁸⁸ and increasing lipolysis and fatty acid mobilization^{244,328,357,396,398,400,424} during exercise. Prolonged exercise and progressive glycogen depletion will result in more pronounced changes in glucose counterregulatory hormones⁴⁰⁴, whilst CHO supplementation during such exercise has been shown to diminish these hormonal responses^{44,180,275,277}. Furthermore, metabolic and hormonal alterations elicited by preexercise CHO intake (short- or long-term) may bring about alterations in parts of the gluco-regulatory system (such as altered insulin, cortisol, catecholamines; altered hepatic glucose output, glycolysis, glycogenolysis), as well as alterations in the availability of alternative oxidative substrates (e.g. FFA) which may alter the fuel selection and relative contribution of substrate oxidation within the muscle³⁹⁹. It is clear that factors such as the status of the endogenous CHO stores, substrate availability and exercise intensity and duration play an important role in altering the neuro-humoral milieu, substrate selection and oxidation and ultimately glucose homeostasis. The full extent of the interplay of these factors is not yet fully understood.

Muscle glycogen depletion and hypoglycemia have been related to the development of peripheral and central fatigue^{52,78,163} and CHO ingestion has been shown to delay the onset of fatigue^{52,78} and attenuate the perception of effort, increase CHO oxidation, and reduce plasma cortisol towards the latter stages of prolonged exercise^{48,420}. These findings support a physiological link between RPE and CHO substrate availability as well as selected hormonal regulation during prolonged exercise.

Furthermore, the observation of a temporal association between increased RPE and reduced cerebral glucose uptake and metabolism suggests that fatigue may be related to inadequate substrate availability for the brain³²⁶. In addition, hypoglycemia has been shown to result in reduced CNS activation accompanied by a significantly lower force production³²⁵. Hence, the ergogenic effect of CHO supplementation during prolonged exercise and prevention of hypoglycemia, may be related, in part, to the counteraction of central fatigue³²⁵.

Though a strong association between CHO (liver and muscle glycogen) depletion and fatigue development during prolonged submaximal exercise has been observed, the findings are not universal and the exact mechanisms regarding CHO regulation and its ergogenic potential are still not fully understood. For example, some studies demonstrated that the development of

hypoglycemia^{87,146,228,230,312} had no effect on exercise performance. Furthermore, though carbohydrate loading and CHO supplementation during exercise have been shown to be ergogenic (for review see⁷⁶), it is not always as a result of augmented blood glucose concentrations and/or rates of CHO oxidation compared to the placebo treatments (e.g. refs.^{35,38,440,441}). There seems to be an individual range in metabolic and hormonal responses, and that this ultimately has a variable impact on the individual's sensation of fatigue and endurance capacity.

Factors that may contribute to individual alterations in the metabolic and hormonal milieu may include an adaptation of the HPA-axis to repeated and prolonged exercise-induced increases in cortisol secretion¹²⁵. In this regard, prolonged endurance training may introduce a resetting in the pituitary-adrenocortical component of the endocrine system, expressed either by intensified endocrine functions in some individuals or by suppressed endocrine functions in others⁴²⁶. Furthermore, it has been demonstrated that repeated exposure to physiological stresses that activates the HPA-axis (e.g. hypoglycemia or exercise) may result in a reduction in subsequent metabolic, hormonal, and symptomatic counterregulatory responses in diabetic^{25,96,101,385} and healthy individuals^{102,103,109,160,205}. The exact mechanism(s) responsible for the reduced neuroendocrine and autonomic-adrenomedullary responses observed after repeated exposure to hypoglycemia (or related physiological stresses) are not known, and the impact of these responses on gluco-regulatory responses in healthy, trained athletes remain to be investigated.

It seems clear that fatigue development during prolonged exercise may be the result of a more complex interaction between peripheral and central factors, at times difficult to measure directly with current research techniques. Though alterations in bodily CHO reserves and hormonal milieu (e.g. via dietary manipulation) can affect several metabolic responses and result in a complex interplay between metabolic and hormonal responses, it is not always clear how this may ultimately affect fatigue development or exercise performance. Further research is needed to clarify metabolic, hormonal and performance effects of various dietary and hormonal alterations, and to explore the individual variability in responses.

AIMS AND OBJECTIVES OF THIS THESIS

The main aims of this thesis were to investigate the effect of altered endogenous glycogen availability, achieved primarily by pre-exercise dietary manipulation and antecedent exercise exposure, on inter-individual variability in metabolic and hormonal responses to dynamic, steady-state exercise. Further, this thesis examined the impact of altered glucose availability (and the related metabolic and endocrine responses) on fatigue development during prolonged exercise. We hypothesized that endogenous CHO availability and the associated metabolic sequelae would impact on effort perception during exercise and fatigue development. We further hypothesized that prior fatiguing exercise and local muscle glycogen depletion would provide some neural and/or humoral signal, thereby altering resistance to fatigue. Finally we proposed that antecedent exposure to hypoglycemia will alter the counterregulatory responses (such as an alteration in HPA-axis (cortisol) response) during subsequent (next-day) prolonged exercise, and that this would translate into reduced effort perception and improved exercise performance.

University of Cape Town

CHAPTER 2

~ PRELUDE ~

THE EFFECT OF ALTERING THE GLYCEMIC INDEX OF THE PRE- EXERCISE MEAL ON METABOLISM AND EXERCISE PERFORMANCE DURING PROLONGED EXERCISE

Chapter 2 represents research conducted as part of my first year of post-graduate research and forms the prelude for the hypothesis of this thesis. The idea and supervision of this study were provided by Prof. Louise Burke.

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Burke L.M., Claassen A., Hawley J.H. and Noakes T.D.*

2.1 INTRODUCTION

CHO supplementation immediately before and during prolonged exercise have been identified as strategies that can increase CHO availability during prolonged exercise with potential performance-enhancing benefits ^{188,240,292}. Though preexercise CHO ingestion can help to optimize liver and muscle glycogen, this strategy has raised some concerns, particularly related to CHO consumed during the hour prior to exercise as it may result in hyperglycemia and hyperinsulinemia, and a potential fall in glucose (rebound hypoglycemia) during exercise ^{3,87,152,280,288}. Other potential consequences of relative hyperinsulinemia include a reduction in the rate of fat oxidation ¹⁷¹ and an increase in CHO oxidation ¹⁷¹, which in turn may lead to increased muscle glycogen oxidation ⁸⁰.

In examining the studies which have measured performance after CHO ingestion in the hour prior to exercise, only one study reported a decrement in endurance performance when subjects consumed glucose 30 min before exercise ¹⁵². The results of this study were widely publicized, leading to recommendations that athletes should avoid pre-exercise CHO intake, for fear of rebound hypoglycemia shortly after the onset of exercise, and premature depletion of glycogen stores during exercise. These fears have persisted despite the subsequent publication of several studies that observed no significant effect ^{145,186,389,439} or an improvement in performance during prolonged, moderate intensity exercise with preexercise CHO ingestion ^{171,251,330,378}.

The diversity in results are likely due to differences in the quantity of CHO ingested, and/or to rate of digestion and oxidation of the ingested meal, which will in turn alter glucose and insulin responses. These metabolic disturbances may be attenuated by choosing CHO sources which produce a minimal glycemic-insulinemic response ¹⁹⁵.

The glycemic index (GI) is determined by the rate at which an ingested carbohydrate is made available to intestinal enzymes for digestion and absorption and has been utilized as a system to rank CHO-rich foods according to their glucose- and insulin responses ²²⁶. It has been suggested that the manipulation of the GI of meals may have application in the area of sports nutrition to manipulate metabolic and hormonal responses, thereby optimizing CHO availability particularly during prolonged moderate-intensity exercise. However, results of studies investigating the effect of varying the GI of the pre-exercise meal on exercise metabolism and performance have yielded inconsistent results.

Thomas et al. ⁴⁰⁸ were the first to study the role of the GI of different CHO-containing foods and their effects on metabolism and exercise performance. They reported that the ingestion of a low GI CHO-rich meal (lentils), 1 h prior to exercise increased the time to exhaustion during the

cycling trial at 67% of maximal oxygen consumption ($VO_{2\max}$), compared with the ingestion of an equal amount of CHO eaten in the form of a high GI CHO-rich food (potatoes). These findings were attributed to lower glycemic and insulinemic responses combined with increased FFA availability and fat oxidation with the low GI trial compared with the other CHO feedings⁴⁰⁸. These findings have been widely publicized and have resulted in popular advice that athletes should choose pre-exercise meals based on low GI CHO-rich foods and drinks⁴⁰⁸.

In contrast, a subsequent study by Febbraio and Stewart¹⁴⁵ reported no differences in the performance of a cycle time-trial conducted after 2 h of cycling at 70% $VO_{2\max}$, when preexercise meals eaten 45 min before the bout consisted of either a low GI CHO (lentils), high GI CHO (potatoes) or placebo (diet jelly). They reported no difference in total CHO oxidation between the two CHO treatments, and similar muscle glycogen utilization in all three trials. These findings are more consistent with the view that glycemic differences at the onset of exercise are short-lived and of little practical significance¹⁹⁵. It should be noted though that the lentil and potato meals used in the studies of Thomas et al.⁴⁰⁸ and Febbraio and Stewart¹⁴⁵ were not equivalent in macronutrient (protein) composition, nor were they isocaloric⁴⁰⁹. Hence, it is not known what the contribution of the protein *per se* was to the insulinemic, glycemic and other metabolic responses seen after ingestion of the lentil meal.

Subsequent studies found that the ingestion of a low GI, isocaloric pre-exercise meal reduced CHO oxidation^{141,389,409,439} whilst others found no difference in CHO oxidation^{145,210,251} or rate of muscle glycogenolysis^{141,251} compared to a high GI pre-exercise meal. Whilst some of these studies reported an improvement in exercise performance (time to exhaustion) when the GI of the pre-exercise meal was lowered^{117,249,251,408}, others found no effect on exercise performance when employing time to complete a set amount of work to assess performance^{141,145,389,409,439}. The latter measure of performance is more representative of real-life racing conditions (where there is a defined end-point) as well as being a more reliable and reproducible measure of performance^{232,301}.

Another nutritional strategy commonly employed by endurance athletes is to ingest CHO during the endurance exercise bout or competition. Though the interaction between preexercise CHO intake combined with CHO ingestion during endurance exercise has received some attention⁴⁵⁴, little is known about the interaction between varying the GI preexercise CHO meal combined with CHO ingestion during exercise. Therefore, the purpose of this investigation was to examine whether the GI of pre-exercise CHO intake has any impact on exercise metabolism and subsequent performance when sufficient amounts of CHO are consumed *during* the exercise session. The conditions of the study were chosen to be representative of competitive sport (i.e. performance was measured with a defined end-point time-trial as opposed to being open-ended),

as well as being representative of sport nutrition guidelines for optimal pre- and during exercise feeding. We hypothesized that a low GI preexercise meal would reduce postprandial hypoglycemia and hyperinsulinemia compared to a high GI meal, but when combined with the ingestion of relatively large amounts of CHO during exercise, will have no further impact on exercise metabolism and performance.

2.2 METHODS

2.2.1 Subjects

Six healthy, endurance-trained male cyclists (23 ± 6 yr, 72 ± 10 kg, 67 ± 9 ml/kg/min) volunteered to participate in this investigation, which was approved by the Research and Ethics Committee of the Faculty of Health Sciences of the University of Cape Town (South Africa). All subjects were racing competitively and were included on the basis of completing a local 105 km cycle race in under 3 h. Because tracer amounts of [U- 14 C]glucose were ingested and blood samples were taken, the risks were carefully explained to the subjects before their written consent was obtained. The total radiation dose received by each subject was ~ 20 mrem. The radiation dose accepted as safe in South Africa is 500 mrem/yr or 130 mrem/13 wk³⁵.

2.2.2 Preliminary testing

2.2.2.1 Peak power output (W_{peak}) and maximal oxygen consumption (VO_{2max})

One to 2 weeks prior to the start of the study, subjects were tested for maximal oxygen uptake (VO_{2max}) and peak sustained power output (W_{peak})²¹⁰ on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands) modified with clip-on pedals and racing handle bars. After a ~ 15 min warm-up period, the W_{peak} test was started at a workload equivalent to 3.3 Watts/kg BM and increased first by 50 Watts (W) after 150 sec and then by 25 W every 150 sec until the pedaling frequency dropped below 50 revolutions/min. W_{peak} was defined as the highest exercise intensity the subject completed for 150 sec in W, plus the fraction of time spent in the final workload multiplied by 25 W. This information was used to adjust the intensity and work rate in the experimental trials so that each subject performed at 70% of VO_{2max} .

Throughout the maximal test, subjects wore a face mask attached to an Oxycon Alpha automated gas analyzer (Jaeger, The Netherlands). Before each test the gas analyzer was calibrated using a Hans Rudolf 5530 3-litre syringe and a 5% CO_2 :95% N_2 gas mixture. Analyzer outputs were processed by a computer which calculated minute ventilation (V_i), oxygen consumption (VO_2) and rates of carbon dioxide production (VCO_2) using conventional equations.

2.2.3 Experimental design

Each subject undertook three trials, separated by 7 days, in a randomized, counter-balanced order. On each occasion one of the following test meals was ingested 2 h before cycling at 70% of $VO_{2\text{ max}}$; a high GI CHO-rich meal (HGI) of instant mashed potato (GI=87, where glucose = 100, ref. ¹⁵¹), a low GI CHO-rich meal (LGI) of lightly cooked pasta (GI=37, ref. ¹⁵¹), or a control meal (CON) consisting of low-energy jelly. The CHO-rich meals provided 2 g CHO/kg BM, and the water content of all meal was standardized so that each provided ~ 1100 ml of fluid (Table 2.1).

Table 2.1. Characteristics of the pre-exercise meals

Meal	Description	Nutrient analysis			
		Energy	CHO	Protein	Fat
HGI meal	130 g dried potato mix + 250 ml skim milk + 625 ml hot water + 240 g tomato-based pasta sauce Total fluid: 1070 ml	2730 kJ	143 g	23 g	1.5 g
LGI meal	165 g dry pasta cooked in water + 250 g tomato based pasta sauce + 500 ml water as drink Total fluid: 1068 ml	2770 kJ	139 g	23 g	2 g
CON meal	1 packet low energy jelly + 500 ml water + 570 ml water as drink Total fluid: 1070 ml	135 kJ	1 g	7 g	0 g

These meals are designed for a 70 kg subject.

Food intake and training were standardized for the 24 h before each trial. Subjects were provided with guidelines for CHO-rich meals and were requested to record their dietary intake on the day before the first trial. Identical food was consumed before the subsequent trials, with dietary records being continued to check compliance. Additionally, subjects were asked to abstain from alcohol, caffeine and strenuous exercise for at least 24 h before each trial. On the morning of an experiment, subjects reported to the laboratory in an overnight fasted state, and their food and training diaries were examined to ensure that all instructions had been followed. Thereafter a flexible 18-gauge catheter was inserted into a forearm vein and attached to a 3-way stopcock for the sampling of blood. The catheter was kept patent throughout the experiment with periodic injection of heparinized saline.

2.2.3.1 *Pre-exercise dietary intake*

After a fasting blood sample was taken, the subjects were given 15 min to consume their test meal and rested for 2 h. Fifteen min before exercise, the subjects consumed 4 ml/kg BM of a 10 g/100 ml [U-¹⁴C]glucose solution (Amersham International, Buckinghamshire, UK) with a specific radio-activity of 0.17 μ Ci/g (6.3 kBq/g). The [U-¹⁴C]glucose label was added to the drink so that the rates of ingested glucose oxidation could be calculated. A total of 3.3 ml/kg BM of the labeled drink was ingested every 20 min during the steady-state cycle, for a total of 24 ml/kg BM each trial (2.4 g of CHO/kg BM).

2.2.3.2 *Exercise protocol*

Immediately before the start of exercise, subjects voided, were weighed, and the cycle ergometer was set up to suit their preferred cycling position. Exactly 2 h postprandially the subjects started their steady-state ride at $\sim 70\%$ of $VO_{2\text{ max}}$ (245 ± 18 W). The steady-state ride was performed to allow for metabolic data to be collected at steady state, and at an intensity that is representative of race-pace (moderate-high intensity). As part of the 2 h cycle, a warm-up period was allowed; exercise started at 100 W for 5 min, after which the workload was increased by 50 W/60 sec until the final workload was attained. During the trials, the subjects were cooled with an electric fan, while the laboratory was maintained at a constant temperature (~ 20 °C) and relative humidity ($\sim 55\%$). On completion of the 2 h steady-state ride, the subjects were given 1 min to rest before they started the performance ride, which consisted of the time to complete 300 kJ (time-trial, TT). During the time-trial, subjects were kept blind to time; the only feedback given was the completion of each 50 kJ of work. After subjects had completed 275 kJ they received information about each successive 5 kJ until the end of the ride. No performance results were provided to any subject until the completion of the entire study.

2.2.3.3 *Blood sampling and analysis*

A fasting blood sample was collected before ingestion of the experimental meal where after blood sampling was undertaken 30, 60, 90 and 120 min postprandially. During the steady-state ride, blood was sampled at successive 20 min intervals, commencing after 20 min of the start of the ride. Approximately 6 ml of blood was drawn at each sampling of which 2 ml was placed in a tube containing potassium oxalate and sodium fluoride for the later analysis of plasma glucose concentrations. The remaining 4 ml was placed in a tube containing gel and clot activator and allowed to clot for 15 min at room temperature for the later analyses of serum insulin and FFA concentrations. All samples were kept on ice during the duration of the trial before the plasma and serum were separated by centrifugation (2,000 rev/min) at 4 °C and stored at -18 °C until subsequent analyses. Plasma glucose concentrations were determined by the glucose oxidase

method (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA, USA). Serum insulin concentrations were determined by the use of a commercially available radio-immunoassay kit (Count-A-Coat Insulin, Diagnostic Products Corporation, CA, USA). Serum total FFA concentrations were determined by an enzymatic colorimetric assay (Half-micro test, Boehringer Mannheim, Germany).

2.2.3.4 VO_2 , VCO_2 and $^{14}CO_2$ measurements

Immediately after each blood sample was taken during the steady-state ride, gas exchange (VO_2 , VCO_2) was measured for 5 min. In addition CO_2 was trapped by passing a sample of expired air, collected in a ambulatory bag, through a solution containing 1 ml of 1 N hyamine hydroxide in methanol (United Technologies, Packard, Ill., USA), 1 ml of 96% ethanol (SAARCHEM, Krugersdorp, RSA) and 2-3 drops of phenolphthalein (SAARCHEM). The expired air was bubbled through the trapping mixture until the solution became clear, at which point exactly 1 mmol of CO_2 had been absorbed. Liquid scintillation cocktail (Ready Gel, Beckman Instruments Inc., Fullerton, USA) was added, and $^{14}CO_2$ radioactivity in dpm was counted in an Insorb 460C Automatic Liquid Scintillation Counter (United Technologies, Packard, Ill., USA).

2.2.3.5 Calculation of total and ingested drink carbohydrate oxidation

Instantaneous rates of CHO oxidation during exercise were calculated (g/min) using the formulae of Frayn¹⁵³, assuming a nonprotein respiratory exchange ratio, assuming no increase in basal amino acid and protein degradation during exercise:

$$\text{Total CHO oxidation} = 4.55 VCO_2 - 3.21 VO_2$$

Where VCO_2 is the volume of CO_2 in the expired air in L/min; and VO_2 is the corresponding oxygen uptake in L/min.

Total CHO oxidation during the 120 min of steady-state exercise was estimated from the area under the CHO oxidation versus time curve for each subject. The rates of ingested CHO oxidation were calculated from the following equation:

$$\text{Glucose}_{\text{ox}} = \{^{14}CO_2 \times 6 / [(SA_{\text{CHO}} / \text{CHO}_D) \times 180]\} \times VCO_2 \times 1.35$$

where $\text{glucose}_{\text{ox}}$ is the amount of ingested CHO oxidized in g/min ; $^{14}CO_2 \times 6$ is the $^{14}CO_2$ dpm/mmol value multiplied by 6 as there are 6 carbon atoms per molecule of ^{14}C glucose tracer added to the ingested solution; SA_{CHO} is the specific activity of the ingested solution in dpm/ml; CHO_D is the CHO content of the drink in g/L; 180 is the molecular mass of glucose; VCO_2 is the

volume expired CO₂ in L/min; and 1.35 is the number of grams of glucose oxidized to produce 1 L of CO₂.

2.2.3.6 Rating of perceived exertion

During all three trials subjective ratings of perceived exertion (RPE) were obtained using the modified Borg scale³⁰. At the end of the study subjects were asked by questionnaire which of the pre-exercise meals provided their best performance and which they would choose to consume before a competition.

2.2.4 Statistical analyses

The statistical software package STATISTICA 7.0 (2004; StatSoft, Inc., Tulsa, OK, USA) was used for the statistical analysis of the data. GraphPad PRISM software package, version 3.0 for Windows (GraphPad Software, San Diego, CA, USA) were used to calculate area under the curve. Data are presented as mean±SD, unless where otherwise indicated. Data from the three trials were compared using a two-factor (diet and time) analysis of variance (ANOVA) with repeated measures. Simple main effects analyses and Scheffe's post-hoc tests were undertaken when ANOVA revealed a significant interaction. CHO oxidation over the 2 h of steady-state exercise, and time trial performances were compared using one-way ANOVA with Scheffe's post-hoc tests. Significance was accepted when $p < 0.05$. All data are reported as mean ± SD.

2.3 RESULTS

Records kept by each subject during the 24 h before each trial indicated compliance to the standardized preparation protocol; reported CHO intake on the day before the three trials was 479±125 g, 465±151 g, 460±127 g for the HGI, LGI and CON trial respectively (NS), and all subjects refrained from exercise during that day.

2.3.1 Plasma glucose, serum insulin and FFA concentrations

Figure 2.1 shows plasma glucose, serum insulin and serum FFA concentrations for the 2 h period following ingestion of each test meal and for the subsequent 120 min of steady-state exercise. There was a significant interaction of diet and time for all parameters ($p < 0.05$). At 30 min after ingestion of the HGI meal, plasma glucose concentrations were significantly increased above fasting values ($p < 0.05$) (Figure 2.1A). At this timepoint, plasma glucose concentrations were greater in both HGI and LGI trials (7.9±1.5 and 6.6±1.2 mmol/L) compared to the CON trial (4.4±0.6 mmol/L). Thereafter, plasma glucose concentrations declined and were back to

baseline levels by 60 min (~5.0-5.5 mmol/L) after each of the CHO meals. Plasma glucose concentrations did not fall below baseline values in any of the trials, and were maintained >5.0 mmol/L throughout the study period. Plasma glucose concentrations rose slightly with the ingestion of the CHO drink (starting 15 min before the start of exercise) and at the onset of exercise, and remained at euglycemic levels (5.0-6.0 mmol/L) throughout the steady-state ride in all trials. Plasma glucose concentrations during exercise were similar between trials (Figure 2.1A).

The serum insulin response after ingestion of the HGI meal was significantly greater ($p < 0.05$) during the 2 h postprandial period compared to that after the LGI meal (Figure 2.1B). Serum insulin concentrations at 30 min were higher in the HGI trial than in the LGI trial (74.1 ± 24.2 vs. 43.9 ± 21.4 $\mu\text{U/ml}$), and remained elevated above those in the LGI trial at 60 and 90 min. Serum insulin concentrations remained at fasting values (< 10 $\mu\text{U/ml}$) in the CON trial until 90 min; however at 120 min there was an increase in insulin concentrations in response to the ingestion of the glucose drink 15 min before the start of exercise. In the LGI trial, serum insulin concentration peaked at 30 min after the meal and then declined thereafter, with the feeding of the bolus of the glucose drink causing a small rise. During the bout of steady state exercise, despite the intake of significant amounts of CHO, serum insulin fell to fasting concentrations in all trials. Insulin concentrations in the HGI trial were still elevated above those in the CON trial at 20 min of exercise; thereafter there were no differences in the serum insulin concentrations during exercise between any of the trials.

Both the CHO meals suppressed serum FFA concentrations to < 0.1 mmol/L, which persisted until the start of the exercise (Figure 2.1C). During the CON trial, FFA concentrations were maintained at fasting levels throughout the pre-exercise phase. However, intake of CHO drink at the onset of exercise caused a small drop in FFA concentration after 20 min of exercise. During exercise, despite ingestion of the CHO drink, serum FFA concentrations gradually increased in all three trials so that concentrations during the last 60 min of exercise were significantly greater (~ 0.25 mmol/L) than at the start of exercise ($p < 0.05$).

2.3.2 Substrate oxidation

Metabolic data from 120 min of steady-state exercise are presented in Fig 2.2. There was no significant interaction of diet and time for RER (Figure 2.2A), total CHO oxidation (Figure 2.2B) and oxidation of the ingested CHO drink (Figure 2.2C). The decline in RER over the 120 min of steady-state exercise was not significant. Oxidation of CHO from the glucose drink increased throughout the 120 min of exercise from ~ 0.3 g/min after 20 min to ~ 0.8 g/min at 120 min in all three trials. At 20 min, oxidation of the ingested CHO drink was significantly lower in the LGI trial

than in CON ($p < 0.05$); however, there were no differences at any of the other timepoints. Overall the drink provided ~16% of total CHO oxidation for the 120 minutes of exercise, and was similar for all trials (Figure 2.3). Total CHO oxidation was ~380 g during the 120 min of steady-state exercise and did not differ between trials.

2.3.3 Exercise performance and perceived exertion

Performance during the TT undertaken at the end of the 120 min of steady-state exercise did not differ between trials. Time to complete 300 kJ was 947 ± 56 ; 953 ± 85 ; and 970 ± 63 sec for HGI, LGI and CON, respectively.

Subjects' rating of perceived exertion (RPE) rose steadily throughout the steady-state cycle and did not differ between trials.

The results of the questionnaire showed that 3 subjects felt they performed best on the LGI meal, whereas 2 chose the HGI meal and 1 the control meal. All subjects reported that, from the 3 study meal selections, the LGI meal would be their preferred choice of meal before an important competition / race event. Furthermore, the pre- and during exercise feeding schedules were well-tolerated seeing that there were no reports of gastrointestinal discomfort.

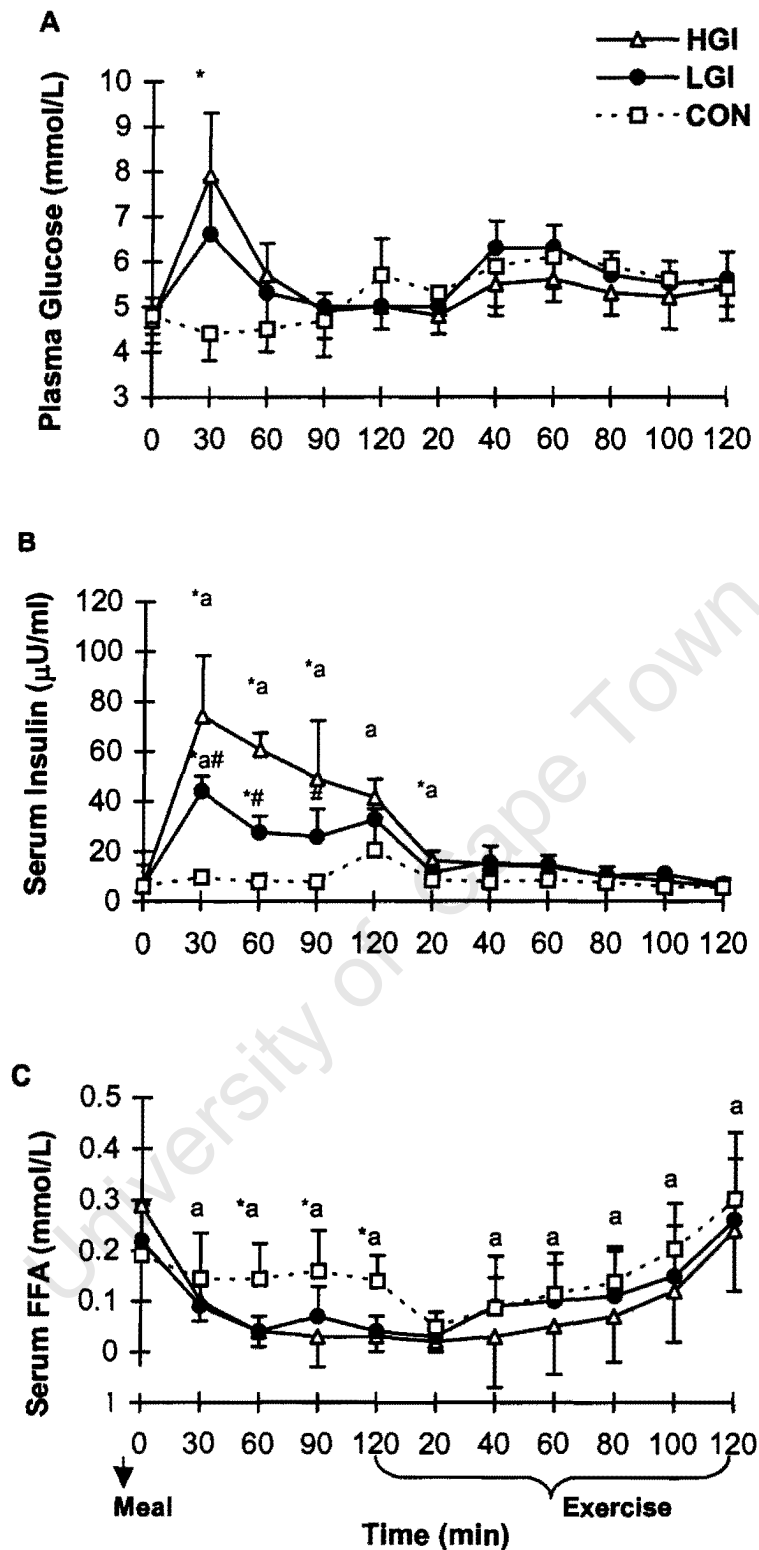


Figure 2.1 Plasma glucose (2.1A), serum insulin (2.1B) and serum free fatty acid (2.1C) concentrations for the 2 h period postprandial and during the 2 h of steady-state exercise *Different from CON; # different from HGI; ^a different from fasting; ^b different from start of exercise; ($p < 0.05$).

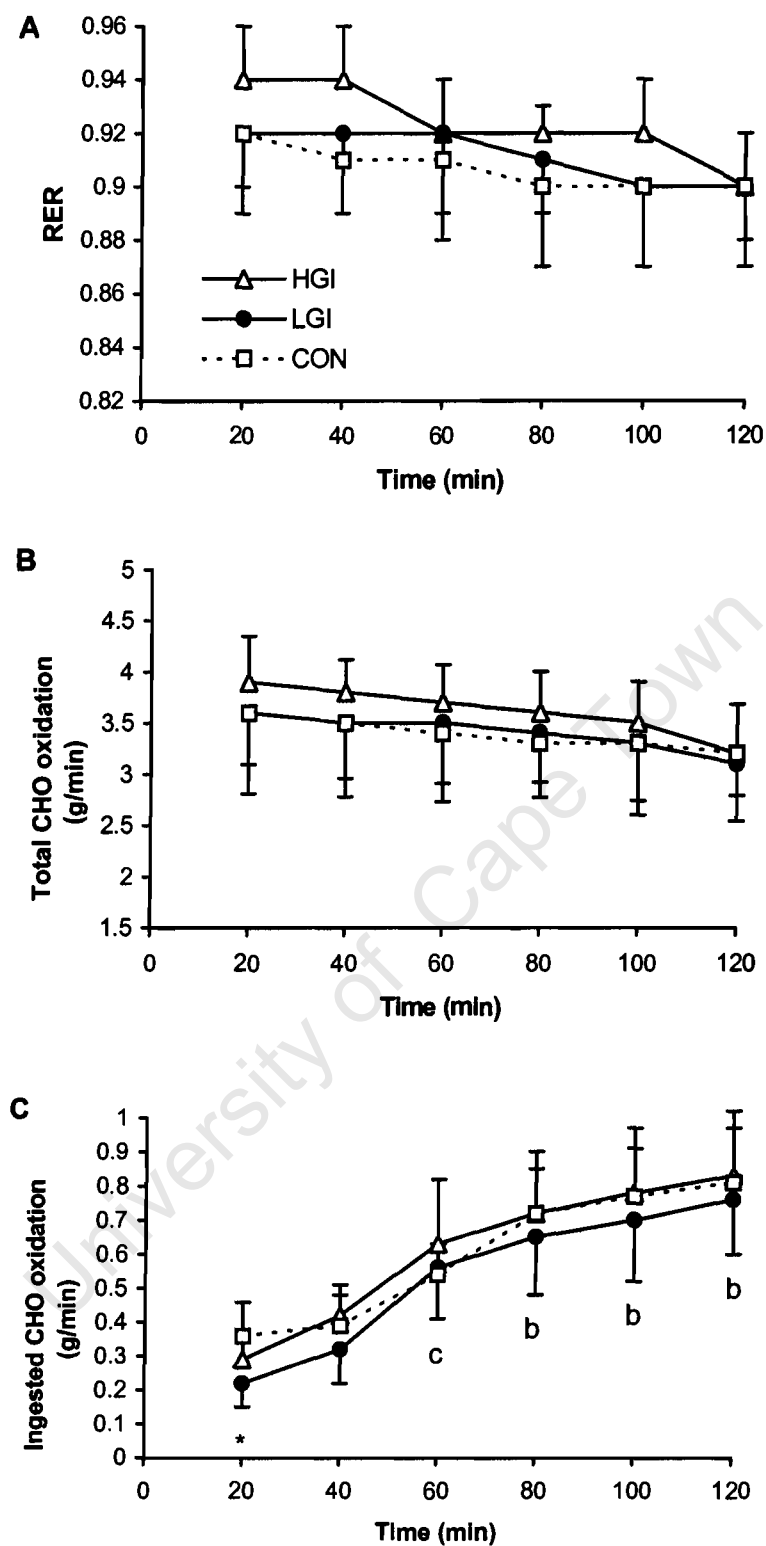


Figure 2.2 Respiratory exchange ratio (RER) (A), total carbohydrate (CHO) oxidation (B) and ingested CHO oxidation (C) during the 2 h steady-state exercise. *Significantly different from CON; ^a different from the start of exercise for HGI; ^b different from start of exercise for HGI, LGI and CON; ^c different from start of exercise for HGI and LGI ($p < 0.05$).

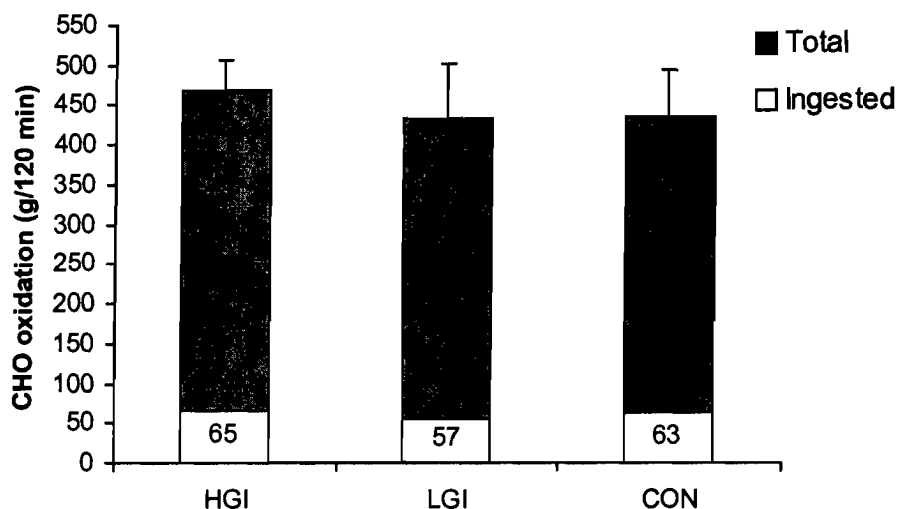


Figure 2.3 Total carbohydrate (CHO) oxidation and ingested CHO oxidation during 2 h of steady-state exercise calculated from area under oxidation curves (Values are mean \pm SD of 6 subjects). NS.

2.4 DISCUSSION

The main finding of the present study was that the ingestion of CHO during exercise in amounts supported by current sports nutrition guidelines^{321,84,196}, minimizes the postprandial metabolic responses elicited by preexercise CHO meals of variable GI. Furthermore, with CHO ingestion during exercise, varying the GI of the preexercise CHO meal has no effect on endurance cycling performance.

This study was carried out in view of the historical controversy regarding CHO intake before prolonged submaximal exercise. There has been considerable focus on the metabolic disturbances caused by the rise in insulin concentration which accompanies pre-exercise CHO intake^{152,171,187,280}. Elevations in plasma insulin have been shown to suppress lipolysis during subsequent exercise²¹¹; and increase CHO oxidation resulting in a decrease in plasma glucose and/or an accelerated rate of muscle glycogen utilization. Indeed, Foster et al.¹⁵² reported impaired cycle time to exhaustion at 80% of $VO_{2\max}$ when 75 g of glucose was fed 30 min before exercise, and based on these findings athletes were advised to avoid CHO intake during the hour before endurance exercise. These recommendations have persisted despite evidence from several more recent studies that CHO intake during the hour before exercise enhances, or at least fails to affect endurance exercise performance (for reviews see^{188,195}).

The recent application of the GI to sports nutrition³⁸⁴ has revived the debate regarding metabolic perturbations associated with pre-exercise CHO intake. Thomas et al.⁴⁰⁸ were the first to propose that consumption of a low GI preexercise meal as a practical means of avoiding the

potential negative effects of a hyperglycemic-insulinemic postprandial response. In their study, exercise time to fatigue was improved when subjects consumed 1 g of CHO/kg BM from a low GI food (lentils), 1 h before cycling at 67% of $VO_{2\max}$, compared with an equal amount of CHO eaten as a high GI food (potatoes). This benefit was attributed to lower glycemic and insulinemic responses to the low GI meal compared with the high GI meal, maintaining blood glucose concentrations during exercise, increasing FFA concentrations and reducing exercise RER values.

Although this study⁴⁰⁸ has led to widespread advice that endurance athletes should choose pre-exercise meals based on low GI CHO-rich foods and drinks⁴³, others investigations have failed to demonstrate that metabolic alterations translate into performance effects when employing time to complete a set amount of work to assess endurance cycling^{141,145,389,409} or running performance⁴³⁹.

A second study conducted by the Thomas et al. group⁹⁷ utilized the same pre-feeding and exercise protocol; on this occasion the test meals consisted of isocaloric low GI and a high GI powdered food, and a low GI and a high GI breakfast cereal. They reported a correlation between the meal GI and the subsequent depression of blood glucose and FFA concentrations during exercise in that low GI meals were associated with higher glucose and FFA concentrations after 90 min than the high GI meals. Thus, low GI meals appeared to provide a sustained source of CHO throughout the exercise bout and later during recovery⁹⁷. However there were no differences in exercise time to exhaustion between trials, and no correlation between exercise time and the GI of the meal⁹⁷. It should be noted that the measurement of performance used in both the Thomas et al. studies (exercise time to exhaustion at a fixed submaximal work rate) has a high (~25%) coefficient of variation^{232,301}, which increases the possibility of Type 2 error.

Subsequent to that study, Febbraio and Stewart¹⁴⁵ found no differences in the performance of a time-trial conducted after 2 h of cycling at 70% $VO_{2\max}$, when pre-exercise meals consisted of either a low GI CHO-rich food (lentils), high GI CHO-rich (potatoes) or a placebo (low-energy jelly). The meals were eaten 45 min before exercise and in the case of the CHO meals, provided an intake of 1 g of CHO/kg BM. Data from Febbraio and Stewart¹⁴⁵ and others showed no differences in total CHO oxidation^{145,210,251} between the two CHO treatments, and similar muscle glycogen utilization in all trials^{145,251}. These findings are consistent with the view that glycemic differences at the onset of exercise are short-lived and unimportant for the performance of most athletes^{409,141,145,389,439}. The advantage of the exercise protocol employed by Febbraio and Stewart¹⁴⁵ is that it provides a more sports-specific and reliable measurement of performance

²³², preceded by a period of steady-state exercise during which comparison of the metabolic responses to treatments can be made.

In agreement with other studies, we found that the ingestion of a high GI CHO-rich meal produced a greater postprandial glycemic and insulinemic response compared with a low GI CHO-rich meal ^{141,145,249,389,408,409}. The rise in insulin concentration following both CHO-rich meals resulted in a suppression of FFA concentrations in the 2 hrs postprandially. The most effective and common strategy used by endurance athletes to promote CHO availability during exercise is to ingest CHO-rich drinks or foods *during* the event. CHO ingestion during exercise is important for endurance performance because it maintains euglycemia and high rates of CHO oxidation when endogenous CHO stores have become limited ⁷⁶. The data from the present study demonstrated that despite alterations in insulin and FFA concentrations induced by the preexercise high and low GI meals, CHO ingestion during exercise minimized any potential differences in either circulating blood metabolites or substrate oxidation during the 2 h bout of exercise. These findings are consistent with the view that glycemic differences at the onset of exercise are short-lived and unimportant for the performance of most athletes ^{141,145,389,409,439}.

Though the large standard deviation (SD) values for the glucose and insulin responses in the 2 h postprandial period suggest an inter-individual range in metabolic responses, there was no evidence of rebound hypoglycemia in any of the subjects, as is typically seen with the pre-exercise intake of glucose ^{69,114,152,186,187,260}, or medium and high GI CHO-rich foods ^{210,211,389,408}. Blood glucose concentrations were maintained >4 mmol/L throughout the 2-h postprandial period (at rest) and throughout exercise in all trials, likely due to the combined effects of CHO ingestion 2 h prior to exercise combined with ingestion of the CHO drink 15 min before, and at 20-min intervals during exercise. Furthermore, CHO oxidation rates were maintained throughout the exercise bout without any differences between trials or over 2 h exercise period. The tracer-determined rates of ingested CHO oxidation were similar to those reported in other studies which have used a pre-exercise bolus feeding and serial feedings throughout exercise (for review, see ¹⁹⁶). Irrespective of the choice of the pre-exercise meal, the ingested CHO drink contributed ~60 g, or ~16% of the total CHO oxidized. Given similar patterns of substrate utilization and availability during exercise between the three trials, it was not surprising to find similar performances during the timed performance rides, which followed the steady-state exercise.

Wright et al. ⁴⁵⁴ examined the interaction of CHO intake before and during exercise. They found that compared with no CHO intake at all, cycling time to exhaustion and total work output at 70% of $\dot{V}O_{2\max}$ were improved by the ingestion of 5 g of CHO/kg BM 3 h before exercise, or by intake of 2.6 g of CHO/kg BM in serial feedings during the trial. Enhancement of the performance measures was ~18% and 33% ($p < 0.05$) for the pre-exercise CHO and during-exercise CHO

intake, respectively. When undertaken together, the two strategies improved performance by ~45%. While this suggests that the combination of CHO intake strategies was superior to either of the feeding strategies alone, performances during the combined trial were not significantly different to the pre-exercise CHO trial or the during-exercise CHO trial⁴⁵⁴. Similarly, in the current investigation, while there was no significant difference in cycling time between trials, 5 of the 6 subjects achieved their best performance with a combination of CHO before exercise (HGI or LGI trial) and CHO intake during exercise, compared with the CON trial (CHO intake during exercise alone).

Although most subjects were able to correctly identify which trial had produced their best performance, they stated that they would not necessarily choose this meal if they were to compete in an important competitive event. The most popular preexercise meal selected was pasta (LGI meal), as it was rated as the most palatable and familiar choice of food. Clearly, practical and personal preferences are important in determining the athlete's choice of pre-event meal.

An important consideration in the interpretation and practical implication of the findings from this study is that the subjects consumed the preexercise CHO meals 2 h prior to the start of exercise, combined with optimal CHO intake during exercise in terms of amount (~170 g/120 min or ~1.4 g CHO/min) and timing (a loading bolus 15 min preexercise and at 20 min intervals throughout exercise). This feeding strategy maintained plasma glucose concentrations during exercise, negated preexercise metabolic perturbations due to HGI and LGI CHO intake, and resulted in similar performance effects. Furthermore, all subjects reported that for an important competition they would choose to eat a preexercise meal that was most familiar. Hence, in practical terms and contrary to popular advice that low GI CHO-rich foods are the preferred pre-exercise choice⁴³, athletes may be advised that in the light of an optimal CHO feeding strategy during exercise, the choice of the preexercise CHO-rich meal may be of HGI or LGI, depending on their personal preference and previous experience.

In conclusion, this study demonstrated that the ingestion of sufficient amounts of CHO during prolonged moderate-intensity exercise according to current sports nutrition guidelines minimizes any differences in the metabolic and performance responses arising from the choice of pre-event meal.

CHAPTER 3

EFFECTS OF ANTECEDENT FATIGUING EXERCISE ON SUBSTRATE METABOLISM, EFFORT PERCEPTION AND EXERCISE PERFORMANCE DURING SINGLE-LIMB EXERCISE

University of Cape Town

3.1 INTRODUCTION

In the previous chapter, we examined the effects of CHO ingestion, prior to and during exercise on substrate oxidation, and specifically, exercise performance. Despite differences in the endocrine response to high or low glycemic index preexercise meals, the provision of exogenous CHO throughout exercise negated any specific effects of the differences in glycemic index. In the present study, we examined the effect of local muscle glycogen depletion following single-leg exercise to exhaustion.

Previous studies have attempted to determine the factors affecting rating of perceived exertion (RPE) and fatigue development during dynamic submaximal, steady-state exercise. No single mechanism has been identified, and in fact, evidence suggests that multiple factors may be involved (for review see ref. ¹⁴⁸). Despite this, fatigue during submaximal steady-state exercise has commonly been attributed to reduced CHO availability during exercise, specifically muscle glycogen depletion and/or development of hypoglycemia (for review see refs. ^{220,240}).

A strong relationship between muscle glycogen depletion and the onset of fatigue have been documented in the literature ^{148,240}. Furthermore, an interesting observation from the literature is that the typical level of muscle glycogen below which fatigue usually sets in, is ~40 mmol/kg wet weight (w.w.). It has long been believed that the main association between fatigue and glycogen depletion is the reduction in available fuel to sustain muscle contraction. Yet, ATP (fuel) depletion at the point of fatigue during dynamic exercise has not been demonstrated, in fact, minimal ATP depletion in the light of substantially depleted muscle glycogen and creatine phosphate stores has been reported ⁴³². Another suggested mechanism for the link between reduced glycogen concentrations and fatigue may be the concomitant increase in metabolites such as inorganic phosphate (P_i), which is known to have an inhibitory effect on both Ca^{2+} release from the SR ^{159,241} as well as the contractile apparatus ¹⁴⁸. It has also been hypothesized by Barnes et al. ¹² that the glycogen concentration *per se* exerts a protective action on excitation-contraction coupling ^{12,403}. These factors may explain the development of fatigue under conditions where blood glucose availability and CHO oxidation are maintained. However, studies in support of these suggestions in intact human muscle are lacking.

More recently, other causes of fatigue have also been hypothesized such as the circulation of humoral substances that may act as afferent signal(s) to the brain, which may in turn reduce central nervous system activation and ultimately reduce force output ^{148,322}.

The use of the single-limb exercise model provides a potential means to investigate the role of local muscle glycogen depletion and/or production of a circulating humoral response in fatigue

development during dynamic steady-state exercise under physiological conditions. Previous single-limb steady-state exercise models have documented the effects of single-limb exercise on resting muscle metabolism and substrate dynamics⁵; compared single-limb exercise responses to that of two-legged exercise³¹⁴; or compared responses between a trained and untrained limb³⁶⁷. No previously published study has to our knowledge considered single-limb exercise to exhaustion performed in sequential manner, to assess the impact of the previous exercise bout and the concomitant physiological changes on the exercise performance of the second, previously rested limb.

A study from this laboratory (Bosch et al., unpublished observations) employed a single-limb exercise model to assess whether dynamic exercise to exhaustion with one limb (and passive rotation of the opposite limb) and the consequent metabolic responses affected the exercise time to fatigue during a similar exercise task in the previously rested, second limb. This study design allowed assessment of the impact of lowered local muscle glycogen concentration reached at exhaustion with the first exercising limb, on exercise capacity of the second limb. The main finding was that exercise time to fatigue was significantly longer with the first leg compared to the second. Muscle biopsies (performed at the point of exhaustion for each limb) revealed that exhaustion was reached at similarly low muscle glycogen concentrations (~40 mmol/kg w.w.), and it was concluded that fatigue was related to muscle glycogen depletion. However, EMG recordings revealed partial recruitment in the passively revolving leg i.e. it was not completely inactive. Hence, one possible explanation for the results is that there was increased muscle glycogen utilization in the "passively" revolving leg. Another possible explanation may be the release of a humoral substance during exercise with the first limb, circulating throughout the body and perhaps causing increased glycogen depletion in the inactive muscle. This suggestion is supported by evidence from Ahlborg et al.⁵ indicating that several metabolic alterations take place in the non-exercising muscle during submaximal low intensity arm or single-leg exercise such as an increase in blood flow and oxygen uptake, partly as a consequence of motor activation; and a shift in substrate utilization from predominant FFA uptake in the basal state to a greater utilization of CHO.

Based on the findings of the investigation by Bosch and colleagues (unpublished data) and Ahlborg et al.⁵, the aim of this study was to further explore the impact of local muscle glycogen depletion on fatigue development and perceived exertion. Furthermore, to assess whether the local muscle glycogen depletion and metabolic responses in consequence to exercise with the first leg (and "seen" by the whole body) set up events that predisposes the second limb to fatigue prematurely. A single-limb, open-looped exercise protocol, where one leg cycles at a set intensity to fatigue, followed by exercise to exhaustion of the second, previously rested leg would be used. This model allows the opportunity to assess, within a physiological milieu, what effect

the metabolic responses elicited during exercise with the first leg (and circulating throughout the whole body) have on RPE, and exercise capacity of the second, previously rested leg. We hypothesized that exercise and concomitant local muscle glycogen depletion will result in a neural and/or hormonal signal that will reduce the exercise capacity of the second, previously rested limb.

3.2 METHODS

3.2.1 Subjects

Six healthy, physically active males aged between 18 and 20 years were recruited to participate in this study (age 19 ± 1 yrs; Weight 87 ± 7 kg, body fat 15 ± 2 %). The subjects were all recruited from a local university rugby (football) club. Exclusion criteria included any chronic diseases, intercurrent illnesses, history or current signs of knee pathology that would influence exercise performance or be negatively affected by the exercise. Each volunteer gave their written, informed consent before participation in the study, which was approved by the Research and Ethics Committee of the Faculty of Health Sciences of the University of Cape Town (South Africa).

3.2.2 Study design

Each subject performed a single trial consisting of cycling to exhaustion at 30% of individual pre-determined peak sustainable workload (W_{peak}) with one leg at a time on a stationary recumbent cycle ergometer. The starting leg was randomly assigned.

3.2.3 Preliminary testing

3.2.3.1 Anthropometry

Mass and stature were predetermined and body mass index (BMI) was calculated for subjects participating in the study. Body fat was measured from the sum of seven skinfolds (biceps, triceps, subscapularis, abdominal, thigh and calf) and calculated using the equation of Durnin & Womersley¹²⁷.

3.2.3.2 Peak sustained power output (W_{peak}) test

Subjects performed an incremental cycle test to exhaustion (with both legs) in order to determine each subject's individual working capacity (peak sustained power output (W_{peak}) test). On arrival at the cycle laboratory, a full anthropometric analysis was performed (as mentioned above),

followed by a 10 min warm-up exercise bout on an electronically-braked cycle ergometer (Lode, Groningen, The Netherlands). After the warm-up, the W_{peak} test was started at a workload equivalent to 3.3 Watts/kg body mass and increased first by 50 Watts (W) after 150 sec and then by 25 W every 150 sec until the pedaling frequency dropped below 50 revolutions/min. PPO was defined as the highest exercise intensity the subject completed for 150 sec in W, plus the fraction of time spent in the final workload multiplied by 25 W. This information was used to adjust the work rate in the experimental trial so that each subject performed single limb cycling corresponding to 30% of W_{peak} .

3.2.4 Experimental procedure

Subjects reported to the laboratory in the morning after a 10-12 hour overnight fast. Upon arrival, an 18-gauge Teflon cannula (Jelco; Johnson and Johnson, Halfway House, South Africa) was placed into the subject's right forearm vein and connected to a three-way stopcock (Uniflex; Mallinckrodt Medical, Hennef-Sieg, Germany), and a baseline blood sample was collected.

3.2.4.1 Single leg exercise protocol

Before exercise was started, the seating position on the recumbent cycle ergometer was adjusted to that chosen by the subjects as most comfortable for his cycling performance. Each subject was given 5 minutes to warm up and settle into a comfortable seating position (3 min at 20 W followed by 2 min at 40 W). After 5 min, the workload was set at 30% of each subject's individual W_{peak} and single-limb cycling to exhaustion followed. While the one leg was strapped into the pedal (around the toe and heel using inelastic adhesive tape), the other leg remained in a rested state, positioned in a comfortable position on a small bench placed next to the cycle ergometer. Care was taken that the resting limb should remain as inactive as possible. Measurement of EMG activity of the quadriceps muscle of the inactive leg revealed muscle recruitment of <5% of pre-determined maximal voluntary contraction. At exhaustion of the starting leg (Leg 1), a muscle biopsy was performed (explained below) in that leg. Immediately thereafter, the same protocol was performed on the previously rested leg (Leg 2). Exhaustion was defined as the point at which the subject could no longer maintain 30% of individual W_{peak} power and were unable to raise the power output after two verbal warnings. During exercise, subjects were cooled by an electric fan, and could consume water ad libitum. Thirty minutes into the exercise time of each limb, 300 ml of a flavored 10% glucose polymer drink (i.e. 30 g CHO) was provided in order to prevent the development of hypoglycemia.

3.2.4.2 Muscle biopsy

A muscle biopsy was obtained from the vastus lateralis muscle at the point of exhaustion of each limb, using the method of Bergstrom¹⁶ as modified by Evans et al.¹³⁶. The muscle biopsy samples were immediately placed in liquid nitrogen, then stored at -80°C for later determination of muscle glycogen concentration (as per wet weight) using conventional methods³⁵.

3.2.4.3 Blood sampling and analysis

Blood sampling was repeated at 15 min intervals during the single-leg cycling protocol. After each blood sample was taken, the cannula was kept patent by flushing it with sterile saline. Aliquots of the blood sample were separated into tubes containing potassium oxalate and sodium fluoride (Midran; Novo Nordisk, Johannesburg, South Africa) for later analysis of plasma glucose and lactate concentrations; tubes containing gel and clot activator (Beckton-Dickinson) for determination of serum free fatty acid (FFA) concentrations; and tubes containing lithium heparin (Beckton-Dickinson, South Africa) for determination of plasma catecholamine concentrations. Tubes were immediately placed on ice and, after 60 min, centrifuged at 3 500 rpm for 12 min at 4°C , and the supernatants were then stored at -80°C for later analysis. Plasma glucose concentrations were determined by the glucose oxidase method using a glucose analyzer (Glucose analyzer 2; Beckman) and lactate concentrations were determined by spectrophotometric (model 35; Beckman, Fullerton, CA) enzymatic assays (Lactate PAP; Bio Mérieux, Marcy-L'Étoile, France). FFA concentrations were measured using an enzymatic colorimetric assay (Half-micro test; Boehringer Mannheim). Catecholamine concentrations were determined by high-pressure liquid chromatography (HPLC) with electrochemical detection using the method described by Forster and Macdonald¹⁵⁰. (HPLC column specifications: Phenomenex Hypersil 3μ ODS C18 100mm X 2.0 mm, Part no. 00D-0145-BO).

3.2.4.4 VO_2 and VCO_2 measurements

At baseline (fasting) and at 15 min intervals during exercise, VO_2 and VCO_2 were determined on-line using a computerized system (Oxycon Alpha, Jaeger-Mijnhart, The Netherlands). Prior to each test, the flow meter of the Oxycon Alpha analyzer was calibrated using a Hans Rudolph 3 liter syringe, and the gas analyzer was calibrated using a two-point calibration of fresh air and a 4% CO_2 , 96% N_2 gas mixture as per manufacturer specifications. The reliability of the Oxycon Alpha analyzer was tested on a weekly basis using the combustion of absolute ethanol (99 % Analytical Report, Associated Chemical Enterprises (Pty.) Ltd., Glenvista, South Africa) and its concomitant respiratory exchange ratio (RER) as a reference.

Rates of total CHO and fat oxidation were calculated (g/min) using the formulae of Frayn¹⁵³, assuming a nonprotein respiratory exchange ratio, assuming no increase in basal amino acid and protein degradation during exercise:

$$\text{Total CHO oxidation} = 4.55 \text{ VCO}_2 - 3.21 \text{ VO}_2$$

$$\text{Total fat oxidation} = 1.67 (\text{VO}_2 - \text{VCO}_2)$$

Where VCO_2 is the volume of CO_2 in the expired air in L/min; and VO_2 is the corresponding oxygen uptake in L/min.

3.2.4.5 Perceived exertion and Heart Rate

Each subject's perception of effort (rate of perceived exertion, RPE) was measured at 5-min time intervals from the start to the end of the single-limb exercise protocol. Two scales were used to quantify the subject's level of exertion: 1) the validated Borg 15-point RPE scale, and 2) the validated Borg category-ratio 10-point scale³⁰. The subjects were asked to use these scales to give a subjective rating of 1) general or "whole-body" exertion, and 2) give a subjective rating of exertion pertaining particularly to the exercising leg. A printed scale and instructions were given to familiarize subjects, and a verbal explanation of each scale and a description of how the scales should be used were given. Heart rate was recorded at 5-second intervals throughout exercise by means of a Polar™ heart rate monitor.

3.2.5 Statistical analysis

The statistical software package STATISTICA 7.0 (2004; StatSoft, Inc., Tulsa, OK, USA) was used for the statistical analysis of the data. All results are presented as means \pm SD. Statistical significance ($p < 0.05$) of between-limb differences was assessed by a two-way analyses of variance (ANOVA) for repeated measures over time for the first 30 to 40 min (complete data sets, $n=6$), depending on the variable measured. In order to determine which means were significantly different, Tukey's HSD post-hoc analysis was used. Subjects fatigued and dropped out at different time points after 40 min. Paired t -Tests were performed for between limb comparisons of variables beyond 40 min, and for comparisons at the point of exhaustion (end of exercise).

3.3 RESULTS

As indicated in Table 3.1, the subjects had moderate $\text{VO}_{2 \text{ max}}$ and peak work rate (W_{peak}) values, consistent with being moderately trained.

Table 3.1: Subject characteristics

n=6	
Age (years)	19±1
Body mass (kg)	87±7
Body fat (%)	15±1
Peak sustained power output (W_{peak}) (Watts)	274±26
VO_{2max} (ml/kg/min)	42±3

3.3.1 Exercise duration (Table 3.2):

The cycling time to fatigue in Leg 1 was ~5 min longer compared to that of Leg 2 (60±6 vs. 55±7 min, Leg 1 vs. Leg 2, respectively, $p=0.03$). All subjects completed at least 50 min of exercise with Leg 1 and 40 min with Leg 2.

3.3.2 Muscle glycogen concentrations (Table 3.2)

Muscle glycogen concentrations at the point of exhaustion in Leg 1 were significantly lower than at the point of exhaustion in Leg 2 (43±25 vs. 51±21 mmol/kg w.w., Leg 1 vs. Leg 2, respectively, $p=0.04$).

Table 3.2: Exercise time and muscle glycogen concentrations at exhaustion

n=6	Leg 1	Leg 2	*p
Exercise time to exhaustion (min)	*60±6	55±7	0.03
Muscle glycogen concentration (mmol/kg w.w.)	*43±25	51±21	0.04

Values presented as Mean ± SD. p = level of statistical significance. *Significant difference between Leg 1 vs. Leg 2 ($p<0.05$).

3.3.3 Blood glucose concentrations (Figure 3.1A)

Plasma glucose concentrations at the start of exercise in Leg 1 were ~4 mmol/L and increased over time to 4.5 mmol/L at the point of exhaustion (NS over time). Plasma glucose concentrations at the start of exercise in Leg 2 were significantly higher (5.3±0.7 mmol/L) than that at the start of Leg 1 ($p=0.007$). This was likely due to the cessation of exercise (point of exhaustion) in Leg 1 and a decline in glucose uptake⁴⁵⁷, with a resultant increase in blood glucose concentrations between cessation of exercise in Leg 1 (~10 min) and start of exercise in Leg 2. The increased catecholamine concentrations at the start of exercise in Leg 2 may also have increased blood glucose concentrations via its effects on increasing hepatic glucose output (glycolysis and gluconeogenesis) and reducing glucose uptake^{214,222}. However, as soon as exercise was started in Leg 2, blood glucose concentrations declined to similar concentrations to

that observed after 15 min of exercise in Leg 1, likely due to contraction-induced uptake of blood glucose uptake by the working muscle⁴⁵⁰.

At the 15 min timepoint, glucose concentrations were similar between legs (~4.1 mmol/L), where after glucose concentrations displayed a similar decline to ~3.7 mmol/L at 30 min of exercise. In Leg 1, glucose concentrations then returned to baseline levels (4.0 ± 0.5 mmol/L) at 45 min of exercise, most likely due to the ingestion of 300 ml of the 10% glucose polymer drink at 30 min of exercise. However, ingestion of an equivalent amount of CHO drink at 30 min of exercise in Leg 2 did not result in an increase in glucose concentration between the 30 and 45 min period as it did in Leg 1. Hence, glucose concentrations remained at $\sim 3.6 \pm 0.3$ mmol/L between the 30 and 45 min exercise period in Leg 2. Glucose concentrations in Leg 2 tended to be lower than that of Leg 1 at the 45 min timepoint, but these differences did not reach statistical significance. After the 45 min timepoint, glucose concentrations in the remainder of subjects increased to ~4.5 mmol/L in both legs (NS). At the point of exhaustion, glucose concentrations were slightly lower in Leg 2 (4.1 ± 0.6) compared to Leg 1 (4.5 ± 0.4), but were not significantly different.

The individual data were examined to explore the potential contribution of hypoglycemia on fatigue development (exercise capacity) (Table 3.3).

Table 3.3: Individual data for plasma glucose concentration at the point of fatigue and exercise time for Leg 1 and 2, respectively.

Subjects	Glucose at point of exhaustion (mmol/L)		Exercise time (min)		Performance time differences
	Leg 1	Leg 2	Leg 1	Leg 2	% Decline
A	5.1	3.4	60	60	0
B	4.4	4.9	67	64	- 4
C	4.3	3.6	53	51	- 4
D	4.7	4.8	61	53	- 13
E	3.9	4.2	67	59	- 12
F	4.5	3.8	52	41	- 21

Note: % Decline = decline in exercise capacity with Leg 2 compared to Leg 1.

Hypoglycemia is typically defined as blood glucose concentrations below 3.5 mmol/L²⁷. When applying this definition, then one subject (subject A) was hypoglycemic at the point of exhaustion in Leg 2. However, his exercise capacity was apparently unaffected by the presence of hypoglycemia as his exercise time for Leg 2 was identical to that of Leg 1. In two of the 6 subjects (C and F), exhaustion was reached earlier than in Leg 1 and at lower plasma glucose concentrations (a 4% and 21% decline in exercise capacity with Leg 2 for subject C and F,

respectively). In contrast, in two of the subjects (B and E), exhaustion was reached earlier in Leg 2 and at higher plasma glucose concentrations (a 4% and 12% decline in exercise capacity for subject B and E, respectively). Despite the small sample size, these data show that there is no clear relationship between low blood glucose concentrations and fatigue development (or exercise capacity) in the current experimental model.

3.3.4 Serum FFA concentrations (Figure 3.1B)

Both legs started exercise with similar resting FFA concentrations. During exercise with Leg 1, FFA concentrations remained fairly constant over time with a slight, yet non-significant increase towards the point of exhaustion. FFA concentrations in Leg 2 were similar to Leg 1 over the first ~30 min of exercise, but increased significantly over time ($p=0.000$) from 30 min onwards and were significantly higher than that of Leg 1 at 45 min ($p=0.07$), 60 min ($p=0.03$, $n=4$) and at the point of exhaustion ($p=0.05$).

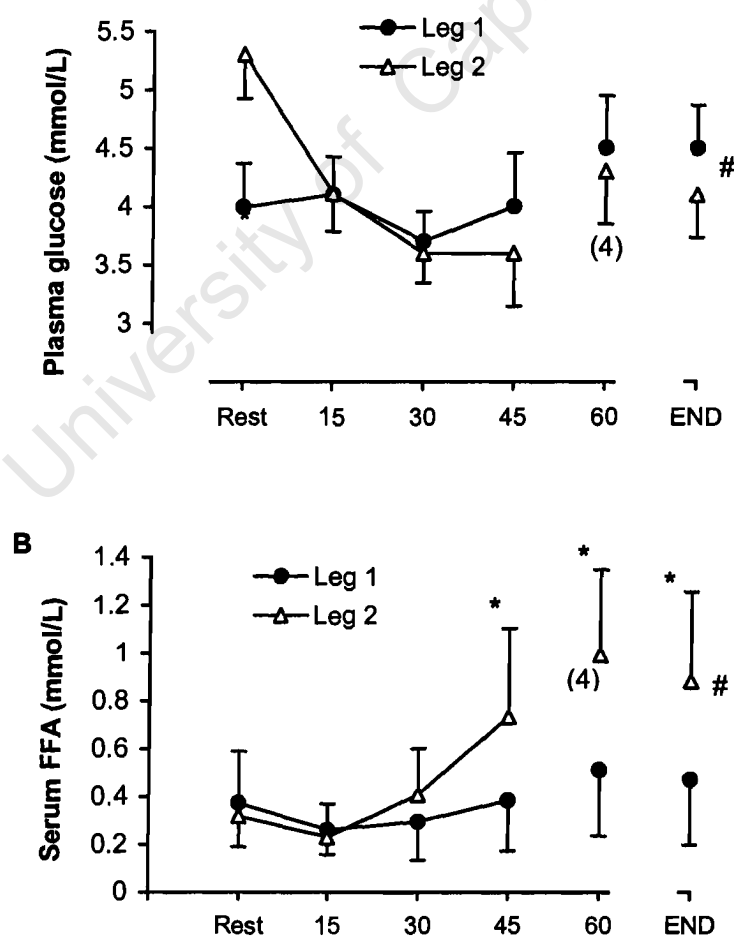


Figure 3.1 A and B: Plasma glucose (A) and Serum FFA (B) concentrations at rest and during single-leg exercise to exhaustion. *Significant difference between Leg 1 vs. Leg 2 ($p<0.05$). #Significant change over time from rest. $n=6$, otherwise as indicated in brackets.

3.3.5 Plasma lactate concentration

Apart from a significant change over time, there was no significant difference in plasma lactate concentrations between Leg 1 and 2. Plasma lactate concentrations increased from ~1.5 to 3.8 mmol/L in Leg 1 and from 2.5 to 4.0 mmol/L in Leg 2 from rest to exhaustion (NS).

3.3.6 Serum epinephrine and norepinephrine concentrations (Table 3.3)

Serum epinephrine concentrations were lower at the start of exercise (0.22 ± 0.16 vs. 0.65 ± 0.55 nmol/L, $p=0.06$) and at 30 min of exercise (0.66 ± 0.37 vs. 1.30 ± 0.43 nmol/L, $p=0.002$) in Leg 1 compared to Leg 2, respectively. Serum norepinephrine concentrations were significantly lower before the start of exercise in Leg 1 compared to Leg 2 (2.06 ± 0.43 vs. 4.05 ± 1.52 nmol/L, respectively, $p=0.02$).

Table 3.3: Serum epinephrine and norepinephrine concentrations

n=6	Leg 1			Leg 2		
	Rest	30 min	END	Rest	30 min	END
Epinephrine (nmol/L)	* 0.22 ± 0.16	* 0.66 ± 0.37	0.81 ± 0.26	* 0.65 ± 0.55	* 1.30 ± 0.43	1.00 ± 0.40
Norepinephrine (nmol/L)	* 2.06 ± 0.43	5.81 ± 0.37	7.95 ± 3.04	* 4.05 ± 1.52	5.96 ± 1.76	7.72 ± 2.43

Values presented as Mean \pm SD. *Significant difference between Leg 1 vs. Leg 2 ($p < 0.05$). END = at the point of exhaustion

3.3.7 Gas exchange data and rates of substrate oxidation

Apart from a significant increase over time in both legs, there were no significant differences between legs in VO_2 and RER (CHO and fat oxidation). In both legs, RER decreased from ~0.93 measured at 15 min of exercise to ~0.87 at the point of exhaustion. In both legs, fat oxidation increased from 0.2 g/min at 15 min of exercise to 0.4 g/min at exhaustion. CHO oxidation during exercise decreased similarly in both legs from ~1.8 g/min (15 min) to ~1.6 g/min at exhaustion (NS).

3.3.8 Ratings of perceived exertion (RPE) (Figure 3.4 A & B)

At the start of exercise (5 min timepoint), whole body RPE was significantly higher in Leg 1 (11 ± 2) compared to that of Leg 2 (8 ± 2 , $p=0.013$), but were comparable between legs thereafter (Figure 3.4 B). Whole body RPE during exercise increased significantly over time in both legs, and was rated at ~17 at the point of exhaustion (the maximum rating being 20).

Leg RPE tended to be lower in Leg 2 over the first 30 min of exercise compared to Leg 1, but did not reach significance. In both Leg 1 and Leg 2, Leg RPE increased significantly over time ($p < 0.05$) and was rated at maximum (a rating of 10) at the point of exhaustion. These data demonstrate that despite shorter exercise time in Leg 2, the rate of increase in RPE was similar between legs

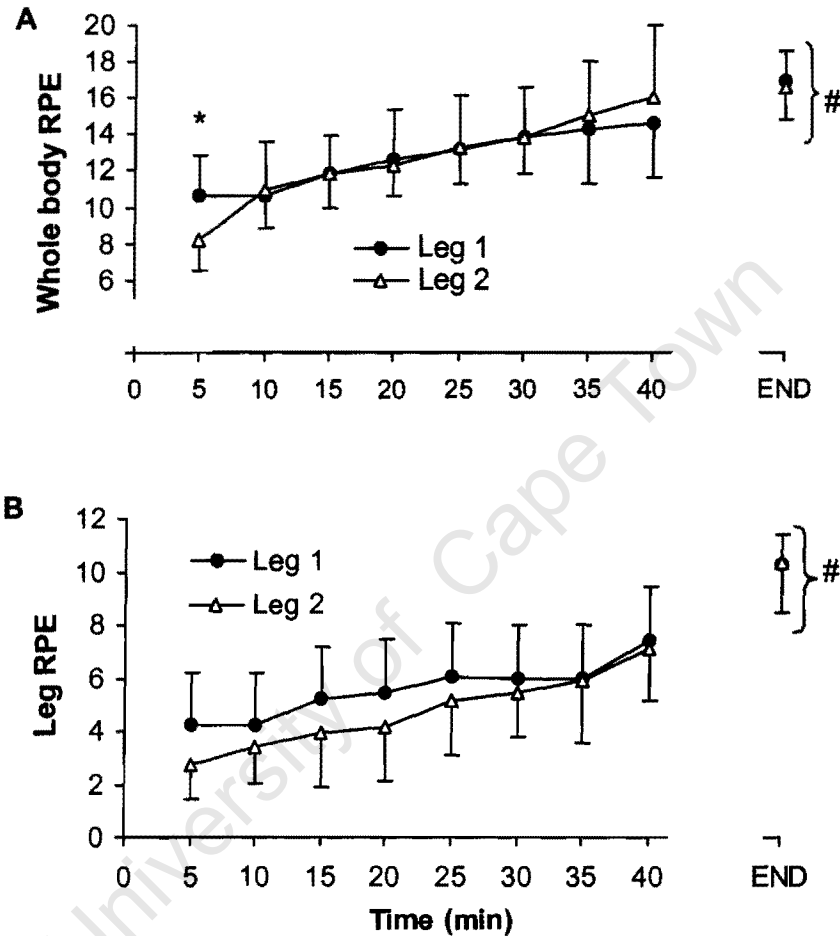


Figure 3.4 A & B: Whole body RPE (A), Leg RPE (B) during single-limb exercise to exhaustion (END). Complete data sets ($n=6$) up to the 40 min timepoint. *Significant difference between Leg 1 vs. Leg 2 ($p < 0.05$). #Significant change over time for both Leg 1 and Leg 2 ($p < 0.05$).

3.3.9 Heart rate (Figure 3.3)

The heart rate response of both Leg 1 and Leg 2 remained fairly constant and comparable (124 ± 23 vs. 121 ± 18 beats/min for Leg 1 vs. Leg 2, respectively).

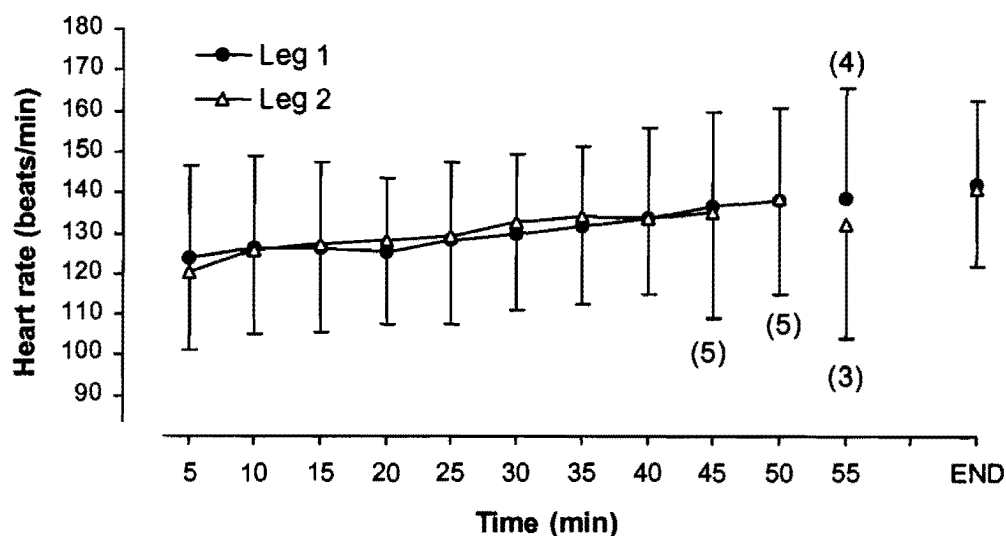


Figure 3.3: Heart rate response during single-limb exercise to exhaustion (END). Complete data sets up to 40 min of exercise ($n=6$), where after subjects fatigued at different timepoints as indicated by the subject numbers in brackets. *Significant difference between Leg 1 vs. Leg 2 ($p<0.05$).

3.4 DISCUSSION

This study aimed to investigate whether the local muscle glycogen depletion, and metabolic and hormonal response elicited during single limb, steady-state exercise to exhaustion affects the rating of perceived exertion and exercise performance during exercise to exhaustion of the other, previously rested limb. The key finding of this investigation was that exercise performance of the second leg was significantly shorter, whilst exercise in the second limb was terminated with a significantly higher muscle glycogen content than that at exhaustion during the first limb. This suggests that local muscle glycogen played a minor role in fatigue development of the second limb. The performance difference may be related, in part, to increased sympathetic nervous system activation, and possible differences in muscle recruitment (reported elsewhere).

In the present study subjects fatigued at plasma glucose concentrations ranging from 3.4 to 5.1 mmol/L and with no clear pattern between plasma glucose concentration and exercise time to exhaustion. Though the sample number is small, the results indicated that fatigue development in this exercise setting could not simply be ascribed to low plasma glucose concentrations or the development of frank hypoglycemia alone.

Fatigue in Leg 1 was reached at a muscle glycogen concentrations of ~ 40 mmol/kg w.w. Fatigue in Leg 2 was reached at a significantly higher muscle glycogen concentration of ~ 50 mmol/kg w.w., and after a significantly shorter exercise time. Again, there was no clear pattern between muscle glycogen content at the point of fatigue and exercise capacity of individual

subjects. In the literature it has commonly been noted that fatigue coincided with muscle glycogen concentration of ≤ 40 mmol/L^{35,37,73,89}. Hence, whilst local muscle glycogen depletion to a level of ~ 40 mmol/kg w.w. could be linked to fatigue development in Leg 1, local muscle glycogen depletion *per se* (in Leg 2) cannot explain the earlier fatigue development observed in Leg 2. Recent evidence from Rauch et al.³⁵⁰ suggested that muscle glycogen content may act as a feed-forward metabolic signal that informs the individual at what point they should slow down or stop exercise to prevent the development of absolute substrate depletion. It may be that the local muscle glycogen depletion in Leg 1 contributed to fatigue development in Leg 2 by augmenting central command, perhaps via stimulation of Group III and IV afferents (shown to be sensitized by metabolic byproducts during muscle contraction)^{198,363,383}.

In the present study, catecholamine concentrations were significantly higher prior to the start of exercise in the second compared to the first leg. Though this might be due, in part, to the stress response following the muscle biopsy procedure, the epinephrine concentrations remained significantly higher after 30 min of exercise in Leg 2. The 30 min of exercise that had lapsed would most likely have overridden the stress response caused by the muscle biopsy procedure⁶⁸. It is well known that sympathoadrenal activation and increased epinephrine concentrations are increased with exercise intensity and duration⁶⁸.

It has previously been shown that increased sympathoadrenal activation results in increased intramuscular glycogen utilization⁶⁸, most likely due to enhanced glycogen phosphorylase activity as a result of β -adrenergic stimulation³⁵⁶. Augmented effects of raised epinephrine concentrations on CHO oxidation and lactate production have also been observed^{142,437}. In the present study, the raised epinephrine concentrations had no effect on altering CHO oxidation and circulating lactate concentrations. It is, however, difficult to evaluate the effect of increased sympathoadrenal activation on muscle glycogen utilization in the second limb compared to the first, as we only measured muscle glycogen at the point of exhaustion, and subjects cycled for ~ 5 min shorter with the second leg. It seems unlikely that the raised epinephrine concentrations enhanced glycogenolysis in the present study as glycogen concentrations were significantly (~ 10 mmol/kg w.w.) higher at exhaustion in Leg 2 compared to Leg 1, and total CHO oxidation and lactate concentrations similar between legs throughout exercise. This is in accordance with three studies where epinephrine infusion (at physiological levels) during exercise did not enhance muscle glycogenolysis^{63,436,442}, nor RER⁴³⁶. However, it should be noted that these studies employed a different mode of exercise (2-legged cycling), higher exercise intensities (85% and 70% of $\text{VO}_{2\text{max}}$) and different exercise duration (90 min and 40 min)^{63,436,442}, and in two of the studies subjects started exercise in a non-fasted state^{63,442}. The reason for the disparate results in the literature is not yet clear. Nevertheless, from these observations it seems unlikely that the earlier fatigue development of the second leg was related to a substrate-depletion mechanism.

Specifically, fatigue development in the second leg was unrelated to local muscle glycogen depletion, and unrelated to alterations in CHO oxidation or lactate metabolism.

In the present study, it may be that the increased sympathetic activation that occurred during exercise (as judged by the increased nor-epinephrine concentrations), combined with local muscle glycogen depletion and the sensation of fatigue with Leg 1 (but circulated and "experienced" by the whole body), elicited an alteration in central command and motor unit activity to maintain the same (set) force production with the second limb. This may also explain the observation that RPE followed the same rate of increase in Leg 2, and ended at the same maximal levels as Leg 1, despite a significantly shorter exercise time. The measurements of muscle recruitment patterns during exercise (to be reported in detail elsewhere) may aid in verifying the likeliness of the suggested alteration in central command and muscle recruitment mentioned above, and may ultimately provide a further explanation for the reduced exercise capacity with the second limb.

FFA concentrations were maintained between 0.3 – 0.5 mmol/L in Leg 1. This is comparable to values found during single-limb cycling at 40% of 2-legged $\text{VO}_2 \text{ max}$ ⁵. However, FFA concentrations increased significantly over time from 0.3 – 0.9 mmol/L in Leg 2, and were significantly higher than during exercise with Leg 1 from 45 min to the point of exhaustion. This was unexpected since 30 g of CHO was ingested at 30 min of exercise in both legs, which is known to suppress FFA release⁴³⁸. Hence, equivalent amount and timing of CHO ingestion in Leg 2 did not seem to have any effect on FFA release during exercise in Leg 2. This may suggest that CHO ingestion during exercise in Leg 2 was insufficient to over-ride the overall effect of exercise duration and the metabolic and hormonal responses elicited on FFA release. Epinephrine has been shown to be a potent stimulus for lipolysis and could therefore have accounted for the increased FFA concentrations seen during exercise with Leg 2²⁹⁰. However, despite significantly higher circulating FFA concentrations during exercise with Leg 2, fat oxidation remained comparable to that of Leg 1. A possible explanation for this may be that due to the low exercise intensity and low energy demand, and due to CHO ingestion and maintenance of euglycemia, the need to oxidize lipid for fuel remained low and similar between trials.

In order to make a distinction between the subject's sensation of whole-body and muscle-specific (local) fatigue, we employed a 20-point validated Borg RPE scale to assess whole-body RPE and the 10-point validated category-ratio Borg scale to assess leg (local) RPE. Surprisingly, whole body RPE was significantly lower at 5 min of exercise in Leg 2 compared to that of Leg 1. One possible explanation for this might be related to the significantly higher blood glucose concentrations immediately prior to the start of exercise in Leg 2 compared to Leg 1. An inverse

relationship between glucose availability and RPE has been demonstrated before²⁴³, however, mechanisms for this effect still remains to be fully elucidated. It has been shown that a number of sensory cues (both cardiopulmonary and peripheral) may control effort perception. Kang et al.²⁴³ demonstrated that ingesting CHO vs. placebo during prolonged exercise significantly lowered overall ratings of perceived exertion, however ratings were not different between trials at the end of the exercise session²⁴³. This study suggests that in endurance exercise, CHO availability may be a sensory cue, but there are other physiological processes contributing to effort perception that may contribute to perceptual cues towards the end of endurance exercise. Despite the significant differences in blood glucose concentrations and whole body RPE that existed at the onset of exercise in the present study, these differences were minimized within the first 15 min of exercise and remained similar thereafter.

Leg RPE tended to be lower in Leg 2 compared to Leg 1 but did not reach statistical significance. Interestingly, there was a linear relationship between RPE and exercise duration, and both legs exhausted at maximal exertion levels (rating of 10), whilst whole body RPE was rated at high, yet submaximal levels (~17, maximal being 20). This suggests localized fatigue and therefore the local leg RPE seems to be the dominant effort sensation for this dynamic type of exercise. It appears that perceived exertion does not reflect whole body metabolism but is rather related to local metabolic or mechanical changes. It has been shown that sensations of strain in the exercising limbs play a role in fatigue, suggesting proprioceptor (such as mechanoreceptor) feedback within the exercising muscle that relates a message of strain and ultimately fatigue³⁰². Furthermore, studies have reported RPE to be higher during cycling at lower cadences despite similar values for ventilation, VO_2 and heart rate^{54,332}. Subjects in the present study were unaccustomed to single-leg cycling and found it difficult to maintain a smooth rhythmic cadence at the point of exhaustion. Though speculative, a contributing factor to the development of fatigue in this study may have been mechanical strain, which was sensed independent of any metabolic changes.

In conclusion, from these observations it seems unlikely that the earlier fatigue development of the second leg was related to a substrate-depletion mechanism *per se*. Specifically, fatigue development in the second limb was unrelated to local muscle glycogen depletion, nor to alterations in CHO oxidation or lactate metabolism. The overall RPE in the previously rested limb (Leg 2) followed the same pattern of increase and ended at the same near-maximal RPE rating despite a significantly shorter exercise time. This study demonstrates that antecedent single-limb exercise to fatigue elicits an altered physiological milieu (altered catecholamine concentrations at the onset of exercise and significant increase in sympathetic activation), which may be responsible for the reduced exercise capacity of the second limb.

4.1 INTRODUCTION

In the previous study we demonstrated that fatigue development during sequential single limb exercise was unrelated to blood glucose concentrations, in fact, blood glucose concentrations at the point of exhaustion between limbs were highly variable between subjects. Furthermore, the reduced exercise capacity of the second limb was unrelated to local muscle glycogen depletion, nor to alterations in CHO oxidation or lactate metabolism. In this study, we aimed to further investigate the impact of whole-body glycogen depletion (achieved by following a low CHO diet) on inter-individual exercise metabolism and endurance capacity when blood glucose concentrations are maintained (~5 mmol/L) with glucose infusion, or allowed to decline with placebo (saline) infusion in endurance-trained men.

Research in the field of metabolism and performance during prolonged exercise has highlighted a strong relationship between the status of bodily carbohydrate (CHO) stores (blood glucose, liver and muscle glycogen) and endurance exercise capacity^{35,73-75,89,440}. Muscle glycogen contributes approximately 85% of the total CHO oxidation early in exercise^{35,89}, but as exercise duration increases and muscle glycogen is depleted, there is a gradual shift toward blood glucose as the predominant carbohydrate energy source^{35,73,89,440}. However, this effect may be limited by declining plasma glucose concentrations late in exercise^{73,89}.

Whereas CHO-loading diets are associated with improved endurance capacity or performance, ascribed mainly to increased endogenous CHO availability and maintenance of a high rate of CHO oxidation throughout exercise, low CHO diets have been shown to impair endurance exercise capacity, which has been ascribed to the reduction of endogenous CHO availability and decreased rate of CHO oxidation^{73,74,89,162,190,223,234,440}. However, the relationship between muscle glycogen availability and blood glucose uptake remain unclear.

Muscle glycogen concentration at the onset of exercise is an important determinant of muscle glycogenolysis during exercise. Reduced muscle glycogen availability results in a lower rate of glycogenolysis during submaximal exercise¹⁹⁰, and similarly, increased glycogen stores are associated with higher rates of glycogenolysis^{35,190,375}. Additionally, Hargreaves et al.¹⁹¹ observed a significant inverse relationship between muscle glycogen concentration and glucose uptake during 40 min of cycling exercise (65-70% $VO_{2\text{ max}}$), and suggested a possible regulatory influence of muscle glycogen on glucose uptake during the early stages of exercise. However, subsequent studies found that tracer-determined rates of glucose uptake were unaffected by pre-exercise muscle glycogen availability during a similar¹⁹⁰ and more prolonged exercise protocol (180 min, 70% $VO_{2\text{ max}}$)³⁵. The above studies were, however, performed in the absence of exogenous CHO supplementation during the exercise bout.

A number of studies have found that exogenous CHO supplementation, especially when muscle glycogen concentrations are low, can maintain blood glucose and total CHO oxidation and delay the onset of fatigue and thereby, in part, negate or 'over-ride' the potential ergolytic effects of low endogenous glycogen stores^{36,60,73,74,88}. Furthermore, CHO supplementation during exercise appear to minimize the performance differences in studies in which subjects ingested different pre-exercise diets and therefore began exercise with different muscle and liver glycogen concentrations^{53,445}. In studies employing steady-state exercise regimes, this effect is more likely the result of changes in blood glucose concentration than in the rate of muscle glycogen oxidation, since evidence suggests that CHO supplementation during steady-state exercise (~70% $VO_{2\text{ max}}$) has minimal effect on the rate of muscle glycogen oxidation³⁵. Evidence of muscle glycogen sparing with exogenous CHO supplementation has typically only been observed in studies where a variable intensity or self-paced exercise protocol were employed⁴⁴⁵.

Yet, results from Coyle et al.⁹¹ and Bjorkman et al.¹⁹ have indicated that CHO supplementation improved endurance exercise capacity only in those subjects who became hypoglycemic during the placebo treatment. In other studies, however, CHO supplementation during exercise improved endurance capacity, lowered ratings of perceived exertion and improved performance in individuals who did not demonstrate significant decreases in blood glucose concentrations and CHO oxidation rates during the placebo trial^{60,293,313}. Perhaps the most intriguing findings are from those studies that found that the development of hypoglycemia^{87,146,228,230,312} or reversal of hypoglycemia¹⁶² (plasma glucose <3.5 mmol/L) during exercise had little effect on exercise capacity. Similarly, a series of studies investigating the effect of type, amount and timing of pre-exercise carbohydrate intake have indicated that some subjects are prone to the development of rebound hypoglycemia during subsequent exercise (for reasons yet unclear), yet the development of hypoglycemia had no effect on their exercise performance^{228,230,312}.

Thus, the specific effects of alterations in blood glucose concentrations on endurance exercise capacity remain unclear, especially under conditions where endogenous glycogen stores are low. Furthermore, the effect of low starting muscle glycogen concentration on glucose uptake and substrate metabolism also remains unclear. Clarity on these aspects may assist with development of specific dietary advice and strategies to enhance athletic performance, especially in strenuous training or competition situations where recovery time is limited and endogenous CHO stores may be compromised.

Accordingly, the aim of this study was to examine the effect of glucose infusion to maintain euglycemia, vs. saline (placebo) infusion on endurance exercise capacity in persons whose glycogen stores had been lowered by prior exercise and a low CHO diet. We chose glucose infusion as the mode of CHO administration (as opposed to ingestion) partly to negate inter-

individual differences in the rate of intestinal glucose absorption, as well as being able to ensure constant euglycemia. We expected that the saline infusion would result in a decline in blood glucose concentration during exercise and offer an opportunity to examine the effect of declining blood glucose concentrations on metabolism and exercise capacity as compared to euglycemic conditions. Differences in exercise metabolism and endurance capacity provide a measure of the impact of altered blood glucose concentrations have on liver and muscle glycogen metabolism and to the development of fatigue in persons who begin exercise with low muscle and liver glycogen concentrations. Furthermore, if the metabolic effects of lowered glycogen stores persist despite the maintenance of euglycemia, then it would indicate that the responses are related specifically to reduced intramuscular or intrahepatic CHO availability. It was hypothesized that the declining blood glucose concentrations would reduce CHO oxidation rates and limit exercise capacity, whereas euglycemia would maintain CHO oxidation and enable trained subjects to complete 2.5 h of moderate-intensity exercise despite reduced muscle glycogen availability.

4.2 METHODS

4.2.1 Subjects

Nine endurance-trained male cyclists volunteered to participate in the study, which was approved by the Research and Ethics Committee of the Faculty of Health Sciences of the University of Cape Town (South Africa). Cyclists were selected who regularly trained ≥ 200 km/wk and who had completed a 105-km cycle race in < 3.5 h. Subject characteristics are given in Table 1. The experimental procedures and potential risks of the study were explained to the subjects, and their informed, written consent was obtained. Each subject repeated the experimental procedure in random, single blind fashion on two separate occasions, at least one week apart: One trial with glucose infusion during the experimental ride in order to maintain euglycemia, and a second with saline (placebo) infusion.

4.2.2 Preliminary testing and manipulation of muscle glycogen content

Two days prior to the experiment proper, subjects reported to the laboratory in the morning after ingestion of their habitual breakfast. On arrival the subject was weighed, where after they began a 10 min warm-up exercise bout on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands). This was followed by an incremental test to exhaustion in order to determine each subject's peak sustained power output (W_{peak}). The W_{peak} test was performed on the electronically braked cycle ergometer at a workload equivalent to 3.3 W/kg body mass and increased first by 50 W after 150 sec and then by 25 W every 150 sec until the pedaling

frequency dropped below 50 revolutions/min. This information was used to adjust the work rate in the subsequent phases of the trial so that each subject exercised at an intensity corresponding to 70% of peak rate of oxygen consumption ($\text{VO}_{2\text{max}}$), which this corresponds to ~63% of W_{peak} .

After the W_{peak} test, subjects rested for 20 min and then cycled for a further 90 min at 63% of W_{peak} . Every 20 min, a 5 min interval was performed at 83% of W_{peak} (90% of $\text{VO}_{2\text{max}}$) to reduce the muscle glycogen content of the exercising muscles. After this procedure, subjects were instructed to follow a low carbohydrate diet for the next 48 h. They were given a list of food choices that would provide an energy intake of ~6,800 kJ (16% CHO). They were also instructed to perform only 1 h of light training (e.g. low-intensity cycling) on the second day. The low CHO diet and light training were designed to limit muscle and liver glycogen resynthesis, while allowing recovery from the fatiguing effects of the depletion ride.

4.2.3 Experimental trial

After the 48 h period on the low CHO diet, subjects were instructed to eat a small breakfast (~1,200 kJ, 30 g CHO) 3 h before arrival at the laboratory. On arrival at the laboratory, an 18-gauge Teflon cannula (Jelco; Johnson and Johnson, Halfway House, South Africa) was placed into the subject's right forearm vein and connected to a three-way stopcock (Uniflex; Mallinckrodt Medical, Hennef-Sieg, Germany) and a resting blood sample was drawn (see details of blood sampling later).

4.2.3.1 Leg muscle glycogen disappearance.

After the resting blood sample was collected, a muscle sample was obtained from the vastus lateralis muscle before the start of, and immediately on completion of exercise using the method of Bergstrom¹⁶ as modified by Evans et al.¹³⁶. The samples were immediately frozen in liquid nitrogen and stored at -80 °C for later determination of muscle glycogen concentration using conventional methods³⁵.

4.2.3.2 Exercise protocol

Ten min before the start of exercise, subjects ingested a 300 ml bolus of water containing [^{14}C]glucose tracer (Amersham International, Buckinghamshire, UK) (providing 30 μCi per liter of drink). Subjects cycled on an electronically-braked cycle ergometer for up to a maximum of 150 min at 70% of $\text{VO}_{2\text{max}}$. During the ride subjects ingested 500 ml/h in divided doses of the radio-

labeled drink for determination of the rate of plasma glucose oxidation. Subjects were cooled with an electric fan and environmental conditions in the laboratory were controlled (20 °C).

4.2.3.3 *Blood sampling and analysis*

A baseline blood sample was collected, and then repeated at 20 min intervals during exercise (~12 ml of blood was drawn at each time-point). After each blood sample was taken, the cannula was kept patent by flushing with 1 ml sterile saline. Aliquots of the blood sample were placed into tubes containing potassium oxalate and sodium fluoride (Midran; Novo Nordisk, Johannesburg, South Africa) for subsequent analysis of plasma glucose, [U-¹⁴C]glucose specific activity and lactate concentrations. Another aliquot of blood was placed into a tube containing gel and clot activator (Beckton-Dickinson, South Africa) for determination of serum free fatty acids (FFA), β -hydroxybutyrate, cortisol and insulin concentrations. The tubes were immediately placed on ice and, after 20 min, centrifuged at 3 500 rpm for 12 min at 4 °C, and the supernatants were then stored at -20 °C for later analysis. Plasma glucose concentrations were determined by the glucose oxidase method using a glucose analyzer (Glucose analyzer 2; Beckman, Fullerton, CA). Lactate concentrations were measured by spectrophotometric (model 35; Beckman, Fullerton, CA) enzymatic assays (Lactate PAP; Bio Mérieux, Marcy-L'Étoile, France). Serum insulin concentrations were determined using a radio-immunoassay technique (Coat-A-Count Insulin, Diagnostic products, Los Angeles, CA). FFA concentrations were measured using an enzymatic colorimetric assay (Half-micro test; Boehringer Mannheim). Circulating β -hydroxybutyrate concentrations were analyzed at time points 0 (rest), at 80 min and at the end of exercise (100% of time), and were determined in neutralized perchloric acid extracts of serum using enzymatic spectrophotometric assays. Serum cortisol concentrations were measured using a radio-immunoassay ²⁸⁹.

4.2.3.4 *Euglycemic glucose clamp procedure and placebo (saline) infusion*

A second 18-gauge Teflon cannula was placed in the opposite (left) forearm vein for infusion of glucose (CI) or saline (PI) during the 150 min of cycle ergometer exercise at 70% of $\text{VO}_2 \text{ max}$. Blood samples were obtained at 5 min intervals during exercise in both trials to measure blood glucose concentrations using a pocket glucometer (Accutrend; Boehringer Mannheim, Mannheim, Germany). The accuracy of the glucometer was verified by comparison with a Beckman glucose analyzer (Glucose analyzer 2; Beckman Instruments, Fullerton, CA). Plasma glucose concentration during exercise in CI was maintained at euglycemic level (between ~5.0-6.0 mmol/L) by adjusting the rate of glucose infusion (20% mass/vol glucose solution) ³⁵ by using calibrated automatic syringe pumps (Travenol Laboratories, Hooksett, NJ). The protocol replicated that utilized by Weltan et al. ⁴⁴⁰ where the rate of glucose infusion needed to maintain

euglycemia in the CHO-depleted state ranged from 0.25 to 0.75 g/min towards the end of the exercise bout.

4.2.3.5 VO_2 , VCO_2 , and $^{14}CO_2$ measurements during exercise

At 20 min intervals during exercise, VO_2 and VCO_2 were determined on-line using a computerized system (Oxycon Alpha, Jaeger-Mijnhart, The Netherlands). Prior to each test, the flow meter of the Oxycon Alpha analyzer was calibrated using a Hans Rudolph 3 liter syringe, and the gas analyzer was calibrated using a two-point calibration of fresh air and a 4% CO_2 , 96% N_2 gas mixture as per manufacturer specifications. The reliability of the Oxycon Alpha analyzer was tested on a weekly basis using the combustion of absolute ethanol (99 % Analytical Report, Associated Chemical Enterprises (Pty. Ltd., Glenvista, South Africa) and its concomitant respiratory exchange ratio (RER) as a reference.

Expired air was trapped for the later determination of $^{14}CO_2$ specific activity. The $^{14}CO_2$ trapping mixture consisted of 1 ml 1N hyamine hydroxide in methanol (United Technologies, Packard, Meriden, CT), 1 ml 96% ethanol (SAARCHM, Krugersdorp, South Africa), and 2 drops of 1% phenolphthalein indicator (Saarchem, Krugersdorp, South Africa). Expired air was bubbled through the trapping mixture until the turnpoint of the indicator (solution became clear), at which point 1 mmol of CO_2 has been absorbed by the trap mixture. Liquid scintillation cocktail (10 ml Ready Gel, Beckman, Fullerton, CA) was then added, and $^{14}CO_2$ radioactivity counted in a liquid scintillation counter (Packard Tri-Carb 4640, Downer's Grove, IL). All ^{14}C counts were automatically corrected for quenching and background radioactivity.

4.2.3.6 Rates of plasma glucose oxidation

Rates of plasma glucose oxidation were measured using HPLC-purified [U- ^{14}C]glucose tracer (Amersham International, Buckinghamshire, UK). Three hundred μL of tracer (containing a negligible amount of glucose) was added to 2 L of water, giving a specific activity of 2.2 MBq/300 μL of tracer (60 μCi /300 μL). This amounted to 30 μCi per liter of drink. Five min before the start of exercise, subjects drank 300 ml bolus of the drink, then 500 ml/h in divided doses during the 150 min of exercise. A 0.5 ml aliquot of each of the plasma samples collected at 20 min intervals for glucose determination (described above), were used for the determination of plasma [U- ^{14}C]glucose specific activity and rate of plasma glucose oxidation⁴⁴⁰. The 0.5 ml aliquot of each of the plasma samples used for glucose determination was deproteinized by addition of 35 μl of $HClO_4$ (3.5 M), which also served to drive off [^{14}C]bicarbonate as $^{14}CO_2$. The samples were then centrifuged at 4 °C, and the protein-free supernatant was removed and kept cold. The precipitate was then twice resuspended in 0.35 ml of 0.13 M $HClO_4$ and recentrifuged,

and the supernatant was added to that previously saved. The pH of the combined supernatant was then adjusted to between pH 7.0 and 8.0 with ~75 μl of 3 M K_2CO_3 in 0.01 M Tris buffer (pH 8), resentrifuged and passed through 500 mg exchange columns (SAX; Bakerbond, Cape Town, South Africa) that were preconditioned with 20 ml ethanol followed by 20 ml distilled water, adjusted to pH 8 with trace amounts of NaOH. Glucose was fully eluted into a scintillation vial with 3 ml of distilled water (pH 8). Lactate was subsequently eluted into a second scintillation vial with 2 ml CaCl_2 (1 mol/L) adjusted to pH 2 with HCl. Eluates were evaporated to near dryness (~0.3 ml) at 60 °C over ~20 h, before liquid scintillation cocktail (Ready Gel; Beckman) was added for ^{14}C radioactivity determination (disintegrations per minute (dpm)) using a liquid scintillation counter (Packard Tri-Carb 4640). During each round of the analysis procedure, a non-labeled plasma sample was spiked with a known quantity of [^{14}C]glucose and analyzed simultaneously to correct the measured dpm values for the percent recovery. Such recoveries exceeded 80% for all samples. Since there were negligible counts in the lactate fraction, no corrections were made for the contribution to V^{14}CO_2 from ^{14}C -lactate oxidation. The rate of glucose oxidation (R_{ox}) was calculated from the equation:

$$R_{\text{ox}} = [({}^{14}\text{CO}_2 \times 6) / \text{SA}_{\text{Glu}}] \times \text{VCO}_2 \times 1.35$$

Where ${}^{14}\text{CO}_2 \times 6$ is the expired CO_2 specific activity (dpm/mmol) multiplied by 6 (6 carbon atoms per molecule of glucose), SA_{Glu} is the plasma [^{14}C] glucose specific activity (dpm/mmol); VCO_2 is the volume of expired CO_2 (L/min); and 1.35 is the number of grams of glucose oxidized to produce 1 liter of CO_2 .

4.2.3.7 Rates of total CHO and fat oxidation

Rates of total CHO and fat oxidation were calculated (g/min) using the formulae of Frayn¹⁵³, assuming a nonprotein respiratory exchange ratio, assuming no increase in basal amino acid and protein degradation during exercise:

$$\text{Total CHO oxidation} = 4.55 \text{ VCO}_2 - 3.21 \text{ VO}_2$$

$$\text{Total fat oxidation} = 1.67 (\text{VO}_2 - \text{VCO}_2)$$

Where VCO_2 is the volume of CO_2 in the expired air in L/min; and VO_2 is the corresponding oxygen uptake in L/min.

4.2.4 Statistical Analyses

The statistical software package STATISTICA 7.0 (2004; StatSoft, Inc., Tulsa, OK, USA) was used for the statistical analysis of the data. All results are presented as means \pm SD. Statistical

significance ($p < 0.05$) of between-group differences was assessed by a two-way analyses of variance (ANOVA) for repeated measures over time for the first 80 min (complete data sets, $n=9$). In order to determine which means were significantly different, Tukey's HSD post-hoc analysis was used. Subjects fatigued and dropped out at different time points after 80 min. Statistical analyses were not performed beyond the 80 min timepoint due to declining subject numbers. Paired t -Tests were performed for comparisons between groups of variables at the point of exhaustion (end of exercise or 100% of time). Pearson's correlations were performed between continuous variables such as plasma glucose and serum cortisol concentrations. However, for correlations with endurance time, which was censored data, the Kendall τ technique was used, which calculates the differences between the probability that the observed data are in the same order for the two variables versus the probability that the observed data are in different orders for the two variables.

4.3 RESULTS

As indicated in Table 4.1, the subjects had moderately high $\text{VO}_{2\text{max}}$ and peak work rate values, consistent with being well-trained, but not elite cyclists.

4.3.1 Exercise duration (Table 4.1)

The time to fatigue in the CI trial was significantly longer than in the PI trial (137 ± 14 vs. 112 ± 29 min, $p < 0.05$). All subjects completed at least 80 min of exercise in PI and 120 min in the CI trial, where after subjects stopped exercising at different timepoints. The overall percentage improvement in endurance time with the CI trial was $28 \pm 26\%$, showing a large range between subjects. Only 2 of 9 subjects in the PI, and 4 of 9 in the CI trial completed 150 min of steady-state exercise. Exhaustion was determined as the inability to maintain the required power output whilst pedaling at a rate of >40 rpm after two verbal warnings; or if the subjects stopped cycling outright.

Table 4.1: Subject characteristics

n=9	
Age (years)	29 ± 8
Body mass (kg)	72 ± 7
Peak sustained power output (watts)	356 ± 34
$\text{VO}_{2\text{max}}$ (ml/kg/min)	63 ± 7
Endurance time (min):	
CI trial	$*137 \pm 14$
PI trial	$*112 \pm 29$
% Improvement	28 ± 26

Values presented as Mean \pm SD. *Significant difference between trials ($p < 0.05$).

4.3.2 Muscle glycogen concentrations (Table 4.2)

Muscle glycogen concentrations were equally low at the start (74 ± 21 vs. 83 ± 15 mmol/kg w.w.) and at the end of exercise (39 ± 12 vs. 44 ± 15 mmol/kg w.w.) for the CI and PI trials, respectively. The total amount of muscle glycogen used was similar in both trials (40 ± 15 vs. 41 ± 12 mmol/kg w.w.).

Table 4.2: Muscle glycogen concentrations

Muscle glycogen concentration (mmol/kg w.w.)	PI	CI
Pre-exercise	83 ± 15	74 ± 21
Post-exercise	44 ± 15	39 ± 12

4.3.3 Blood glucose concentrations (Table 4.1A)

Blood glucose concentrations at the start of the trials were similar in both groups. Blood glucose concentrations were maintained between 5-6 mmol/L throughout the CI trial (due to glucose infusion), but declined in the PI trial to 4.2 ± 0.7 mmol/L over the first 80 min, with a significantly decline over time to the point of exhaustion (3.8 ± 0.7 mmol/L; $p<0.05$). Blood glucose concentrations were significantly higher in the CI trial between 20 to 80 min and at exhaustion than in the PI trial ($p<0.05$).

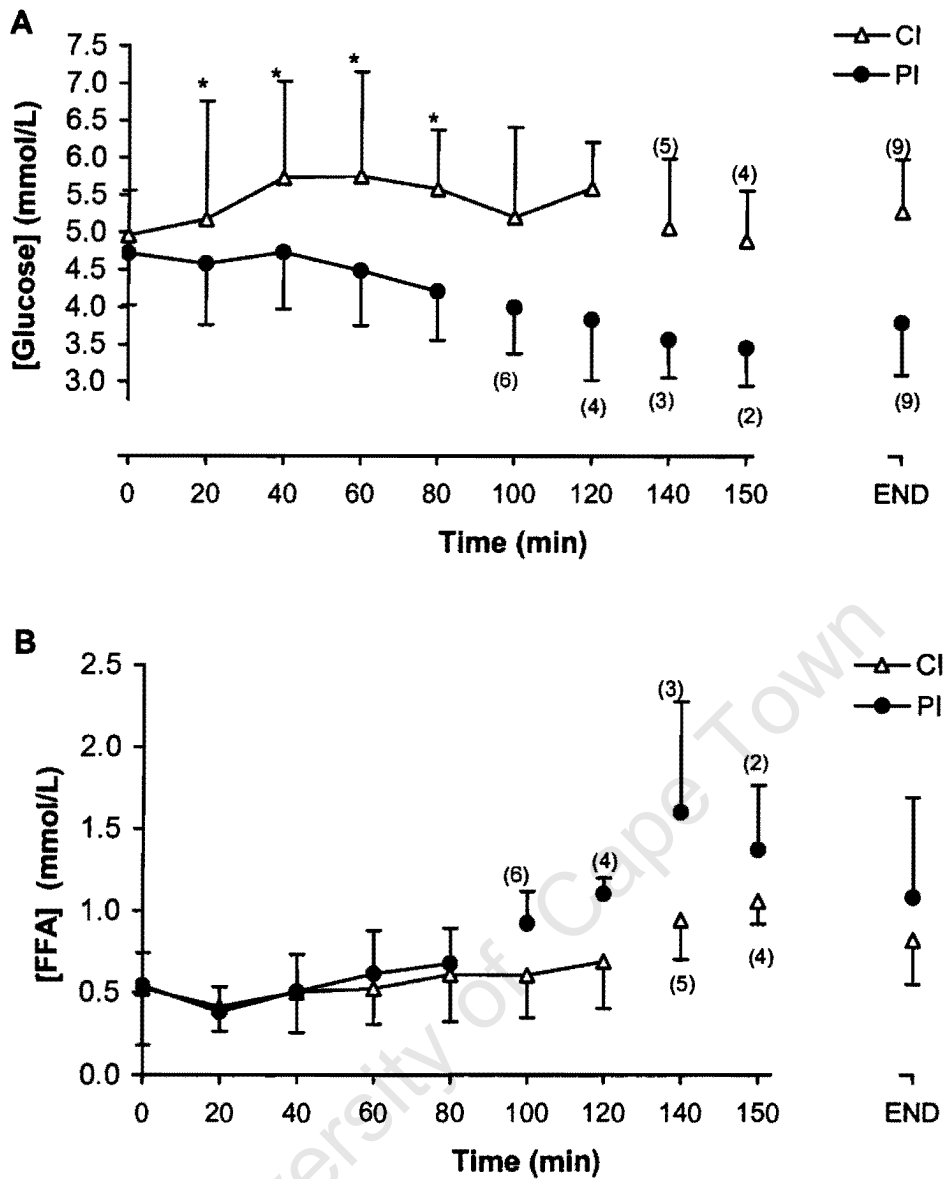


Figure 4.1 A and B: Plasma glucose (A) and serum free fatty acid (FFA) (B) concentrations during steady-state exercise. $n=9$ up to end of solid line, subject numbers indicated in brackets thereafter. *Significant difference between treatments ($p < 0.05$). END = value at exhaustion (100% of time).

There was a significant inverse relationship between exercise duration and plasma glucose concentrations at exhaustion in PI (Figure 4.2) ($\rho = -0.63$; $p = 0.02$). Hence, subjects who exercised the longest, tended to terminate exercise with the lowest plasma glucose concentrations. This was true even after covarying for performance time.

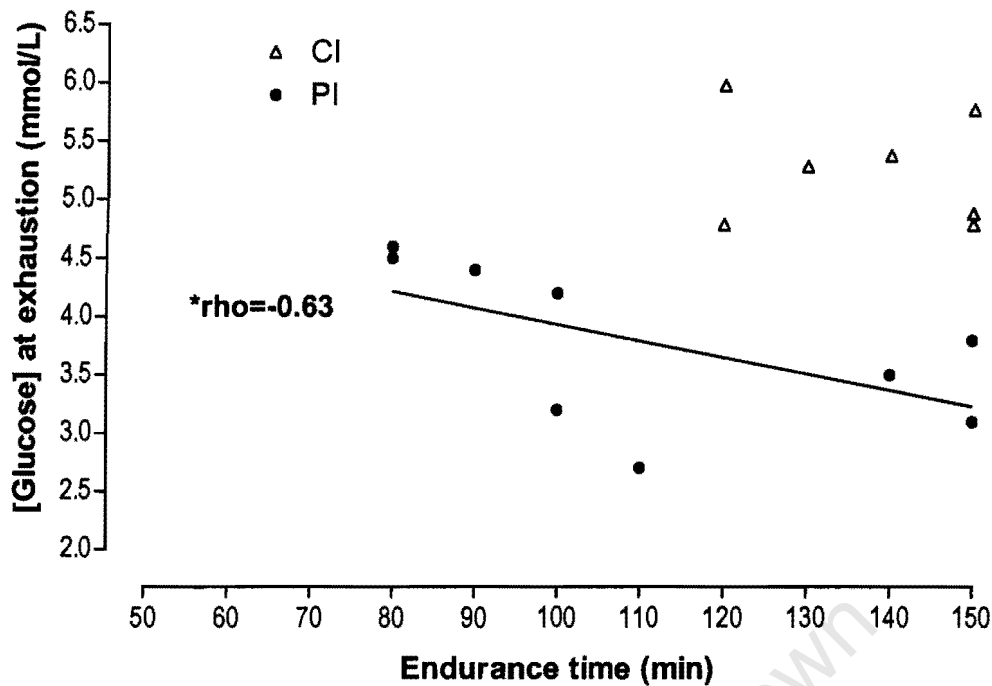


Figure 4.2: Relationship between plasma glucose concentrations at exhaustion and endurance time. $n=9$. (Values of individual subjects). *Significant inverse relationship between endurance time and plasma glucose concentration at exhaustion in the PI trial ($p<0.02$).

4.3.4 Serum insulin and cortisol, and plasma lactate concentrations

Apart from a significant change over time, there were no significant differences between trials in the concentrations of serum cortisol (Table 4.3) or plasma lactate (Figure 4.3). Plasma lactate concentrations increased from ~1 to 3 mmol/L during exercise in both trials.

Resting serum cortisol concentrations were above normal values (~500 nmol/L) in both trials, most likely due to the established effects of a low CHO diet⁴⁴⁰. Over the first 80 min of exercise, serum cortisol increased by 30% in the CI trial compared to 48% in the PI trial, and by 93 and 102% at exhaustion, in CI and PI trials, respectively (non significant, NS). Serum cortisol concentrations at exhaustion were significantly inversely correlated with plasma glucose concentrations reached at exhaustion in the PI trial ($r= -0.71$; $p<0.05$). Subjects in the PI trial who completed 150 min of exercise tended to have lower serum cortisol concentrations throughout the exercise period.

Serum insulin concentrations (Table 4.3) were similar between trials over the first 80 min of exercise, but were significantly higher at the end of exercise in the CI compared to the PI trial (4.2 ± 1.3 vs. 3.2 ± 1.0 $\mu\text{U/ml}$, $p=0.009$).

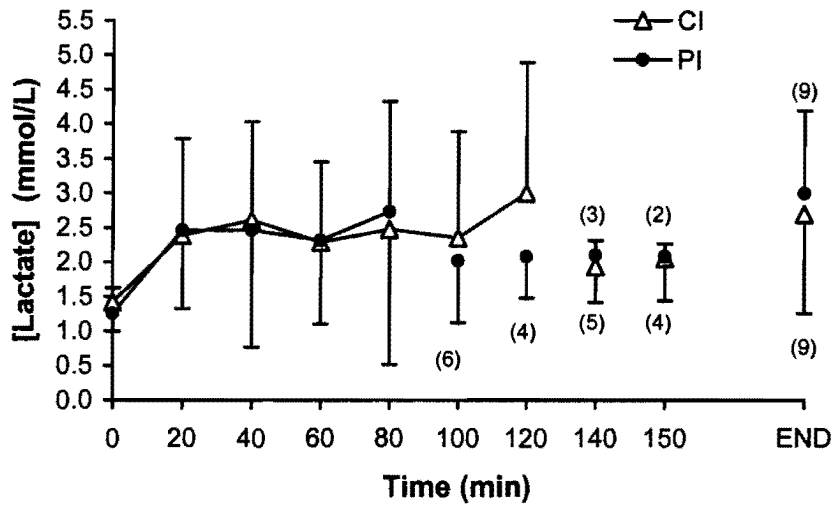


Figure 4.3: Plasma lactate concentrations during steady-state exercise. $n=9$ up to end of solid line, subject numbers indicated in brackets thereafter. END = value at exhaustion (100% of time).

Table 4.3: Serum insulin and serum cortisol concentrations during steady-state exercise.

	Time (min)									
	0	20	40	60	80	100	120	140	END ($n=9$)	
Insulin ($\mu\text{U/ml}$)										
CI	9.9 \pm 7.8	6.2 \pm 2.2	5.6 \pm 1.8	5.2 \pm 1.1	5.7 \pm 1.7	5.0 \pm 1.9	4.6 \pm 1.3	5.2 \pm 2.0 ⁽⁵⁾	*4.2 \pm 1.3	
PI	8.8 \pm 2.8	4.6 \pm 1.5	4.4 \pm 1.3	4.3 \pm 1.1	3.5 \pm 1.1	3.8 \pm 1.0 ⁽⁶⁾	3.9 \pm 0.1 ⁽⁴⁾	3.3 \pm 0.4 ⁽³⁾	*3.2 \pm 1.0	
Cortisol (nmol/L)										
CI	507 \pm 200	431 \pm 170	430 \pm 207	481 \pm 192	596 \pm 265	708 \pm 307	866 \pm 336	792 \pm 249 ⁽⁵⁾	906 \pm 312	
PI	473 \pm 165	450 \pm 157	466 \pm 156	547 \pm 240	663 \pm 254	711 \pm 346 ⁽⁶⁾	805 \pm 332 ⁽⁴⁾	846 \pm 71 ⁽³⁾	901 \pm 234	

END = value at exhaustion (100% of time). *Significant difference between trials ($p<0.05$). $n=9$, otherwise as indicated in brackets.

4.3.5 Serum free fatty acid (FFA) concentrations (Figure 4.1B)

Serum FFA concentrations were similar in both groups at rest (~ 0.5 mmol/L) and up to 80 min of exercise (~ 0.6 mmol/L). At 100 min, FFA concentrations were significantly higher in PI than CI (0.9 ± 0.2 vs. 0.6 ± 0.2 mmol/L, respectively; $p<0.05$, $n=6$), and increased progressively thereafter to concentrations of above 1.0 mmol/L. FFA concentrations at exhaustion were ~ 0.4 mmol/L higher in the PI (1.1 ± 0.6 mmol/L) than the CI trial (0.8 ± 0.3 mmol/L) ($p=0.09$). The FFA concentrations at exhaustion were significantly correlated to endurance time in both the PI ($\rho=0.87$; $p=0.001$) and CI trials ($\rho=0.83$; $p=0.005$). Hence, in both trials, the subjects with the highest FFA concentrations (>0.8 mmol/L) had the longest endurance times.

4.3.6 Serum β -hydroxybutyrate concentrations (Figure 4.4)

Serum β -hydroxybutyrate concentrations were significantly higher ($p=0.02$) in PI (0.26 ± 0.08 mmol/L) than in CI (0.18 ± 0.05 mmol/L) at 80 min, and tended to remain higher in PI at the end of exercise ($p=0.07$). Additionally, there was a significant inverse relationship between β -hydroxybutyrate and plasma glucose concentrations reached at exhaustion in the PI trial ($r = -0.70$; $p < 0.05$). Endurance time was significantly correlated to serum β -hydroxybutyrate concentrations at 80 min in PI trial ($\rho = 0.94$; $p = 0.0001$) and to serum β -hydroxybutyrate concentrations at exhaustion in both CI and PI trials ($\rho = 0.65$ and $\rho = 0.80$, respectively, $p < 0.05$).

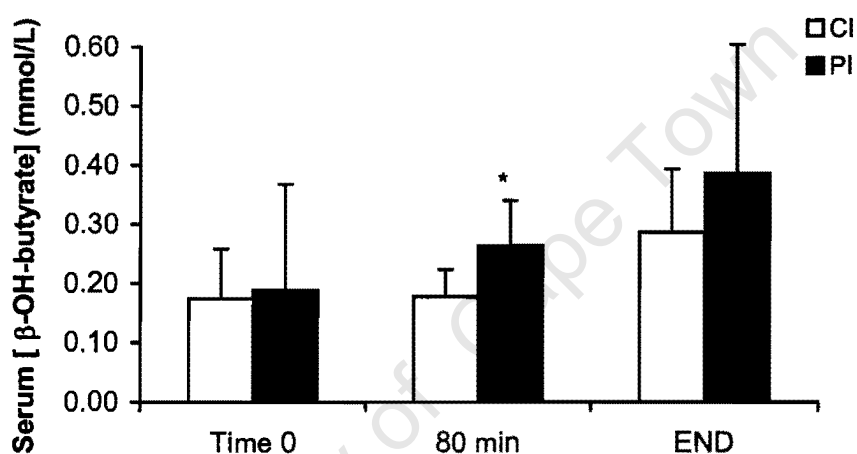


Figure 4.4: Serum β -hydroxybutyrate concentrations at rest, at 80 min during steady-state exercise and at END (value at exhaustion). $n=9$. *Significant difference between treatments ($p < 0.05$).

4.3.7 Gas exchange data (Figure 4.5) and rates of substrate oxidation (Table 4.4)

RER (Figure 4.5) remained between 0.86 and 0.88 within the first 80 min of exercise in both the CI and PI trials. RER were maintained in this range beyond 80 min of exercise in the CI trial, whilst it declined to between 0.84 and 0.86 in those subjects who exercised beyond 80 min in the PI trial, however, values were not significantly different between trials.

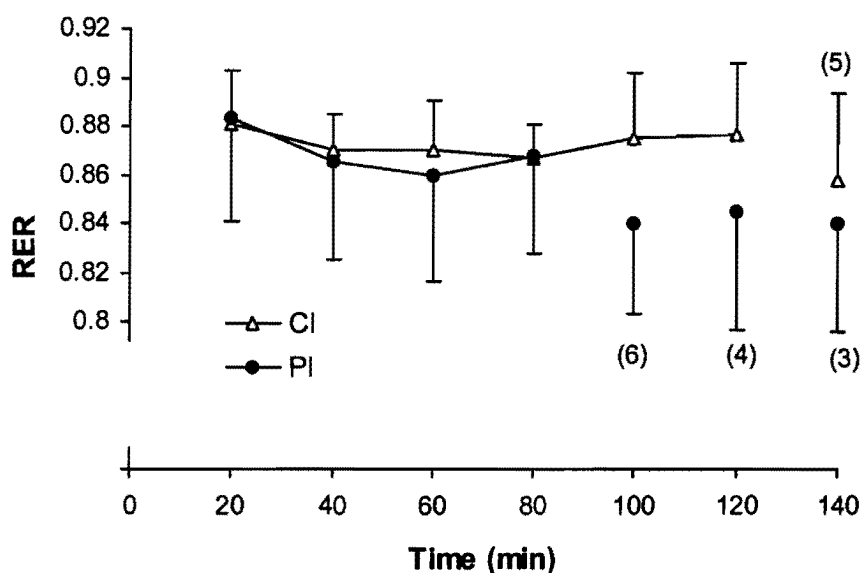


Figure 4.5: Respiratory gas exchange ratio (RER) during exercise. $n=9$ up to end of solid line, subject numbers indicated in brackets thereafter. *Significant difference between treatments ($p<0.05$). END = value at exhaustion (100% of time).

VO_2 (Table 4.4) remained constant during the CI trial, but increased significantly over time in PI ($p<0.05$), reaching significantly higher values compared to CI from 40 to 80 min and at exhaustion ($p<0.05$). The rate of total CHO oxidation (Table 4.4) remained constant between ~2.2-2.4 g/min during both exercise trials. In the CI trial, the rate of fat oxidation (Table 4.4) remained fairly constant (~0.6 g/min) throughout the first 120 min of exercise, with an increase to 0.8 g/min at 140 min in the 5 subjects who sustained that duration of exercise. During the first 20 min of exercise, the rate of fat oxidation in the PI trial was similar (~0.6 g/min) to that of the CI trial, but showed a significant increase over time ($p=0.004$) to 0.9 ± 0.3 g/min towards the end of exercise. From 40 min onwards, the average rate of fat oxidation was significantly higher in the PI compared to the CI trial ($p=0.03$).

Table 4.4: Steady-state gas exchange data and rates of fat and total CHO oxidation during exercise.

	Time (min)							
	20	40	60	80	100	120	140	END
VO_2 L/min								
CI	3.1±0.6	3.0±0.6	3.0±0.6	3.1±0.6	3.1±0.6	3.1±0.5	3.3±0.4 ⁽⁵⁾	3.2±0.5
PI	3.0±0.4	*3.3±0.4	*3.3±0.3	*3.4±0.2	3.3±0.3 ⁽⁶⁾	3.5±0.3 ⁽⁴⁾	3.5±0.4 ⁽³⁾	*3.3±0.3 [#]
Fat oxidation (g/min)								
CI	0.6±0.2	0.7±0.2	0.7±0.2	0.7±0.1	0.7±0.2	0.6±0.2	0.8±0.2 ⁽⁵⁾	0.7±0.2
PI	0.6±0.3	0.8±0.3	0.8±0.3	0.9±0.3	0.9±0.2 ⁽⁶⁾	0.9±0.3 ⁽⁴⁾	0.9±0.3 ⁽³⁾	0.8±0.3 [#]
CHO oxidation (g/min)								
CI	2.4±0.4	2.2±0.4	2.2±0.5	2.3±0.5	2.4±0.7	2.4±0.7	2.2±0.6 ⁽⁵⁾	2.4±0.8
PI	2.3±0.5	2.2±0.5	2.2±0.6	2.3±0.6	2.1±0.6 ⁽⁶⁾	2.2±0.7 ⁽⁴⁾	2.1±0.7 ⁽³⁾	2.3±0.8

END = value at exhaustion (100% of time). * Significant difference between trials. #Significant change over time ($p<0.05$). $n=9$, otherwise as indicated in brackets.

4.3.8 Plasma glucose oxidation

In both trials, plasma glucose oxidation (Figure 4.6) rose steadily from ~0.2 g/min at 20 min, to 0.4 g/min in PI and 0.5 g/min in CI at 80 min of exercise. Thereafter, the rate of plasma glucose oxidation increased significantly over time towards the end of exercise in the CI trial (~0.8 g/min), reaching significantly higher values compared to that of the PI trial from 40 min onwards (up to 100% of time; $p < 0.05$). In the PI trial, the rate of plasma glucose oxidation were maintained around 0.3 g/min from 80 min onwards and at end of exercise (100% of time). Endurance time tended to be inversely correlated to the rate of plasma glucose oxidation at exhaustion in the PI trial ($\rho = -0.63$; $p = 0.07$). This suggests that plasma glucose oxidation is low in those subjects who are able to complete 140 min of exercise under PI conditions.

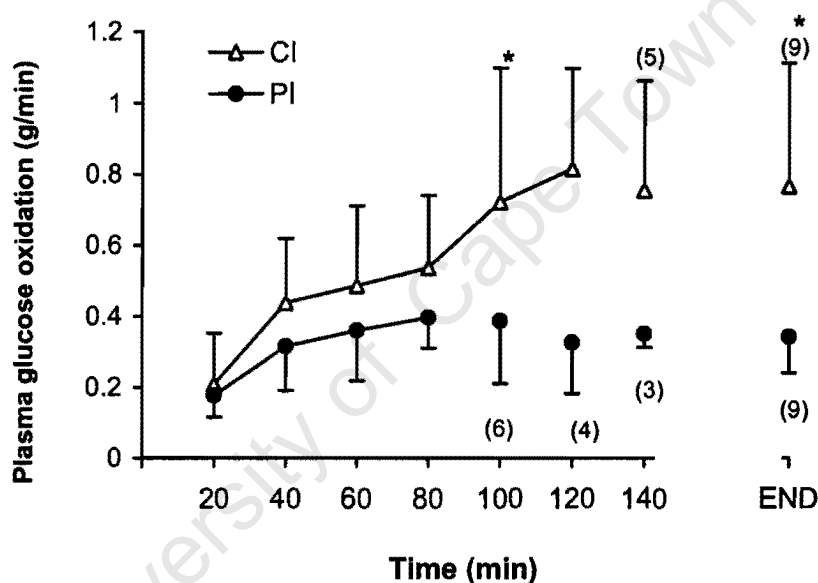


Figure 4.6: Tracer-determined rate of plasma glucose oxidation during exercise. $n = 9$ up to end of solid line, subject numbers indicated in brackets thereafter. *Significant difference between treatments ($p < 0.05$). END = value at exhaustion (100% of time).

4.4 DISCUSSION

The study was designed to investigate the effect of declining blood glucose concentrations compared to euglycemic conditions on metabolism and exercise capacity in subjects starting exercise with low glycogen stores. Thus, the first important finding of this study was that glucose infusion increased the mean endurance time of subjects starting exercise with low glycogen stores by an overall $28 \pm 26\%$ ($p < 0.05$). Only 2 (of 9) subjects completed the 150 min of exercise in the PI trial. Yet, despite the maintenance of euglycemia in the CI trial, the majority of subjects (5 of 9) still could not complete the given 150 min of exercise at $70\% \text{ VO}_{2 \text{ peak}}$, a task that would

be regarded as manageable for an endurance-trained cyclist⁴⁴¹. This indicates that aspects independent of blood glucose concentration *per se* hampered exercise capacity under these exercise conditions. Interestingly, the individual range in endurance time improvement was large, varying from 0% (in the 2 subjects that finished both the PI and CI trial) to 63%. It is conceivable that the measured effect of glucose infusion on endurance time might have been even greater if the duration of the exercise bout was longer or had not been censored (to 150 min). However, we specifically chose a defined limit time trial since open-ended trials are notoriously unreliable due to the large coefficient of variation (~27%) in performance that they produce²³².

Muscle glycogen concentrations were equally low in both trials at the start and end of exercise (Table 4.2). Hence, despite a significantly longer exercise time in CI (~26 min), a similar amount of muscle glycogen was used. One possible explanation for this result may be that the glucose infusion and increased glucose availability resulted in a slowing of muscle glycogenolysis in the CI trial. However, with muscle biopsies taken only at the start and end of exercise, it is difficult to speculate on the potential impact of the glucose infusion on altering the rate of muscle glycogen utilization. Furthermore, the majority of studies employing similar steady-state exercise protocols (~70% VO_2 peak) demonstrated that CHO supplementation during exercise has a minimal effect on the rate of muscle glycogen oxidation^{36,89}. Interestingly, Coyle et al.⁸⁹ demonstrated that CHO ingestion (compared to placebo) during exercise did not influence the rate of muscle glycogen oxidation, so that muscle glycogen concentrations were equally depleted (~40 mmol/kg w.w.) after 3 h of exercise despite CHO ingestion. However, CHO ingestion extended the exercise by a further ~60 min, with little reliance on muscle glycogen. It was concluded that with CHO feeding, highly trained endurance athletes are capable of oxidizing CHO at relatively high rates from sources other than muscle glycogen during the latter stages of prolonged exercise and that this postpones fatigue⁸⁹. Therefore, similar to Coyle et al.⁸⁹, the rate of glycogen utilization in the present study may have been similar between trials, but, whereas subjects in the PI trial started fatiguing after ~80 min and with muscle glycogen of ~40 mmol/kg w.w., the euglycemic glucose infusion extended exercise by an additional ~26 min, with little further reliance on muscle glycogen oxidation.

As expected, saline infusion in subjects with lowered glycogen stores resulted in a gradual decline in plasma glucose concentrations during the exercise bout compared to the euglycemic glucose infusion. A decline in blood glucose concentrations during prolonged exercise has traditionally been associated with a reduction in exercise capacity, mainly ascribed to a concomitant decline in total CHO oxidation^{73,75,89}. However, in the present study, despite an increase in plasma glucose oxidation late in exercise when euglycemia was maintained, the overall rate of CHO oxidation remained unaltered. More recent studies support the finding that

the frequently-quoted increase in CHO oxidation with CHO vs. placebo supplementation is not always observed ^{36,100,298,416}. Alternative explanations for the ergogenic effect of CHO supplementation have come from McConell et al. ²⁹⁸, suggesting that CHO supplementation (compared to placebo) may enhance endurance exercise performance by lowering muscle inosine monophosphate levels (a marker of the rate of ATP degradation vs. resynthesis), thereby improving muscle energy balance. Similarly, though still controversial, it has also been suggested that CHO supplementation may enhance endurance exercise performance in some subjects via alternations in central nervous system function ^{100,326,394}.

In the present study, it is difficult to draw conclusions from the observed data beyond the 80 min timepoint due to declining subject numbers and a censored end-point. Nevertheless, comparison between trials of the values reached at the point of exhaustion ($n=9$), indicate that glucose infusion resulted in a significant increase in insulin concentrations and a significant (two-fold) increase in plasma glucose oxidation from ~ 0.3 - 0.4 g/min (at 80 min in PI and CI, and at exhaustion in PI), to ~ 0.8 g/min (at exhaustion in CI), which might have contributed, at least in part, to the increased endurance capacity ⁸⁹. However, despite the significant increase in plasma glucose oxidation in CI, the majority of subjects still failed to complete the exercise task. Furthermore, there were no notable differences in the rates of plasma glucose oxidation between subjects whose exercise capacity was increased most (relative to PI) and those who did not show a large improvement as a result of glucose infusion. Peak rates of plasma glucose oxidation of ~ 0.8 g/min with euglycemic infusion in subjects starting exercise with low glycogen status have been noted previously ^{440,441}, whilst plasma glucose oxidation rates of 1.0-1.5 g/min have been reported in studies where euglycemia was maintained via CHO ingestion ³⁸ or with hyperglycemic glucose infusion ⁴⁴⁰ and with concomitant insulin concentrations of >5 μ U/ml. Thus it is possible that glucose infusion at the rate to merely maintain euglycemia in subjects starting exercise with low glycogen reserves (as in the present study), is inadequate to increase insulin and plasma glucose oxidation sufficiently (or above 0.8 g/min), so as to enable all subjects to finish the exercise task. Unfortunately, results on the individual rates of glucose infusion needed to maintain euglycemia are not available. This data could have provided more insight into the individual responses observed in exercise capacity with glucose infusion.

The gradual decline in plasma glucose during the PI trial (and significantly lower rate of plasma glucose oxidation at exhaustion) likely reflects the inability of hepatic glucose production to keep pace with glucose uptake by the working muscle. Liver glycogen content would be low, as it has been shown to decrease to 22-55 mmol/kg w.w. after 3 days on a low CHO diet ³¹⁹. Even with ingestion of 30 g of CHO 3 hours prior to the trial, if 29% ²⁴⁷ of the CHO ingested was taken up by the splanchnic tissues, with a liver mass of 1.8 kg, liver glycogen content would remain low at 49-82 mmol/kg w.w. Under such conditions, glucagon, ACTH and cortisol concentrations are

known to rise, providing a stimulus for enhanced hepatic glycogenolysis and gluconeogenesis^{223,307,440,441} and a shift towards fat oxidation. However, the euglycemic glucose infusion had no effect on the concentrations of lactate, insulin, FFA and cortisol during the initial 80 min of exercise compared to saline (placebo) infusion. These findings can be explained by Weltan et al.⁴⁴¹, who demonstrated that only when insulin was infused or hyperglycemia maintained (in subjects starting exercise after a similar glycogen lowering protocol), were the metabolic effects of a low CHO diet and lowered glycogen counteracted. This indicates that, at least during the initial 80 min of exercise, euglycemic glucose infusion had little effect on altering the hormonal and metabolic effects that are typically seen as a consequence of low glycogen content at the onset of exercise^{162,190,194,204,223,440}.

The variability in endurance capacity when glycogen concentrations are low at the start of exercise could be the individual's ability to oxidize alternative substrates, specifically FFA and β -hydroxybutyrate, to contribute to the energy demand. This is strengthened by the significant correlation between serum FFA concentrations at exhaustion and endurance time in both the trials. A large variability in substrate (particularly fat) oxidation and fatigue resistance between individuals have been noted previously^{2,172}. This variability may be linked to the existence of a metabolic phenotype, but may also be influenced by diet and/or training status¹⁷².

An important, but unexpected finding, was that the glucose infusion did not enhance endurance exercise capacity simply by preventing a decline in blood glucose concentrations. There are two lines of reasoning to explain this conclusion. First, there was a surprising inverse relationship between exercise duration and blood glucose concentrations at exhaustion in PI (Figure 4.2). Hence, subjects who exercised for the shortest duration during PI, terminated exercise with the highest blood glucose concentrations and their exercise capacity was enhanced the most by glucose infusion, and vice versa. Indeed, some subjects (4 of 9) who terminated exercise early (~80 min) in the PI trial had blood glucose concentrations of >4 mmol/L and hence were not hypoglycemic according to the usual definition⁷. Blood glucose concentrations at exhaustion in the placebo infusion trial ranged from 2.7 to 4.5 mmol/L. The results of this study are in contrast to those studies supporting the hypothesis that increased resistance to fatigue associated with CHO-loading or CHO supplementation during exercise, or both, may result from the prevention of hypoglycemia^{53,73,242,445}. It is also in contrast to those studies which have demonstrated that CHO supplementation improved endurance exercise capacity only in the individuals who became hypoglycemic during the placebo treatment^{19,91,92}. For example, Coyle et al.⁹¹ reported that CHO ingestion during prolonged exercise at 74% $\text{VO}_{2\text{max}}$ increased endurance time by ~17%, but only in those subjects who developed hypoglycemia during the placebo trial. The present findings are in agreement with studies demonstrating that CHO supplementation during exercise improved endurance performance in individuals who did not become hypoglycemic during the

placebo trials^{60,293,313}, and demonstrating that CHO supplementation did not alter total CHO oxidation rates during exercise^{36,60,100,132,298,313,416}. Furthermore, these results are supported by studies in which the development of hypoglycemia had no effect on exercise capacity or performance^{87,146,228,230,312}. Collectively taken, these results suggest that the “fatiguing effects” of declining blood glucose concentrations differ between individuals. The nature of this difference is not clear, but may relate to differences in cerebral sensitivity to reductions in blood glucose concentrations⁷, and/or phenotypic differences in metabolism during exercise^{2,172}. The phenotypic differences seen might be related to genetic differences, or to differences in habitual dietary and training regimes^{102,172}.

Secondly, the majority of subjects (5 of 9) terminated exercise prematurely in the CI trial even when euglycemia was maintained. When euglycemia is maintained by glucose infusion in subjects with lowered glycogen stores, then glucose that disappears from the circulation is replaced with infused glucose, and hence the CHO energy demand is met. Thus, the impaired exercise capacity cannot be attributed to a shortage of available CHO for oxidation. Furthermore, glucose infusion should prevent any metabolic or hormonal effects as a consequence of inadequate extramuscular and extrahepatic CHO availability. Yet, the metabolic and ergolytic effects of low glycogen reserves described in this and other studies^{162,204,223,440} persisted despite the maintenance of euglycemia, which indicates that the responses might rather be related to reduced intramuscular or intrahepatic CHO availability. Interestingly, in both the CI and PI trials the subjects fatigued at muscle glycogen concentrations of ~40 mmol/kg w.w. This has also been observed in other studies employing steady-state cycling protocols performed to exhaustion at similar exercise intensities (~70% $VO_{2\max}$)^{73,89}.

Though still speculative, the above observations might be explained by recent evidence suggesting that muscle glycogen may act as a feed-forward metabolic signal that informs the individual at what point they should slow down or stop exercise to prevent the development of absolute substrate depletion^{340,350,383,440,441}. It has been suggested^{328,440,441} that peripheral inhibitory input is conveyed from metabo- and chemo-receptors that are sensitive to chemical changes within the muscle, which may, via group III and IV afferents, affect supraspinal parts of the central nervous system which may then reduce central motor drive. Evidence in support of this suggestion comes from studies demonstrating, for example, that group III and IV muscle afferents invoke circulatory and ventilatory reflexes during exercise³⁰⁸ and provide inhibitory feedback from metabolic disturbances such as lactic acid production³⁸³, pain⁸⁵ and mechanoreception^{308,340}. In addition, there is also evidence to suggest that apart from a metabolic role, muscle glycogen availability may also have a structural role in that it may be critical in the regulation of excitation-contraction coupling^{12,64}.

In summary, the findings of this study provide additional evidence for the ergogenic effect of CHO supplementation during prolonged exercise in subjects with reduced glycogen content, however, this effect is highly variable between individuals and independent of changes in the rate of total CHO oxidation. Furthermore, despite euglycemic glucose infusion and an increase in plasma glucose oxidation, the majority of these well-trained endurance subjects still could not complete the prescribed 150 min of exercise. This study establishes that some aspect of intramuscular and/or intra-hepatic glucose metabolism influences the endurance capacity of subjects who begin exercise with low glycogen content. The exact link between plasma glucose concentration and endurance capacity remains unclear as subjects whose blood glucose concentrations did not change markedly during placebo infusion, demonstrated the greatest benefit from glucose infusion with regard to increased endurance capacity. It appears that those individuals with a high rate of fat oxidation (>1 g/min) and a decreased sensitivity to declining blood glucose concentrations and hypoglycemia are more likely to sustain exercise for longer than 80 min under these conditions. Further investigation into the existence of a metabolic phenotype and the influence of diet and training status in altering individual substrate metabolism and sensitivity to altered blood glucose concentrations and fatigue resistance warrants further investigation.

CHAPTER 5

**HORMONAL, METABOLIC, COGNITIVE AND EXERCISE
PERFORMANCE RESPONSES AFTER ANTECEDENT EXPOSURE TO
HYPOGLYCEMIA IN HEALTHY, WELL-TRAINED MEN**

University of Cape Town

5.1 INTRODUCTION

There is a substantial body of scientific and medical literature concerning carbohydrate (CHO) metabolism during exercise, particularly in relation to the onset of fatigue during more prolonged exercise bouts (for reviews see ²²⁰). However, there are many key aspects of the regulation of CHO metabolism, and the importance of maintaining euglycemia during exercise in delaying the onset of fatigue that remain unresolved and in some cases, appear paradoxical.

A number of studies have found that maintenance of blood glucose concentrations, especially towards the latter stages of prolonged (>90 min) exercise, is a key factor for delaying fatigue and improving performance ^{35,73,74,89}, and that a decline in blood glucose (from 5.2 to 2.9 mmol/L) after 3 h of exercise is related to an increase in perceived exertion ³²⁶. Conversely, others ^{146,162} found that the prevention or reversal of hypoglycemia (plasma glucose <2.5 mmol/L) during exercise had little effect on endurance capacity or perceived exertion. For example, in the study of Felig et al. ¹⁴⁶ plasma epinephrine was inversely related to blood glucose concentrations ($p < 0.01$) and was three times higher in the hypoglycemic subjects ($p < 0.05$). The ingestion of glucose (40 or 80 g/h) prevented the hypoglycemia and resulted in a smaller rise in plasma epinephrine concentrations, but did not alter perceived exertion or consistently delay exhaustion. These results demonstrated that hypoglycemia resulted in an exaggerated rise in plasma epinephrine concentrations, but failed to effect endurance capacity, and its prevention did not consistently delay exhaustion ¹⁴⁶. Thus, the exact effects of alterations in blood glucose concentrations on exercise performance remain unclear.

The previous study (Chapter 4) demonstrated that glucose infusion increased the endurance capacity of CHO-depleted subjects by an average of 28% ($p < 0.05$) compared to a placebo (saline) infusion. However, the inter-individual variability for improvement in endurance capacity was large, ranging from either no improvement (i.e. similar endurance time for both trials), up to 63% improvement with glucose infusion. The most surprising finding was the inverse relationship between exercise duration and blood glucose concentrations at exhaustion in the placebo infusion trial ($r = -0.63$, $p = 0.02$). Hence, subjects who became the most hypoglycemic managed to exercise the longest, whilst others who exercised for the shortest duration, terminated exercise with blood glucose concentrations of >4 mmol/L (i.e. not hypoglycemic). These results suggested that the fatiguing effects of hypoglycemia may differ between individuals. The nature of this difference is not clear, but may relate to phenotypic differences in metabolism ¹⁷² or perhaps to different nutritional strategies before and during exercise which can predispose the individual to develop hypoglycemia. It is not uncommon for endurance athletes to experience low blood glucose levels during long, strenuous training sessions due to insufficient CHO supplementation.

It has been shown that mild, recurrent, hypoglycemia impairs or reduces the capacity of the individual to recognize symptoms of hypoglycemia at the physiological threshold of ~ 3 mmol/L²⁶. The *in vivo* mechanisms responsible for this phenomenon have not been fully clarified, but reduced neuro-endocrine and symptomatic responses to subsequent hypoglycemia after one (recent) episode of hypoglycemia have been demonstrated in patients with type 1 diabetes, at rest^{39,95,101,349,444} and during exercise^{39,370}, as well as in nondiabetic, healthy individuals²⁰⁵. Several studies have now shown that a variety of other stresses, sufficient to result in raised glucocorticoid concentrations, have the ability to blunt neuroendocrine and autonomic nervous system (ANS) responses during subsequent exposures^{106,261,267,341}. Raised cortisol has been shown to play a key role in the pathogenesis of deficient counterregulatory responses to subsequent hypoglycemia in healthy individuals^{106,205}. As hypoglycemia and exercise can both lead to elevations in glucocorticoids and elicit similar neuro-endocrine and ANS responses, it has been suggested that these two forms of stress may reciprocally blunt their respective counterregulatory responses¹⁶⁰.

In a study by Kuipers et al.²⁶⁶ some subjects (6 out of 19) developed rebound hypoglycemia (plasma glucose < 3.0 mmol/L) after ingestion of 50 g of CHO, 30 min prior to starting 40 min of exercise at 60% of maximal power output. These subjects (n=6) displayed a significantly lower norepinephrine response throughout most of the exercise bout compared to the group of subjects who did not develop hypoglycemia (n=13), which reflects a lower activation of the sympathetic-mediated counterregulatory response in the hypo-group. Insulin concentrations remained similar between groups, however, insulin sensitivity *per se* was not measured. Furthermore, though epinephrine concentrations were similar between groups, a large inter-individual variation was noted²⁶⁶.

A further interesting observation in the literature is that endurance-trained men might develop an adaptation of the HPA-axis to repeated and prolonged exercise-induced increases in cortisol secretion (or repeated stimulation of the HPA-axis)¹²⁵. These adaptive mechanisms may include decreased sensitivity to cortisol to protect muscle and other glucocorticoid-sensitive tissues against the increased cortisol secretion commonly observed during and up to 2 h after exercise^{124,125}. The findings by Viru et al.⁴²⁸ suggested that fatigue from prolonged endurance activity may introduce a resetting in the pituitary-adrenocortical component of the endocrine system, expressed either by intensified endocrine functions in some individuals or by suppressed endocrine functions in others. The potential impact of these alterations in HPA-axis responsiveness on glucoregulatory hormonal responses and maintenance of glucose homeostasis have, to our best knowledge, not been fully investigated.

Reduced counterregulatory responses may, in part, explain the observation noted in the literature that some individuals show altered metabolic and hormonal responses in relation to alterations in blood glucose concentrations ²⁶⁶. Furthermore, the reduced symptomatic responses (referred to as “hypoglycemia unawareness”) that may accompany the deficient counterregulatory responses may explain the situations where exercise performance, particularly during prolonged exercise, remains unaffected despite the development of hypoglycemia (as described in Chapter 4).

Indeed, Davis et al. ¹⁰² reported that antecedent exposure to hypoglycemia resulted in significant blunting of neuro-endocrine (glucagon, insulin, catecholamine) and metabolic (endogenous glucose production, lipolysis, ketogenesis) responses during a 90-min exercise bout (the following day) in healthy subjects. The group exposed to antecedent hypoglycemia required a 10-fold higher rate of glucose infusion in order to maintain euglycemia during the exercise bout, compared to the antecedent euglycemic group. However, the euglycemic glucose infusion during exercise makes it difficult to elucidate whether the neuro-endocrine and metabolic responses were caused by, or resulted in the high rate of glucose infusion required to maintain euglycemia.

In a subsequent study, Galassetti et al. ¹⁶⁰ demonstrated that antecedent prolonged exercise under euglycemic conditions blunted neuro-endocrine and metabolic counterregulatory responses to subsequent hypoglycemia. Furthermore, Galassetti et al. ¹⁶¹ demonstrated a sexual dimorphism in the counterregulatory response to antecedent hypoglycemia, with a significantly greater attenuation of response in men, when compared to women. Again, in both these studies, glucose was infused during exercise to maintain euglycemia, which complicates interpretation of the blunted counterregulatory response. It is unclear what the metabolic and hormonal responses would be if blood glucose concentrations during exercise were left free to vary and without exogenous manipulation. In addition, the responses of well-trained individuals, with presumably enhanced insulin sensitivity (for review see ³⁴) and altered sympathetic and parasympathetic activation as a result of regular exercise training ^{62,266}, are unknown.

In the above-mentioned studies by the Davis group, exercise performance *per se* was not measured. Seeing that glucose is a major fuel for the brain and important to sustain cognitive metabolism, hypoglycemia may impair cognitive performance ¹⁵⁶. Nybo et al. ³²⁶ recently investigated whether hypoglycemia-induced fatigue during 3 h of cycling at 60% $VO_{2\max}$ could be related to alterations in cerebral metabolism. A temporal association between increased perceived exertion and reduced cerebral glucose uptake suggested that fatigue was related to inadequate substrate availability for the brain ³²⁶.

Measurement of cognitive function *per se* (such as choice and simple reaction time) has been shown to be impaired during exposure to hypoglycemia^{135,158}, but a single episode of mild antecedent hypoglycemia (3.1 mmol/l) in healthy men can reduce the magnitude of cognitive impairment during a subsequent exposure to hypoglycemia (within 18-24 h)¹⁵⁸. This might be explained by data indicating that cerebral adaptations may occur that allow for normal brain glucose uptake and cerebral function to be maintained during recurrent systemic hypoglycemia⁴².

The limited available literature seem to indicate that prolonged (2-3 h), moderate-intensity (<60-75% VO_2 max) exercise may be associated with an increase in cognitive performance^{81,177,352}. This effect is suggested to be the result of an increase in metabolic load, specifically, to the rate of increase in catecholamine concentrations which increases CNS activation and in turn, results in an increase in arousal level^{46,66,177}. The increase in arousal and cognitive functioning have also been linked to an increased heart rate and an increased rating of perceived exertion⁴⁶. Prolonged exercise may also increase cerebral blood flow, or neurotransmitter (catecholamines / endorphin) release, however, the exact mechanism for the enhanced cognitive functioning is still unknown⁴⁶.

During prolonged exercise, the development muscle glycogen depletion and hypoglycemia have been related to the development of peripheral and central fatigue^{52,76} which may also impair cognitive functioning⁴⁶. Conversely, CHO ingestion has been shown to delay the onset of fatigue^{52,76} and attenuate the perception of effort towards the latter stages of prolonged exercise^{46,420}, and minimize the negative effect of central fatigue induced by prolonged exercise⁴⁶. The findings of Grego et al.¹⁷⁷ recently suggested a combined effect of arousal and central fatigue on electrocortical indices of cognitive function during 3 h of exercise¹⁷⁷, however, more research is needed to investigate the role of central factors on cognitive performance⁴⁶.

It is yet unclear if the blunted neuroendocrine and metabolic responses seen after antecedent exposure to physiological stress (e.g. hypoglycemia or exercise^{102,160}) may affect muscle recruitment, perceived exertion, cognitive function and ultimately endurance exercise performance. Specifically, It is also not known if the altered HPA-axis activation (and reduced sympathetic activation) may result in reduced central command, thereby potentially affecting force production (muscle recruitment), perceived exertion cognitive function and exercise performance.

It is also conceivable that if the symptomatic response to hypoglycemia is blunted during subsequent stressful exposures³¹¹, then exercise performance *per se* may actually be improved as the individual will be able to exercise for longer before "sensing" symptoms of hypoglycemia

which may translate into symptoms of fatigue. Effort perception may primarily be a feedforward system that results from the interpretation of afferent sensations around the body. It is unclear how the brain interprets this feedback, but it has been suggested that an integration of these sensory cues may indirectly and unconsciously influence effort perception during exercise, and thereby fatigue^{182,331}.

In summary, there is disparate evidence as to how the development of fatigue during prolonged exercise is affected by decreased CHO availability and the associated neuro-hormonal response. It is also not clear as to how these central and peripheral (feedforward and feedback) responses are integrated, and to what extent it may vary between individuals. Accordingly, the aim of this study was to determine the effect of an antecedent bout of hypoglycemia on counterregulatory responses, metabolism, liver glucose output, perception of effort and endurance performance during endurance exercise. We hypothesized that antecedent exposure to hypoglycemia would reduce neuroendocrine and metabolic responses to prolonged exercise, and in addition, reduce effort perception and thereby improve exercise performance.

5.2 METHODS

5.2.1 Subjects

Ten healthy, endurance-trained males aged between 19 and 35 years were recruited to participate in this study (subject characteristics are presented in Table 1). None were taking any medication or had any previous significant medical history, nor had any personal or family history of diabetes mellitus. Each volunteer gave their written, informed consent before participation in the study, which was approved by the Research and Ethics Committee of the Faculty of Health Sciences of the University of Cape Town (South Africa).

5.2.2 Research design

Each subject underwent two, 2-day experimental trials separated by at least one month. The order of the experimental trials was randomized and performed in a single-blind, counter-balanced fashion. On day 1, subjects were either exposed to two 80-min bouts of hypoglycemia (HYPO trial) separated by 40 min of euglycemia, on the other occasion, euglycemia was maintained throughout (EU trial). On day 2, subjects performed 90 minutes of cycling exercise at 70% of $\text{VO}_{2\text{max}}$ followed by a cycling time trial consisting of time to complete 200 kJ of work. No blood glucose manipulations were done during day 2. Subjects ingested water only (*ad lib*), and a primed, continuous infusion of [3-³H]glucose was started at -60 min and continued for 90 min in order to measure hepatic glucose output during steady-state exercise. Studies were identical

other than the plasma glucose profiles on day 1 of testing (detailed below). Subjects were blinded to the order of the studies as well as their plasma glucose concentration at all times. On the day prior to each test day, subjects were required to abstain from alcohol and exercise, and were asked to keep a detailed food diary, which were to be repeated the day prior to the second experimental trial. During the course of the study subjects were instructed to maintain their habitual exercise schedule and diet.

5.2.3 Preliminary testing

5.2.3.1 Anthropometry

At least one week prior to the experiment proper, each subject's height and weight were recorded and body mass index (BMI) was calculated. Body fat was determined from the sum of seven skinfolds (biceps, triceps, subscapularis, abdominal, thigh and calf) and calculated using the equation of Durnin & Womersley¹²⁷.

5.2.3.2 Peak sustained power output (W_{peak}) test

The anthropometric assessment was followed by a 10-min warm-up exercise bout on a stationary cycle ergometer (Lode, Groningen, The Netherlands). This was followed by an incremental test to exhaustion in order to determine each subject's peak sustained power output (W_{peak}). The W_{peak} test was performed on the electronically braked cycle ergometer at a workload equivalent to 3.3 W/kg body mass and increased first by 50 W after 150 sec and then by 25 W every 150 sec until the pedaling frequency dropped below 50 revolutions/min. This information was used to adjust the work load during the exercise trial so that each subject exercised at an intensity corresponding to 70% of peak rate of oxygen consumption ($VO_{2\ max}$), which this corresponds to ~63% of W_{peak} ¹⁹⁷.

5.2.3.3 Cognitive function baseline testing

A computerized software program (*CogState™*) was used in the cognitive testing. *CogState™* is a software program comprising five non-verbal computerized neuropsychological tests assessing reaction time, decision making, sustained and divided attention, working memory and new learning⁸². This test battery requires the participant to respond manually (via the keyboard) to playing cards presented on the computer screen. Although the physical response remains consistent between tasks (the same 2 keys are used), each of the tasks is set in a different context and requires use of different cognitive processes. For example, the simple reaction time (SRT) task requires participants to press a key as soon as the presented card turns face-up. The choice reaction time (ChRT) task requires participants to press one of two keys depending upon

the color of the presented card. Thirty accurate responses are required to complete each task. These computerized tasks are highly reliable when administered serially to healthy adults and children⁸³, and have documented sensitivity to mild cognitive changes caused by concussion²⁸⁴, fatigue¹³⁷, alcohol²⁸⁴, early neurodegenerative disease⁹⁹, coronary surgery³⁸¹ and childhood mental illness³¹⁰. The effects of practice⁸² and its correlation with conventional paper-and-pencil tests⁸³ have been documented. Table 5.1 lists the cognitive assessment tasks employed in the present study.

Table 5.1. Cognitive assessment battery used in this study.

Task name	Abbreviation	Cognition assessed
Simple Reaction Time	SRT	Motor function
Choice Reaction Time	ChRT	Decision making
Monitoring task	DIVA	Divided attention
One-Back	OBK	Working memory
Continuous Learning	LEARN	Learning & memory

One to two weeks prior to the experiment proper, all participants completed a baseline cognitive test consisting of a practice trial and a baseline trial. Eight of ten participants completed a second baseline test, performed 2 weeks after completion of the study. Baseline testing was conducted in a quiet, well-lit environment and was supervised by one of the study investigators.

5.2.4 Day 1 experiments: Blood Glucose Clamping Procedure

On the morning of each trial (8:00 A.M.), subjects reported to the testing laboratory after a 10-hour overnight fast. Two intravenous catheters were inserted into the non-dominant arm. One cannula was placed in retrograde fashion distally in the forearm for blood sampling and kept patent with a slow infusion of normal saline. The other cannula was placed in the antecubital fossa to enable administration of insulin and glucose. The forearm was then placed in a heated box (55-60 °C) to arterialize the venous blood samples. The heated box was exchanged for a heating blanket during the 5-min it took to perform the computerized cognitive test. A primed, continuous intravenous infusion of regular insulin (Human Actrapid, Novo Nordisk), which was made up to 55 ml in 0.9% sodium chloride to which 2 ml of the subject's blood had been added, was infused at a maintenance rate of 1.5 mU/kg BM/min. A stopwatch was started as soon as 0.5 ml of the insulin solution had been delivered. Plasma glucose (measured at the bedside every 5 minutes (Glucose analyzer 2; Beckman Instruments, Fullerton, CA)) was maintained at the required concentration during HYPO and EU using a variable-rate intravenous infusion of 20% dextrose⁹⁴. Plasma glucose was maintained at euglycemia (5 mmol/L) for the first 40 minutes of each of the glucose clamping trials (HYPO and EU). In the HYPO trial plasma glucose was reduced (by reducing the glucose infusion rate) to 2.9 mmol/L and maintained at

this level for 80 min. This was followed by 40 min of euglycemia (5 mmol/L), and a subsequent second 80-min bout of hypoglycemia (2.9 mmol/L). The clamp lasted a total of 240 min. In the EU trial, euglycemia (5 mmol/L) was maintained throughout the 240 min.

On completion of the trials (at 240 min), the insulin infusion was stopped. The plasma glucose was restored to euglycemia if necessary, and subjects were given a meal to consume. Plasma glucose monitoring continued until euglycemia was maintained spontaneously, after which all lines were withdrawn and subjects could return home.

5.2.4.1 Measurements during Day 1 experiments

5.2.4.1.1 Blood sampling

Apart from bed-side determination of plasma glucose concentration every 5 min, a ~12 ml blood sample was collected at baseline and at 20-min intervals and separated into tubes containing gel and clot activator (Beckton-Dickinson, South Africa) for determination of serum insulin and cortisol concentrations, and a tube containing lithium heparin (Beckton-Dickinson, South Africa) for determination of plasma catecholamine concentrations. The tubes were immediately placed on ice and, after 20 min, centrifuged at 3 500 rpm for 12 min at 4 °C, and the supernatants were then stored at -80 °C for later analysis. Serum insulin concentrations were determined using a radio-immunoassay (RIA) technique (Coat-A-Count Insulin, Diagnostic products, Los Angeles, CA). Serum cortisol concentrations were assayed using the Clinical Assays Gamma Coat RIA kit (Diagnostic Products, Los Angeles, CA). Plasma catecholamines were measured using HPLC with electrochemical detection using the method described by Forster and MacDonald¹⁵⁰ (however, due to technical delays the catecholamine data will not be reported here).

5.2.4.1.2 Blood pressure

Systolic blood pressure remained similar between the HYPO and EU trials, and remained unchanged throughout the clamping experiments. Diastolic blood pressure was significantly lower in the HYPO compared to the EU trial at the 100, 220 and 240 min timepoints ($p < 0.05$). In the HYPO trial, diastolic blood pressure was significantly lower at 100 and 120 min (end of the first HYPO exposure; $\sim 58 \pm 7$) and at 200, 220 and 240 min (end of second HYPO exposure; $\sim 58 \pm 5$) compared to baseline (68 ± 8).

5.2.4.1.3 Symptomatic responses

Subjective ratings of hypoglycemic symptoms were assessed by the use of a questionnaire (administered every 20 min), asking subjects to rank sweating, warmth, palpitations, tingling,

anxiety, trembling, hunger, blurred vision, drowsiness, confusion, weakness, headache, difficulty speaking, dizziness, and irritability individually on a linear analog scale. Autonomic symptom scores were derived from the first 7 symptoms listed, and neuroglycopenic scores from the latter 8 symptoms with symptoms being ranked from 0 (not at all) to 6 (very severe/maximal) throughout each of the trials²⁰⁷.

5.2.4.1.4 Cognitive testing

Two of the cognitive tests, SRT and ChRT, were administered towards the end of the first 80 min bout of hypoglycemia (HYPO 1, at time 115 min), and again at the end of the second bout of hypoglycemia (at time 230 min), before euglycemia was restored, and at equivalent time points in the EU trial. The administration of the SRT and ChRT tests took ~5 min. These two tests were included to enable comparisons of cognitive function between the two bouts of hypoglycemia (HYPO 1 vs. HYPO 2), combined with comparisons to euglycemic clamp experiment (EU) at the equivalent time points. Only two of the full battery of 5 tests was administered during the clamp (at 115 min) as it was short enough to minimize subject strain and minimize disruption of the clamping procedure. The full battery of 5 tests was administered towards the end of each glucose clamp experiment, i.e. at 225 min during the HYPO and EU experiment, respectively and took ~12-15 min to complete.

5.2.5 Day 2: Exercise experiment

The morning after the glucose clamp procedure, subjects reported to the laboratory after a 10-hour overnight fast. One 18-gauge cannula was placed in a forearm vein to enable blood sampling. A second 21-gauge cannula was placed in the contralateral arm to enable infusion of the [3-³H]glucose tracer for isotopic determination of glucose production and utilization during steady-state exercise. After insertion of the cannulae, subjects remained at rest for 60 min during which the primed (35 μ Ci), continuous (0.25 μ Ci/min) infusion of the [3-³H]glucose tracer was started (Amersham International, Buckinghamshire, UK). The isotope infusion was continued during the 90 min of steady-state exercise and stopped prior to the start of the time trial. After resting for 60 min, subjects started a 10-min warm-up cycling bout, 5 min at 100 W followed by 5 min at 150 W, on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands). This was followed by 90 min at cycling at 60% of their individual pre-determined maximal work capacity (W_{peak} ; equivalent to ~70% VO_{2max}). At 15-min intervals (and after blood and respiratory gas exchange measurements were completed), subjects performed a sprint, which consisted of completing 10 kJ of work in as fast a time as possible (~20 sec). At the end of the 90-min ride, subjects rested for 1 min, followed by the performance time-trial consisting of completing 200 kJ of work in as fast a time as possible (~15-20 min). During exercise the

subjects could ingest water *ad lib* and were cooled with an electric fan. Environmental conditions in the laboratory were controlled at 20 °C.

5.2.5.1 *Measurements during Day 2 experiments*

5.2.5.1.1 *Blood sampling and analysis, VO₂ and VCO₂ measurements*

Blood, on-line respiratory gas exchange (done for 5 min at a time; Oxycon Alpha computerized system, Jaeger-Mijnhart, The Netherlands) were sampled at baseline, at 15 min intervals during steady-state cycling (before performing the sprint), and at the end of the time trial. After each blood sample was taken, the cannula was kept patent by flushing with 1 ml sterile saline. Aliquots of the blood sample were placed into tubes containing potassium oxalate and sodium fluoride (Midran; Novo Nordisk, Johannesburg, South Africa) for subsequent analysis of plasma glucose, [3-³H]glucose specific activity, and lactate concentrations. Another aliquot of blood was divided into a tube containing gel and clot activator (Beckton-Dickinson, South Africa) for determination of serum free fatty acids (FFA), cortisol and insulin concentrations, and a tube containing lithium heparin (Beckton-Dickinson, South Africa) for determination of plasma catecholamine concentrations (not reported here). The tubes were immediately placed on ice and, after 20 min, centrifuged at 3 500 rpm for 12 min at 4 °C, and the supernatants were then stored at -80 °C for later analysis. Plasma glucose concentrations were determined by the glucose oxidase method using a glucose analyzer (Glucose analyzer 2; Beckman, Fullerton, CA). Lactate concentrations were measured by spectrophotometric (model 35; Beckman, Fullerton, CA) enzymatic assays (Lactate PAP; Bio Mérieux, Marcy-L'Étoile, France). Serum insulin concentrations were determined using a RIA technique (Coat-A-Count Insulin, Diagnostic products, Los Angeles, CA). FFA concentrations were measured using an enzymatic colorimetric assay (Half-micro test; Boehringer Mannheim). Serum cortisol concentrations were assayed using the Clinical Assays Gamma Coat RIA kit (Diagnostic Products, Los Angeles, CA). Catecholamine concentrations were determined by HPLC (however, due to technical delays the catecholamine data will not be reported here).

5.2.5.1.2 *Rating of perceived exertion and heart rate measurements*

Subjective ratings of perceived exertion (RPE) was measured at 15-min time intervals during exercise (before performing the ~20 sec sprint). Two scales were used to quantify the subject's level of exertion: 1) the validated Borg 15-point RPE scale, and 2) the validated Borg category-ratio 10-point scale³⁰. The subjects were asked to use these scales to give a subjective rating of 1) general or "whole-body" exertion, and 2) give a subjective rating of exertion pertaining particularly to how their head and exercising legs "feel". A printed scale and instructions were

given to familiarize subjects, and a verbal explanation of each scale and a description of how the scales should be used were given prior to the start of exercise.

Heart rate was recorded before and immediately after the ~20 sec sprint that was performed every 15 min during the 90 min cycle and by means of a Polar™ heart rate monitor.

5.2.5.1.3 Analysis of isotope samples

HPLC-purified [3-³H]glucose was infused to assess rate of hepatic glucose output during exercise. Since the radioactivity in the blood samples had to be divided by plasma glucose concentration to determine specific activity (disintegrations/ml plasma ÷ mmol glucose/ml of plasma), blood was collected in the same way as for a determination of plasma glucose concentration. Since proteins would interfere with the efficiency of radioactivity counting, a 1 ml aliquot of the plasma was taken and the concentration of HClO₄ needed to deproteinize the sample with the minimum of added volume was determined by experimentation. Thus 0.1 ml of 3.5 M HClO₄ was added to deproteinize the sample. The sample was then centrifuged at 4 °C and the protein-free supernatant was removed and kept cold. It was found that the precipitate contained around 10% of the total ³H-glucose dpm. Thus it was resuspended in 0.4 ml of distilled water, re-centrifuged and the supernatant added to that previously saved. This resulted in removal of almost all of the radioactivity in the precipitate. The supernatant was finally collected in a 20 ml glass vial. In order to (a) minimize the presence of ³H₂O (which accounted for ~5% of the glucose radioactivity counts) from the metabolism of [3-³H]glucose in the glycolytic pathway and (b) reduce the water/ liquid scintillation cocktail ratio for radioactivity counting, the eluates (~6 ml) were evaporated to near dryness (~0.3 ml) at 70 °C over approximately 20 h. One ml of distilled water was then added to redissolve the residue and this solution was then mixed with 15 ml of Ready Gel (Beckman, Fullerton, USA) liquid scintillation cocktail for ³H determinations (dpm) by dual-channel counting (Packard Tri-Carb 4640, Illinois, USA). As small losses of radioactivity during processing are inevitable, whenever samples were processed, a control plasma sample was spiked with a known amount of [3-³H]glucose and processed concurrently so that the dpm/min values of experimental samples could be corrected for the percentage recovery. Recovery was around 90%. Since the 0.5 ml aliquot of plasma used for radiation counting was from the same plasma sample as previously used for the determination of glucose concentration, the specific activity in dpm/ mmol glucose could be calculated.

5.2.5.1.4 Calculation of glucose rate of appearance

The rate of appearance of glucose from the liver ($R_{a\text{hepatic glucose}}$) was calculated using Steele's equations for non-steady-state exercise³⁹⁵, validated by Radziuk et al.³⁴⁴ and are shown below:

$$Ra_{\text{hepatic glucose}} = (I - (pV \times [\text{Glu}] \times dSA/dt))/SA$$

where, $Ra_{\text{hepatic glucose}}$ is the rate of appearance of glucose from the liver in mmol/min; I is the infusion rate of [3-³H]glucose in dpm/min; p is the pool fraction (0.75) in which rapid changes in glucose concentration and specific activity take place^{224,344}; V is the glucose distribution volume (19.6% of body mass in liters at rest²²⁴; $[\text{Glu}]$ is the mean plasma glucose concentration in mmol/L in consecutive samples; dSA/dt is the change in plasma [3-³H]glucose specific activities in dpm/mmol over the sample interval in minutes; and SA is the mean dpm/mmol [3-³H]glucose specific activity in successive samples.

5.2.5.1.5 Cognitive testing

The full battery of 5 tests (as explained under the preliminary testing section) was repeated at the end of the cycling TT.

5.2.6 Statistical analysis

The statistical software package STATISTICA 7.0 (2004; StatSoft, Inc., Tulsa, OK, USA) was used for the statistical analysis of the data. All results are presented as means \pm SD for $n=10$ subjects.

5.2.6.1 Metabolic, hormonal and exercise performance data

Statistical significance ($p < 0.05$) of between trials (HYPO vs. EU) was assessed by a two-way analyses of variance (ANOVA) for repeated measures over time ($n=10$) during the blood glucose clamp procedure and exercise protocol. In order to determine which means were significantly different, Tukey's HSD post-hoc analysis was used. Paired t - Tests were performed for between trial comparisons of variables such as total amount of glucose infused during clamp experiments, total amount of hepatic glucose production during exercise experiments, average sprint time and TT performance time.

5.2.6.2 Cognitive function data

The mean RT on each task was used to express the speed of performance. For each participant, anticipatory responses (defined as responses faster than 100 ms) were counted as errors and excluded from further analysis. Inspection of the distributions of RTs indicated a positive skew in all distributions. This is a common feature of RT distributions²⁸³. Data for each participant was therefore logarithmic base 10 (\log_{10}) transformed prior to statistical analysis, to ensure that data met the assumptions of normality and heterogeneity of variance.

For each individual participant, change from baseline on each outcome variable was expressed using a difference score. This was calculated by subtracting performance during each experimental condition from performance at the first baseline test, and dividing the difference by the group mean within subject standard deviation (WSD) calculated from the two baseline assessments²¹. The resulting statistic is interpreted as a z-score, with values greater than 1.64 ($p < 0.05$ one-tailed) considered a significant decline. A composite cognitive score was then calculated by summing the z-scores from all 5 individual tasks (Maruff et al, submitted). A series of paired sampled Students' t-tests were then undertaken to compare the performance on each outcome variable under the HYPO and EU conditions.

5.3 RESULTS

As indicated in Table 5.2, the subjects had moderately high $VO_{2\text{ max}}$ and peak work rate values, consistent with being well-trained, but not elite cyclists.

Table 5.2: Subject characteristics

n=10	
Age (yrs)	24±4
Body mass (kg)	73±7
BMI (kg/m ²)	23±1
Sum of 7 skinfolds (mm)	50±18
Body fat (%)	9±3
Peak sustained power output (Watts)	367±26
$VO_{2\text{ max}}$ (ml/kg/min)	64±3

Values presented as Mean ± SD.

5.3.1 Day 1: Glucose clamping experiments

5.3.1.1 Plasma glucose and serum insulin concentrations

Arterialized plasma glucose concentrations during the euglycemic clamp was maintained at 5.1 ± 0.2 mmol/L. During the hypoglycemic clamp, arterialized plasma glucose was maintained at 2.8 ± 0.1 mmol/L during the first bout of hypoglycemia (HYPO 1) and at 2.9 ± 0.1 mmol/L during the second bout of hypoglycemia (HYPO 2). In response to the insulin infusion, serum insulin concentrations reached 81.4 ± 25.3 and 81.8 ± 25.7 $\mu\text{U/ml}$ at the end of HYPO 1 (120 min) and HYPO 2 (240 min), respectively; and 92.0 ± 17.3 (120 min) and 83.6 ± 16.5 (240 min) during the EU clamp.

5.3.1.2 Rate of glucose infusion

Table 5.3: Total amount of glucose infused and glucose infused per kg of body mass during the hypoglycemia (HYPO) and euglycemia (EU) experiment.

Subjects (n=10)	Total amount of glucose infused (g)		Glucose infused per kg body weight (g/kg)	
	HYPO	EU	HYPO	EU
A	82	238	1.2	3.5
B	134	237	1.5	2.9
C	90	225	1.6	3.7
D	144	224	1.5	3.0
E	101	215	1.9	3.0
F	109	307	1.4	3.9
G	114	264	1.4	3.3
H	171	293	2.2	3.7
I	92	207	1.4	3.0
J	100	236	1.5	3.6
	114±28	244±33	1.6±0.3	3.4±0.4

5.3.1.3 Cortisol concentrations

Serum cortisol concentrations were significantly higher during the HYPO compared to EU trial ($p < 0.05$) (Figure 5.1). After 120 min serum cortisol concentrations showed a decline, likely due to re-establishment of euglycemia for 40 min from 120 to 160 min. At 160 min glucose concentrations were again reduced to 2.9 mmol/L whilst serum cortisol continued declining over the next 40 min of hypoglycemia up to 200 min, where after it again increased over the last 40 min of the second 80-min hypoglycemic bout (indicating a delayed response). Apart from the delay in cortisol response during the initial 40 min of the second hypoglycemic bout (and after 40 min of euglycemic restoration), the cortisol response over the last 40 min of HYPO 2 increased to similar levels than that reached toward the end of HYPO 1.

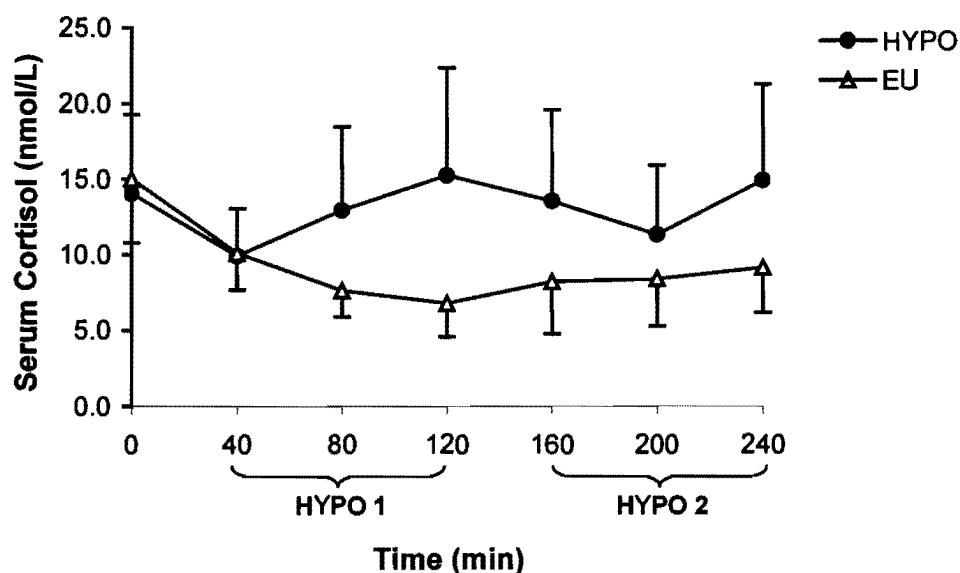


Figure 5.1 Serum cortisol concentrations from arterialized venous blood during the blood glucose clamp experiments on Day 1. HYPO 1 and HYPO 2 represent the 2 bouts of hypoglycemia (plasma glucose 2.9 mmol/L) preceded (0-40 min) and interspersed (120-160 min) by euglycemia (plasma glucose 5 mmol/L).

5.3.1.4 Systolic and diastolic blood pressure

Systolic blood pressure was maintained similarly during hypoglycemia and euglycemia experiments (Table 5.4). Diastolic blood pressure was significantly lower in the HYPO compared to the EU trial at the 100, 220 and 240 min timepoints ($p < 0.05$). In the HYPO trial, diastolic blood pressure was significantly lower at 100 and 120 min (end of the first HYPO exposure; $\sim 58 \pm 7$) and at 200, 220 and 240 min (end of second HYPO exposure; $\sim 58 \pm 5$) compared to baseline (68 ± 8 ; $p < 0.05$).

Table 5.4: Systolic and diastolic blood pressure during Day 1 hypoglycemia (HYPO) and euglycemia (EU) experiments.

Blood pressure	Time (min)												
	0	20	40	60	80	100	120	140	160	180	200	220	240
HYPO:													
Systolic	125	122	124	122	125	125	125	120	119	121	122	123	127
±SD	±10	±10	±12	±11	±12	±10	±10	±11	±16	±10	±9	±11	±8
Diastolic	68	66	64	62	62	58*	*58*	59	59	62	59*	*57*	*58*
±SD	±8	±8	±9	±8	±7	±7	±6	±7	±7	±7	±4	±4	±5
EU:													
Systolic	120	118	121	120	123	122	123	126	122	126	123	124	125
±SD	±10	±9	±12	±9	±10	±10	±10	±11	±9	±7	±11	±10	±11
Diastolic	68	63	65	62	64	64	66	65	65	65	65	66	68
±SD	±6	±6	±10	±7	±6	±7	±9	±10	±5	±8	±6	±9	±9

*Significant difference between HYPO and EU experiments. *Significant difference from baseline.

5.3.1.5 Symptomatic responses

Subjective ratings of the symptoms related to autonomic and neuroglycopenic responses to hypoglycemia remained similar throughout the euglycemic clamp experiment (Table 5.5), whereas it increased over time from 60 min onwards in the hypoglycemic clamp experiment ($p < 0.05$).

Autonomic symptom ratings were significantly higher in the hypoglycemic compared to euglycemic experiment from 60 min onwards, and neuroglycopenic symptoms from 80 min onwards. Neuroglycopenic symptoms were not significantly different between experiments at the 140 and 180 min timepoints, likely due to the 40 min of euglycemia that was re-established in-between the first and second bout of hypoglycemia (from 120 to 180 min).

When comparing the symptom ratings between the first and second bout of hypoglycemia (HYPO), the second bout of HYPO elicited significantly higher ratings of autonomic symptoms compared to the first bout of HYPO ($p = 0.04$). The neuroglycopenic symptom ratings, however, did not differ significantly between the first and second HYPO bout.

Table 5.5: Hypoglycemic symptom ratings during Day 1 hypoglycemic (HYPO) and euglycemic (EU) clamp experiments.

Symptoms	Time (min)													
	0	20	40	60	80	100	120	140	160	180	200	220	240	
HYPO:				*	*	*	*	*	*	*	*	*	*	
Autonomic	2±1	1±1	1±1	5±7	7±7	8±7	8±7	6±4	5±3	4±2	10±10	12±7	12±6	
Neuroglycopenic	1±1	1±1	1±1	4±7	6±7	7±9	7±9	6±7	5±4	3±3	9±10	10±8	9±8	
EU:														
Autonomic	2±2	1±1	1±1	2±2	2±2	2±2	2±1	2±2	2±2	2±2	2±2	2±2	2±2	
Neuroglycopenic	1±1	1±2	1±2	1±2	2±2	2±3	3±2	3±2	3±2	3±3	3±3	3±3	2±3	

Values mean ± SD. *Significant difference between HYPO and EU experiments. #Significant difference from baseline.

There was a large range in inter-individual symptom ratings in the HYPO experiment, as is evident from the large SD's. Table 5.6 displays the sum of symptom ratings for individual subjects, ranging from 28 to 220 for autonomic symptoms and 12 to 239 for neuroglycopenic symptoms. The individual sum of symptom ratings was strongly correlated to the individual area under the curve for serum cortisol concentrations in the HYPO experiment ($r = 0.81$ and $r = 0.71$, for autonomic and neuroglycopenic symptom ratings, respectively, $p < 0.05$). Individual inspection

of the data suggest that those subjects with the lowest symptom ratings, also have a cortisol response that are in the lowest end of the range, and vice versa.

Table 5.6: Sum of symptom ratings and area under the serum cortisol response curve for individual subjects during Day 1 hypoglycemia (HYPO) and euglycemia (EU) experiments.

Subjects (n=10)	Sum of Autonomic symptom ratings		Sum of Neuroglycopenic symptom ratings		Cortisol response curve (AUC)	
	HYPO	EU	HYPO	EU	HYPO	EU
A	35	10	33	8	2407	1641
B	57	23	54	27	1907	2235
C	220	16	239	63	5070	2941
D	52	6	73	11	2321	1908
E	82	24	43	4	2994	1156
F	121	51	116	60	3432	1980
G	100	48	31	41	3166	2819
H	28	5	12	1	2331	1739
I	51	2	30	6	3742	2180
J	62	48	48	56	3632	2715
Mean±SD	81±57	23±19	68±67	28±25	3100±931	2132±568
r	0.81		0.71	0.74		

r=significant (p<0.05) correlation between symptom rating and area under serum cortisol response curve.

In addition to rating the various hypoglycemic symptoms (autonomic and neuroglycopenic), subjects were also asked to answer "YES" or "NO" to whether they feel hypoglycemic (individual data presented in Table 5.7). Based on the large number of "NO" answers and relatively low composite symptomatic scores, subjects were for the largest part "unaware" of being hypoglycemic. The subject with the most number of "YES" answers also had the highest composite symptomatic score. However, no clear individual patterns in responses emerged that allowed for statistical comparisons of this data. Nor was there a clear pattern between the hypoglycemic "awareness" and symptomatic data obtained during Day 1 compared with Day 2 exercise performance data (Table 5.7) (performance data are discussed in more detail under section 5.3.4).

Table 5.7: Subjective symptomatic awareness of hypoglycemia during the Day 1 HYPO clamp procedure.

Time	Awareness of hypoglycemia									
	Subjects (n=10)									
	A	B	C	D	E	F	G	H	I	J
0	No	No	No	No	No	No	No	No	No	Yes
40	No	No	No	No	No	No	No	No	No	No
80	No	No	Yes	No	No	No	No	Yes	No	No
120	No	No	Yes	No	Yes	No	No	No	Yes	No
160	No	No	No	No	No	No	No	No	Yes	No
200	No	No	Yes	No	No	Yes	No	No	No	No
240	No	Yes	Yes	No	Yes	Yes	No	No	No	No
Composite symptomatic score out of 114 (max) during the Day 1 HYPO experiment	11	30	49	16	15	35	20	8	14	9
Day 2 Sprint performance after Day 1 HYPO exposure	Improved	Improved	Improved	Reduced	Improved	Reduced	Improved	Improved	Improved	Reduced
Day 2 time-trial performance after Day 1 HYPO exposure	Improved	Reduced	Improved	Reduced	Reduced	Improved	Reduced	Improved	Improved	Improved

Symptomatic awareness of hypoglycemia: In addition to rating the various hypoglycemia symptoms (autonomic and neuroglycopenic), subjects were also asked to answer YES or NO to whether they feel hypoglycemic.

5.3.1.6 Cognitive function data

Difference scores for each cognitive outcome measure are shown in Table 5.8 (and Figure 5.10). Inspection of Table 5.8 reveals that reaction time slowing occurred under the HYPO condition for all 6 outcome variables. However, only the SRT task were significantly reduced compared to baseline ($p < 0.05$). The magnitude of this slowing was mild for the DIVA and LEARN tasks, moderate for the OBK and SRT tasks, large for the ChRT task and very large for the composite score. In contrast, under the EU condition, reaction time became faster on five of six outcome variables (the exception being the SRT task). These changes were all of mild magnitude. Comparison of difference scores between the HYPO and EU experiment using paired t tests revealed significant differences on the ChRT ($p = 0.023$) and composite variables ($p = 0.015$), and an almost significant difference on the OBK task ($p < 0.056$).

Table 5.8. Change from baseline during Day 1 HYPO and EU conditions on 6 cognitive outcome measures.

	EU condition		HYPO condition		p
	Mean change	SD change	Mean change	SD change	
SRT	0.31	0.71	0.95	1.16	0.114
ChRT	-0.04	0.71	1.43	1.86	0.023
OBK	-0.36	1.71	0.74	1.80	0.056
DIVA	-0.33	1.35	0.32	1.86	0.204
LEARN	-0.09	1.01	0.30	1.13	0.376
COMP score	-0.51	2.37	3.74	4.71	0.015

Note: EU = euglycemia; HYPO = hypoglycemia; SRT = simple reaction time task; ChRT = choice reaction time task; OBK = one-back task; DIVA = divided attention task; LRN = learning task; COMP = composite cognitive score.; p = level of significance comparing HYPO to EU experiment.

Performance on the SRT task at the end of the HYPO clamp were positively correlated to autonomic ($r = 0.7$) and neuroglycopenic ($r = 0.79$) symptom ratings ($p < 0.05$). There was no significant relationship between autonomic and neuroglycopenic symptoms and any of the other cognitive function tests. Furthermore, as was found with the symptomatic responses, there was also a strong positive correlation between performance on the SRT and ChRT tasks and the cortisol response (measured as area under the cortisol response curve).

5.3.2 DAY 2: Exercise experiment

5.3.2.1 Plasma glucose, lactate and serum free fatty acid (FFA) concentrations

Plasma glucose concentrations were equivalent at the start of exercise after Day 1 hypoglycemia (4.8 ± 0.3 mmol/L) or euglycemia (4.6 ± 0.4 mmol/L). During the exercise trial (Day 2), plasma

glucose concentrations were similarly maintained at euglycemic levels (>4.5 - 5.5 mmol/L) after Day 1 HYPO or EU exposure (Figure 5.2 A). Furthermore, antecedent exposure to HYPO compared to EU had no effect on the concentrations of plasma lactate or serum FFA at baseline or during exercise (Figure 5.2 B, C).

5.3.2.2 *Hormonal responses*

Serum insulin concentrations were similar at baseline (7.5 ± 5.9 vs. 6.7 ± 5.1 μ U/ml) and displayed a similar decline over time ($p < 0.05$) after Day 1 HYPO vs. EU exposure, respectively (Figure 5.3).

Day 1 HYPO vs. EU exposure resulted in similar serum cortisol concentrations at baseline (~ 17 nmol/L), followed by a similar decline (~ 13 nmol/L) over the first 30 min of exercise, where after it increased similarly towards the end of 90 min of exercise (17.9 ± 4.8 vs. 19.9 ± 6.2 nmol/L), and at the end of the TT (19.8 ± 5.9 vs. 22.4 ± 6.8 nmol/L, HYPO vs. EU, respectively, $p < 0.05$) (Figure 5.4).

When individual cortisol responses for day 2 were assessed, 5 subjects displayed a significant increase in cortisol responses from baseline to the end of the TT (group A), whilst in 5 subjects the cortisol concentrations were similar between baseline and the end of the TT (group B).

5.3.2.3 *Isotopic determination of hepatic glucose output*

There was no significant difference in hepatic glucose output during exercise after HYPO vs. EU antecedent exposure (Figure 5.5). Hepatic glucose output increased from 18 ± 8 μ mol/kg/min over the first 15 min of exercise to 27 ± 9 μ mol/kg/min at the 45 min timepoint in antecedent HYPO compared to virtually no change from 22 ± 8 to 24 ± 5 μ mol/kg/min at equivalent times in the antecedent EU trial. Over the last 45 min of exercise, hepatic glucose output was increased to 29 ± 8 μ mol/kg/min in the antecedent HYPO trial, whilst it was maintained at $\sim 25 \pm 8$ μ mol/kg/min at the end of exercise in the antecedent EU trial (NS between trials).

The rate of glucose disappearance displayed a significant increase over time from the 45 min timepoint onwards up to the end of the 90 min exercise bout (Figure 5.5.) in the antecedent HYPO trial (from 18 ± 8 at 15 min, to 25 ± 9 at 45 min, to 30 ± 6 μ mol/kg/min at 90 min). In the antecedent EU trial, the rate of glucose disappearance showed a similar yet more gradual increase from 19 ± 8 μ mol/kg/min at 15 min of exercise to 29 μ mol/kg/min at 90 min (end) of exercise, and only reached a level of significance (compared to 15 min) at the 90 min timepoint (NS between trials).

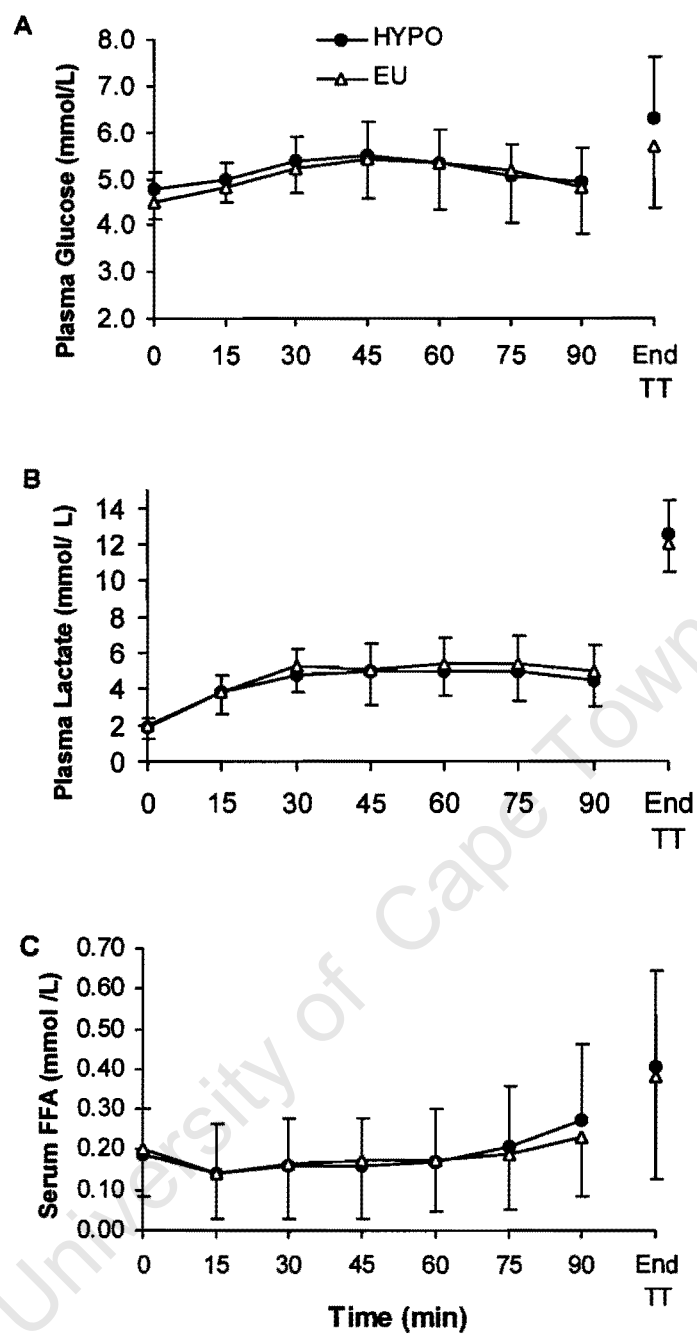


Figure 5.2: Plasma glucose (A), Lactate (B) and serum FFA concentrations during the exercise trial on *Day 2* after *Day 1* hypoglycemic (HYPO) vs. euglycemic (EU) exposure. End TT represent measurements taken at the end of the time trial.

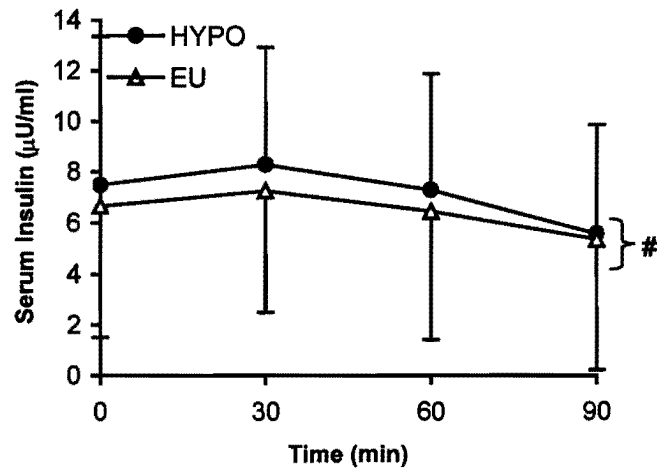


Figure 5.3: Serum insulin concentrations during the exercise trial on *Day 2* after *Day 1* hypoglycemic (HYPO) vs. euglycemic (EU) exposure. End TT represent measurements taken immediately after completion of the time trial (TT). #Significant change from baseline ($p < 0.05$).

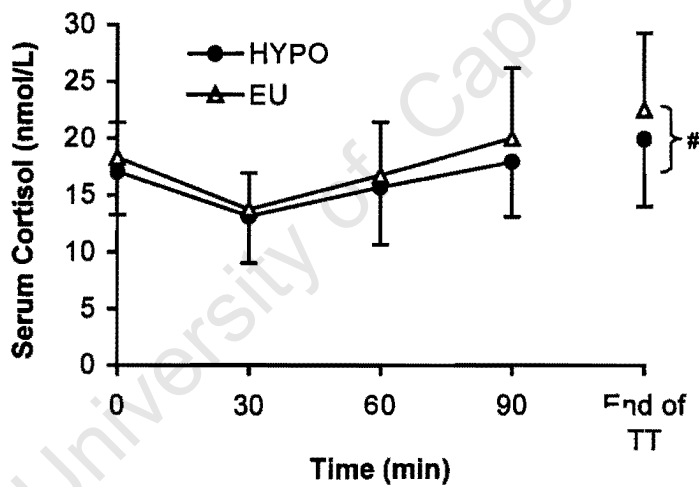


Figure 5.4: Serum cortisol concentrations during the exercise trial on *Day 2* after *Day 1* hypoglycemic (HYPO) vs. euglycemic (EU) exposure. End TT represent measurements taken at the end of the time trial. #Significant change from baseline ($p < 0.05$).

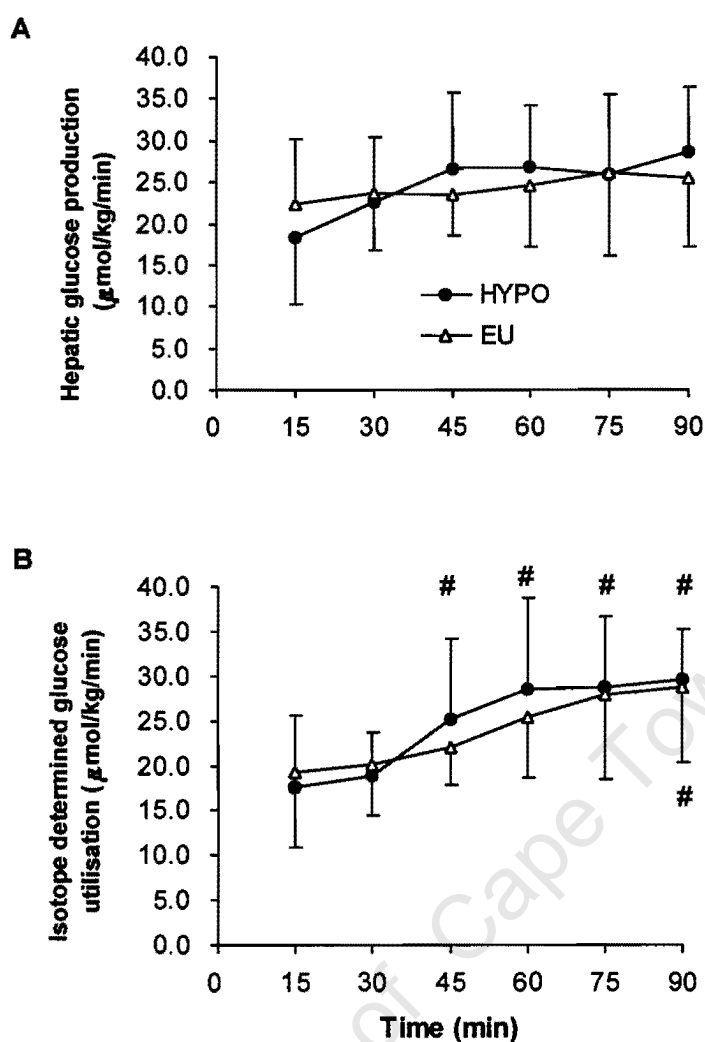


Figure 5.5: Effect of 90 min of exercise at 70% $VO_{2\max}$ on glucose kinetics on Day 2 after Day 1 hypoglycemic (HYPO) vs. euglycemic (EU) exposure. #Significant change from the 15 min value ($p < 0.05$).

5.3.2.4 Gas exchange data and rates of total CHO and fat oxidation

There were no differences in VO_2 , respiratory exchange ratio (RER) or rates of total CHO or fat oxidation during exercise between antecedent HYPO and EU exposures.

5.3.2.5 Exercise performance data

Time-trial performance, measured as time to complete 200 kJ of work, was not significantly different between antecedent HYPO (687 ± 62 sec) compared to EU exposure (696 ± 73 sec).

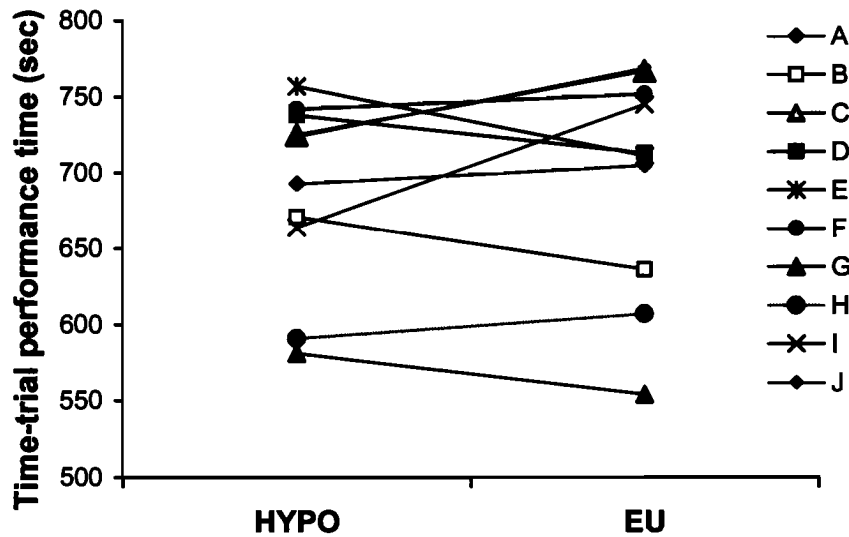


Figure 5.6: Day 2 time trial performance (sec) of individual subjects (n=10) after Day 1 exposure to hypoglycemia (HYPO) compared to euglycemia (EU).

Total sprint time (sum of the 6 sprints, measured as time to complete 10 kJ of work as fast as possible) was not statistically different between antecedent HYPO vs. EU exposure.

Though not statistically significant, n=6 (of 10) TT performance and n=7 subjects' average sprint time performance were improved after the HYPO compared to EU antecedent exposure. Lack of statistical power in this regard is probably due to the much larger magnitude of improvement in TT performance in those 4 subjects who performed better after EU vs. HYPO exposure.

When the performance data are analyzed according to the cortisol groupings (group A and B mentioned under 5.3.2.2), then there was no correlation between the TT performance and cortisol responses between group A (increased cortisol response) and group B (cortisol response unchanged between baseline and end of the TT).

5.3.2.6 Heart rate and RPE

Heart rate responses during the Day 2 exercise experiment were similar between Day 1 HYPO vs. EU exposures (Figure 5.7).

Similar ratings of perceived exertion (RPE) were reported for general, head and legs during exercise after HYPO vs. EU exposure (Figure 5.8).

Table 5.9: Effects of antecedent hypoglycemia (HYPO) and euglycemia (EU) on average sprint time and time-trial (TT) performance the following day.

Subjects (n=10)	Average sprint time (sec)		TT performance (sec)	
	HYPO	EU	HYPO	EU
A	115	122	693	705
B	117	124	671	636
C	129	135	725	767
D	131	94	738	713
E	116	130	757	711
F	148	128	742	752
G	123	126	581	554
H	122	124	591	607
I	99	101	664	745
J	141	133	724	769
	124±14	122±13	689±62	696±73

[‡]=Sum of 6 sprints

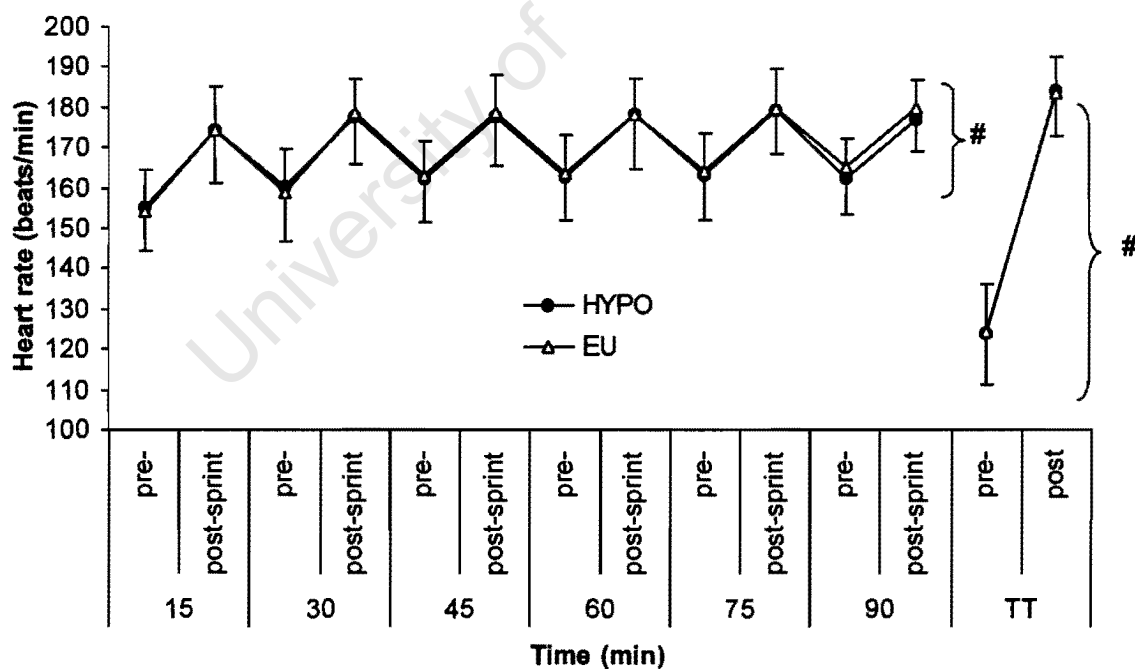


Figure 5.7: Heart rate (beats/min) responses measured before and after the ~20 sec sprints performed every 15 min; and before and after the time-trial (TT). #Significant change from baseline ($p < 0.05$).

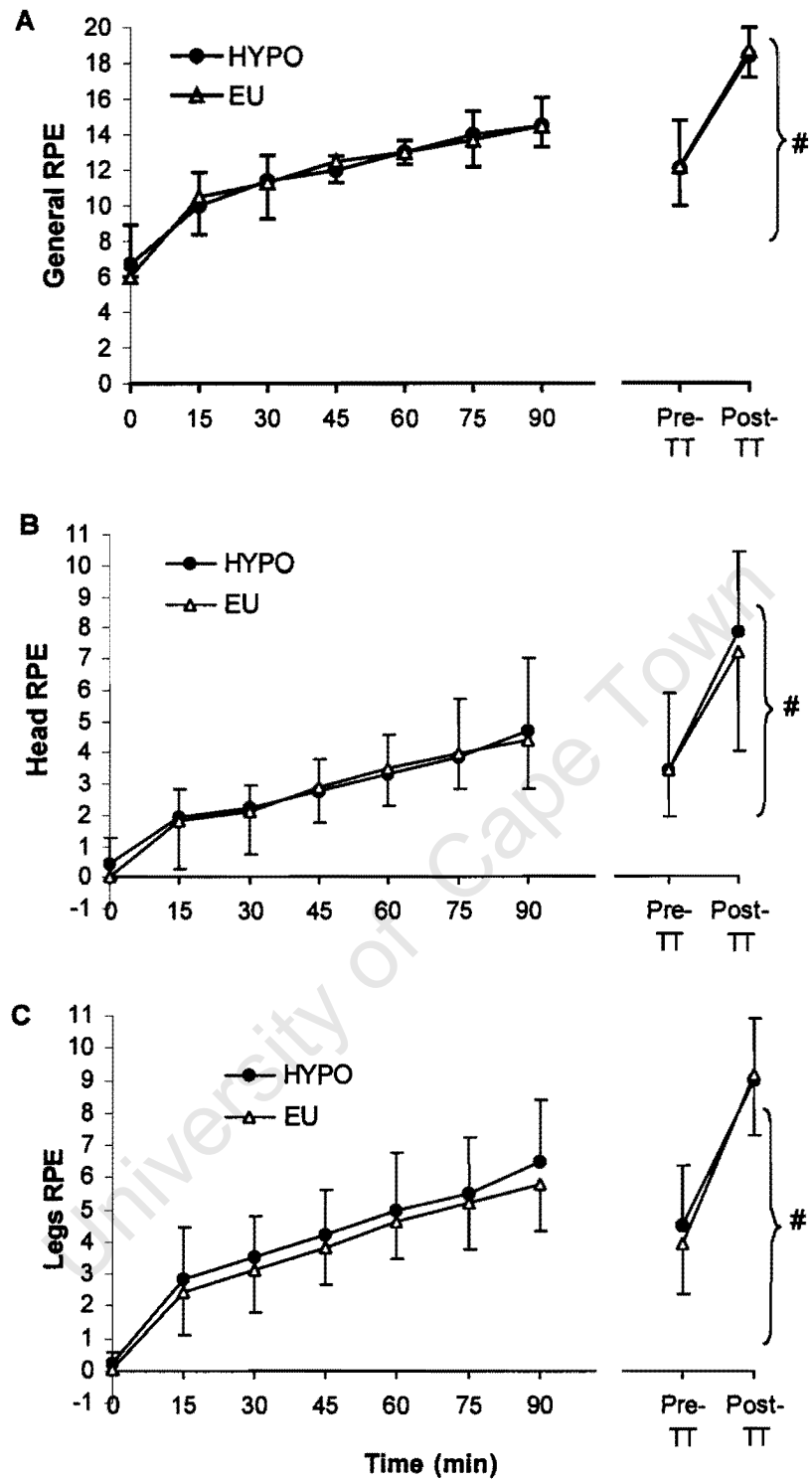


Figure 5.8: Ratings of perceived exertion (RPE) during Day 2 exercise. TT=time-trial. #Significant change from baseline ($p < 0.05$).

5.3.2.7 Cognitive function data

During Day 1 experiments, though performance on all cognitive function tasks showed a decline during HYPO compared to EU exposure, only SRT performance showed a significant reduction compared to baseline performance, and only ChRT performance was significantly reduced at the end of Day 1 HYPO compared to EU exposure (Figure 5.9).

Further inspection of Figure 5.9 reveals that reaction time became faster following Day 2 exercise after both antecedent HYPO and EU exposure (the exception being the SRT task following antecedent hypoglycemia). These changes were all of mild magnitude. Performance on the OBK and LEARN tasks were significantly faster at the end of exercise after Day 1 HYPO compared to baseline; and so was performance on the ChRT, OBK and LEARN tasks at the end of exercise after Day 1 EU compared to baseline ($p < 0.05$). Day 1 antecedent exposure to hypoglycemia compared to euglycemia had no significant impact on cognitive performance tasks performed at the end of exercise on Day 2.

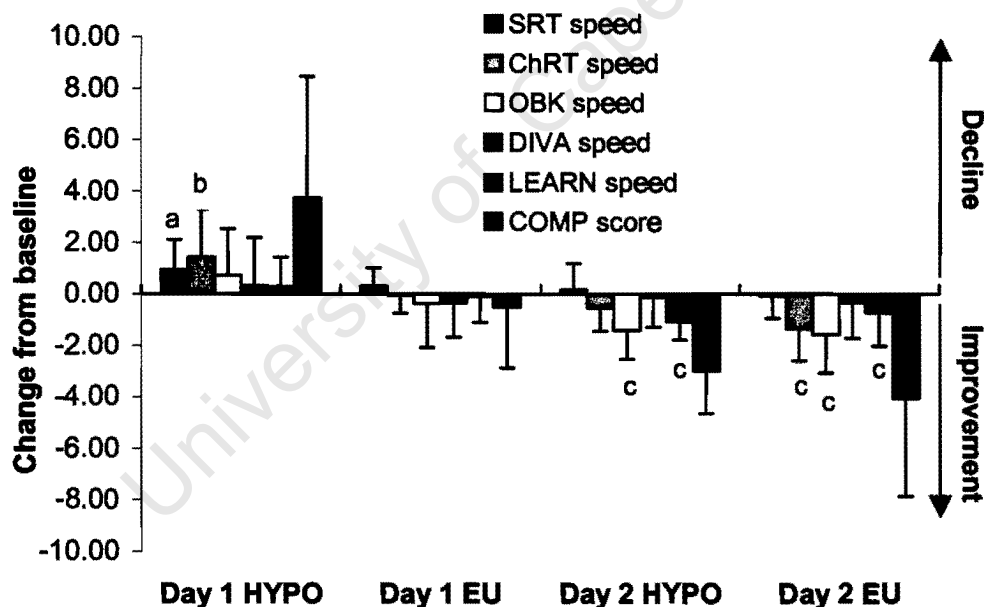


Figure 5.9: Cognitive performance data at the end of Day 2 exercise.

Note: Day 1 = glucose clamping experiments, HYPO = hypoglycemia; EU = euglycemia; Day 2 = Exercise experiment; SRT = simple reaction time task; ChRT = choice reaction time task; OBK = one-back task; DIVA = divided attention task; LRN = learning task; COMP = composite cognitive score; a=Significant decline after Day 1 HYPO clamp compared to baseline; b=significant difference between Day 1 HYPO vs. EU experiment; c=significant improvement after Day 2 exercise experiment compared to baseline, for antecedent HYPO and EU exposure trials, respectively; significance = $p < 0.05$.

5.4 DISCUSSION

The main aim of this investigation was to determine the effects of antecedent hypoglycemic exposure on neuroendocrine and metabolic responses, as well as perceived exertion and exercise performance during moderate to high-intensity exercise performed the following day in well-trained, healthy males. The results indicate that prior, recent exposure to two 80-min bouts of hypoglycemia (and stimulation of the gluco-counterregulatory response) in healthy, well-trained men had no effect on subsequent responses to prolonged exercise, and that the ability to counterregulate and maintain blood glucose homeostasis (in the absence of external blood glucose manipulation) is effectively maintained.

5.4.1 Day 1 blood glucose clamp experiment

In addition to the main aim mentioned above, a secondary aim was to determine whether a second exposure (80-min bout) of moderate hypoglycemia (plasma glucose 2.9 mmol/L) in short succession (40 min) to the first exposure would alter cognitive function and/or symptomatic responses compared to the first exposure. To our knowledge, though other studies have employed similar protocols where two bouts of hypoglycemia (of various durations) were elicited within one day, the symptomatic and cognitive responses thereof have traditionally only been compared to responses during next-day exposure to hypoglycemia^{71,96,443}. These studies reported that prior hypoglycemia had either no effect on thresholds for hypoglycemic symptoms^{71,443} or produced a significant blunting of symptomatic awareness during subsequent exposure (for review see ref.⁹⁶). Additionally, blunting of neuroendocrine responses have been observed whilst cognitive function was preserved^{98,219}. In the present study, each bout of hypoglycemia produced a comparable cortisol response, which was significantly higher than that observed during the euglycemic clamp. Hence, there was no evidence of a blunting effect on cortisol responses. A limitation to the study is that the responses of the other gluco-counterregulatory hormones (glucagon and catecholamines in particular) were not available. These results would have provided a more comprehensive picture of the effect of repeated bouts of hypoglycemia on subsequent counterregulatory responses. However, as shown in previous studies, the effect of the cortisol response seems paramount in the phenomena of counterregulatory failure in healthy humans^{106,107}. In contrast, the present study demonstrated that raised cortisol concentrations during the initial bout of hypoglycemia did not attenuate the cortisol response during the subsequent bout of hypoglycemia.

The present study further indicates that a second 80-min exposure to moderate hypoglycemia, elicited within short (40-min) succession to an identical prior exposure, results in a significant increase over time in autonomic symptom awareness. Furthermore, autonomic symptom scores

were significantly higher at the end of the second bout compared to the first bout of hypoglycemia, whilst neuroglycopenic symptom awareness as well as attentional slowing (indicated by the choice RT cognitive test) were statistically equivalent at the end of the two 80-min exposures to hypoglycemia. These findings are interesting in the light of other studies where prior (previous-day) exposure to hypoglycemia resulted in a blunting of subsequent (next-day) autonomic symptom awareness. The role played by the duration of prior hypoglycemia, the time period between exposures and the differential effects on the magnitude of autonomic and neuroglycopenic symptomatic awareness or "unawareness" thereof needs further investigation.

Neuroglycopenic symptom awareness, on the other hand, was significantly increased above that in the euglycemic trial only after the second 80-min bout of hypoglycemia was induced. This is in agreement with the results by Towler et al.⁴¹⁰ which demonstrated that the awareness of hypoglycemia is largely, perhaps exclusively, the result of perception of autonomic rather than neuroglycopenic symptoms, mediated to a large extent by muscarinic cholinergic mechanisms.

Performance on an attention test (choice RT) completed at the end of 2 consecutive 80-min bouts of hypoglycemia was significantly slower than that observed during both baseline and euglycemic conditions. In contrast, no differences were observed on a psychomotor task (simple RT), suggesting that acute, moderate hypoglycemia affects attention, but not motor speed, in healthy young individuals. These findings are consistent with previous research suggesting that choice RT tasks are more sensitive to exposure to moderate hypoglycemia than simple RT tasks (for discussion see ref.⁸). However, impaired motor function has previously been noted during hypoglycemia^{41,42}, unlike our findings where motor function was unaltered. One explanation for this discrepancy is that the depth of hypoglycemia induced during the two bouts of hypoglycemia in the present study (2.9 mmol/L) was insufficiently low to induce motor impairments. Evans and colleagues¹³⁴ observed that the glucose threshold for motor speed impairment (using the Finger tapping task) was below 2.2 - 2.4 mmol/L during acute hypoglycemia, while others have observed that the blood glucose threshold for dysfunction on choice reaction time tasks to be 2.8-3.1 mmol/L^{133,285}. In the present study, symptoms indicative of neuroglycopenia were significantly increased above that observed in the euglycemic trial only at the end of the second bout of hypoglycemia. Overall, cognitive test results were statistically unrelated to either autonomic or neuroglycopenic symptom responses.

A large inter-individual variability in symptom ratings and cognitive function were noted during the hypoglycemic clamp experiment. This finding is consistent with prior research suggesting that the blood glucose threshold for cognitive impairment during hypoglycemia may vary between individuals¹³⁹, and that prior exposure to hypoglycemia may result in cognitive adaptation¹⁵⁸. A further interesting finding is the strong positive correlation between individual symptom ratings

and individual cortisol responses, indicating that those individuals with the lowest symptom ratings have the lowest cortisol responses (expressed as area under the cortisol curve) and vice versa. This would further lend support to the suggestion that some individuals display altered gluco-counterregulatory hormonal responses combined with altered symptomatic "awareness" of hypoglycemia. However, a larger sample size than that recruited in the present study is required to adequately investigate this hypothesis.

5.4.2 Day 2 Exercise experiment

In this study, the antecedent exposure to two 80-min bouts of moderate hypoglycemia (2.9 mmol/L) compared to euglycemia did not alter the hormonal (insulin, cortisol) and metabolic responses (hepatic glucose production, blood glucose, lactate and FFA concentrations, total CHO and fat oxidation) during 90 min of exercise (at 70% $VO_{2\ max}$) performed the next day. Blood glucose was effectively maintained at euglycemic levels throughout the exercise bout without exogenous manipulation. These results are in contrast to studies suggesting that prior elevations in cortisol may result in blunted subsequent counterregulatory responses in healthy humans^{102,106,107,160,300}. Rather, these results are in agreement with studies showing that prior elevations in cortisol concentrations have no effect on subsequent counterregulatory responses in healthy humans³⁴⁵.

In a similar study to the present, Davis et al.¹⁰² demonstrated that two 2-h episodes of hypoglycemia (2.9 mmol/L) in healthy individuals resulted in a substantial (~50%) reduction in exercise-induced counterregulatory hormone and metabolic responses performed the next day. In this study¹⁰², subjects (n=8 male; n=8 female) underwent (in random order), two bouts of hyperinsulinemic, hypoglycemic clamps (2.9 mmol/L) each lasting 2 h, separated by 2 h of euglycemia, compared to equivalent euglycemic periods (5 mmol/L). The following day, subjects performed 90 min of steady-state exercise at 50% $VO_{2\ max}$, whilst euglycemia was maintained via exogenous glucose infusion. The group exposed to antecedent hypoglycemia displayed significant blunting of neuroendocrine (glucagon, insulin, catecholamine) and metabolic (endogenous glucose production, lipolysis, ketogenesis) responses to exercise and required a 10-fold higher rate of glucose infusion in order to maintain euglycemia during the exercise bout, compared to the antecedent euglycemic experiment¹⁰². The euglycemic glucose infusion during exercise makes it difficult to interpret whether the neuro-endocrine and metabolic responses were caused by, or resulted in the high rate of glucose infusion required to maintain euglycemia.

A subsequent study by the same group¹⁶⁰ further demonstrated that two 90-min antecedent bouts of prolonged exercise (90 min at 50% $VO_{2\ max}$) with euglycemic glucose infusion can

produce a similar reduction in neuro-endocrine and metabolic counterregulatory responses to subsequent (next-day) hypoglycemia (2 h at 2.9 mmol/L).

There may be a couple of explanations for the discrepancy in results between the present study and those of Davis et al.¹⁰². Firstly, there was a substantial difference in training status between the subject populations used in these studies. The subjects of Davis and co-workers^{102,160} ranged from sedentary to actively participating in competitive sports, with an average $\text{VO}_{2\text{ max}}$ of 43 ± 3 ml/kg/min (range 21 to 54), whilst subjects in the present study constituted a homogenous group of lean (9 ± 3 % body fat), endurance-trained cyclists with an average $\text{VO}_{2\text{ max}}$ of 64 ± 3 ml/kg/min (range 60 to 70), indicative of a superior training status. Previous studies have demonstrated that highly trained individuals have enhanced insulin sensitivity (for review see³⁴) and an altered sympathetic and parasympathetic activation in consequence to frequent strenuous exercise bouts^{62,266}. Furthermore, training status have been shown to affect metabolic responses, amongst these are an increased ability to oxidize fat combined with a decreased dependency on the limited CHO fuel stores^{15,179,218}.

Secondly, subjects in the present study exercised at a higher intensity (70% as opposed to 50% $\text{VO}_{2\text{ max}}$ ¹⁰²), and performed a maximal sprint (lasting 15-20 sec) every 15 min. Exercise intensity in itself can strongly activate the sympathetic system¹ and as a result, independently activate the counterregulatory system and enhance blood glucose regulation and maintenance²⁶⁶.

Lastly, an important difference between these studies is that Davis et al.¹⁰² infused glucose to maintain euglycemia during the exercise bout whilst in the present study we chose not to infuse glucose in order to assess the responses under physiological conditions when blood glucose was left free to vary. When exogenous glucose is infused, it seems likely that the normal neuroendocrine and metabolic responses involved in glucose counterregulation would not be elicited (as it is not needed). This could explain the 50% reduction observed in counterregulatory responses¹⁰². An alternate explanation for the near 10-fold higher rate of glucose infusion needed after hypoglycemic compared to euglycemic exposure, may be related to an enhanced insulin sensitivity and glucose uptake by the periphery (muscles) in consequence to hypoglycemia, and not because of counterregulatory failure *per se*. It is interesting to speculate that in highly trained individuals (as in the present study), where insulin sensitivity is already increased and glucose uptake during exercise already optimized (above threshold)^{449,450}, the effect of antecedent hypoglycemic exposure to potentially increase glucose disposal may be less pronounced, and could hence explain the similar responses seen during exercise in the present study after antecedent hypoglycemic compared to euglycemic exposure.

In addition, the higher exercise intensity in the present study may have been sufficient to fully activate the sympathetic system¹, thereby activating the counterregulatory system (as reflected by similar neuroendocrine and metabolic responses between antecedent HYPO and EU exposures) and similar maintenance of blood glucose during subsequent exercise in the present study.

Measurement of cognitive function (such as choice and simple RT) has been shown to be impaired during exposure to hypoglycemia¹³⁵, but a single episode of mild antecedent hypoglycemia (3.1 mmol/L) in healthy men can attenuate several aspects of cognitive dysfunction during subsequent hypoglycemia 18-24 h later¹⁵⁸. In contrast to this finding, the present study demonstrated that cognitive function at the end of the exercise protocol performed within 24 h after antecedent hypoglycemic exposure was improved. This finding is in agreement with evidence indicating that prolonged exercise may be associated with an increase in cognitive performance, possibly due to increase in metabolic load which is associated with increased arousal, as well as a possible increase in cerebral blood flow, or neurotransmitter (catecholamines / endorphin) release, however, the exact mechanism is still unknown^{46,46}.

Grego et al.¹⁷⁷ investigated the effect of prolonged exercise (3 h at ~66% VO_2_{max}) on hormonal, metabolic and cognitive function in trained male cyclists. Exercise resulted in a progressive increase in FFA, heart rate and RPE (after the 108 min), and glycerol, epinephrine and cortisol (after 144 min) and a significant decrease in blood glucose (after 108 min) and insulin (144 min). However, blood glucose remained above 4 mmol/L. The results showed a temporary increase in P300 amplitude between the 1st and the 2nd hour (an indication of an improvement in cognitive function) and an increase in latency after the 2 h of exercise concomitant with some hormonal changes, including an increase in cortisol and epinephrine and a decrease in blood glucose (an indication of an alteration of information processing speed with fatigue). These findings suggest a combined effect of arousal and central fatigue on electrocortical indices of cognitive function during acute physical exercise¹⁷⁷. Very recently, Grego et al.¹⁷⁸ examined the influence of 3 h of cycling at 60% VO_2_{max} on simple and complex cognitive performance in 8 well-trained male subjects before, every 20 min during, and immediately after the exercise task. A significant improvement in speed of response and a decrease in error number during the map recognition task were recorded between 80 min and 120 min when compared with the first 20 min of exercise. After 120 min the number of recorded errors was significantly greater indicating a shift in the accuracy-speed trade-off. These results provide some evidence for exercise-induced facilitation of cognitive function. However this positive effect disappears during prolonged exercise - as evidenced by an increase in errors during the complex task and an alteration in perceptual response (i.e. the appearance of symptoms of central fatigue).

During prolonged exercise, apart from marked increases in metabolic load, symptoms of central (e.g. reduced neural drive) and peripheral (e.g. decreased muscle excitability) fatigue are also commonly reported¹⁷⁷, which may negatively affect cognitive functioning⁴⁶ and attenuate the perception of effort towards the latter stages of prolonged exercise⁴²⁰. However, it is yet unclear what relationship exists between cognitive function, metabolic and hormonal responses, perceived exertion and endurance exercise performance. In addition, it is not yet clear how cognitive function may be affected at the end of prolonged exercise and after prior recent exposure to hypoglycemia (which may alter neuro-endocrine, metabolic, and cognitive responses) in well-trained men. In the present study, antecedent exposure to hypoglycemia compared to euglycemia had no effect on cognitive functioning or effort perception. It may be that any likely effect of antecedent hypoglycemia on cognitive functioning at the end of the exercise bout on day 2 were masked by the overall effect of exercise on improving cognitive function, however, this remains speculative.

Furthermore, there was no association between the perception of effort, cortisol response and exercise performance. Similar to the present study, Viru et al.⁴²⁸ reported that 2 h of running exercise increased the responsiveness of components of the HPA-axis (such as cortisol response) in some subjects, whilst it remained unchanged in others. As a whole, power output measured in a 1-min maximal test performed before and after the 2 h run was not different. However, the higher cortisol responses in the subgroup of subjects were associated with increased performance (power output), whilst power output was significantly lower after compared to before the 2 h run in the group where cortisol concentrations were the same or lower before and after the 2 h run. This may indicate that increased HPA-axis activation is related to a strong nerve influence on the muscle recruitment and performance⁴²⁸. However, in contrast to these findings, the present study found no relationship between TT performance and cortisol responses. In addition, similar to the findings of Viru et al.⁴²⁸, RPE increased progressively over the exercise period, but no relationship between overall or individual cortisol and RPE responses were found. Differences in mode and duration of exercise protocols may explain the discrepancy in results, however, more research is needed to further investigate the potential link between individual responsiveness in HPA-axis activation, RPE and exercise performance.

In conclusion, in overnight-fasted, healthy, well-trained individuals recent antecedent exposure to hypoglycemia and elevation in cortisol concentrations have no effect on counterregulatory responses to subsequent prolonged exercise performed at 70% $\text{VO}_{2 \text{ max}}$, nor is RPE or exercise performance affected.

Acknowledgements: I wish to give special thanks to Sacha West for her assistance during testing throughout this study, as well as to Dr. Jeroen Swart, Zig Gibson, Ryan Jankelowitz and Ryan Kohler for providing medical supervision at various stages of testing.

CHAPTER 6

IL-6 RESPONSE DURING EXPOSURE TO TWO PROLONGED BOUTS OF HYPOGLYCEMIA WITHIN ONE DAY FOLLOWED BY PROLONGED EXERCISE THE NEXT DAY

University of Cape Town

6.1 INTRODUCTION

Traditionally, fatigue during exercise has been attributed mainly to physiological and biochemical changes such as metabolite (lactate) accumulation¹⁴⁸, substrate (glycogen) depletion^{148,240} and pH changes¹⁴⁸. Though a strong association between CHO (liver and muscle glycogen) depletion and fatigue development during prolonged submaximal exercise has been observed, the exact mechanisms regarding CHO regulation and its role in fatigue development is still not fully understood.

Recent evidence indicate that interleukin-6 (IL-6) may play a role as a possible regulator of blood glucose homeostasis and fatty acid mobilization during exercise^{143,424}. IL-6 concentrations have been shown to markedly increase during exercise and skeletal muscle has been identified as the major source of this IL-6 production^{143,336}. The brain³²⁷, peritendon²⁶⁸ and adipose tissue²⁴⁴ have also been identified as sites of IL-6 production in response to exercise. Helge et al.²⁰¹ recently demonstrated that the amounts of IL-6 released from contracting skeletal muscle was positively related to work intensity, glucose uptake and plasma epinephrine concentrations. Furthermore, an inverse correlation between IL-6 release and muscle glycogen content has also been observed^{140,201,397,399}. From these observations, it has been suggested^{170,400} that IL-6 may serve as an energy status hormone in muscle in response to exercise, acting as a signal to the liver or adipose tissue to increase glycogenolysis or lipolysis, respectively, thereby contributing towards glucose homeostasis^{244,246}.

It has been proposed that IL-6 can act in a "hormone-like" manner by regulating glucose homeostasis during exercise¹⁴⁰. Studies have shown that IL-6 can partly mediate the hepatic glucose output necessary to maintain blood glucose homeostasis during exercise³⁹⁴. IL-6 is a potent activator of the hypothalamic-pituitary adrenal (HPA) axis³³⁵, but does not simply mediate blood glucose concentrations through its effect on glucose-regulating hormones. The liver is capable of producing IL-6, which seems to have a regulatory function by directly acting on hepatocytes to increase hepatic glucose release³⁵⁷. Thus, the liver may produce IL-6 to stimulate hepatic glucose output if blood glucose concentrations are compromised. In addition, it has been shown that IL-6 induces an increase in appearance and disappearance of free fatty acids (FFA)²⁴⁴, and it has been suggested that the increased IL-6 production in adipose tissue late in exercise may provide a link from the contracting skeletal muscle to enhance fat metabolism, thereby contributing FFA oxidation to the energy demand (at a time when glycogen stores are reduced).

Furthermore, data obtained from a performance related study has indicated that IL-6 may play a role in affecting the sensation of fatigue during exercise³⁵⁹. In this study, recombinant human IL-

6 was administered subcutaneously prior to a 10 km running time-trial in amounts equivalent to those produced during 2 hrs of running (9 ± 2 pg/ml vs. baseline concentrations of 1 ± 0.5 pg/ml)³⁹⁴. With IL-6 infusion (compared to placebo), subjects reported an increased sensation of fatigue, which ultimately resulted in a decrement in running performance. This has led to the suggestion that IL-6 may possibly act as a circulating "fatiguogen" during exercise. In recent years, the role of the central nervous system (CNS) in the development during exercise has received increasing attention⁵⁹. It is suggested that afferent signals relating the physiological and metabolic status of the body are relayed back to the CNS. Based on these signals, efferent signals from the CNS / brain are related back to the appropriate bodily structures, and in respect to exercising skeletal muscle, result in a de-recruitment of skeletal muscle (reduced exercise capacity) in order to maintain a homeostatic environment¹⁶³. Exercise induced changes in various brain neurotransmitters (serotonin, dopamine and acetylcholine) and neuromodulators (ammonia and various cytokines such as IL-6) have been suggested to be possible mediators in the onset of exercise related fatigue⁵⁹.

The hypothesis of the role of IL-6 as a possible "fatiguogen" is further supported by the findings of Spath-Schwalbe et al.³⁹⁰ where low doses of rh-IL-6, were administered to healthy individuals at rest and the elevated IL-6 levels were associated with increased cortisol and adrenocorticotrophic hormone (ACTH) concentrations and an increased heart rate. In addition, subjects reported an increased sensation of fatigue, altered ability to concentrate and disrupted sleep patterns. Furthermore, when an IL-6 antagonist (humanized anti-IL-6 receptor antibody) was administered to patients diagnosed with IL-6 related immune-inflammatory diseases (Castleman's disease and rheumatoid arthritis) subjects reported an immediate attenuation of fatigue symptoms³²⁰. Changes associated with elevated levels of plasma IL-6 include malaise, fatigue, elevated levels of adrenocorticotrophic hormone and cortisol, and increases in heart rate and core temperature^{217,351,417}. It has therefore, at least in part, been suggested that cytokines, and in particular IL-6, may play a role in the development or perception of fatigue.

Recent exposure to a stressful event such as hypoglycemia and exercise have been shown to alter (blunt) neuro-endocrine and metabolic glucose counterregulatory responses to following (next-day) exposure to a similar stress^{102,160,205}. Elevated IL-6 concentrations are also associated with increased cortisol and adrenocorticotrophic hormone (ACTH) concentrations, yet it is unknown if and how IL-6 responses are affected by antecedent and subsequent exposures to physiological stresses such as hypoglycemia and exercise.

Accordingly, the aim of this study was to determine the effect of an antecedent bout of hypoglycemia on IL-6 and glucose counterregulatory hormone responses, metabolism, liver

glucose output, perception of effort and endurance performance during a subsequent (next-day) bout of endurance exercise.

6.2 METHODS

(As explained in detail in CHAPTER 5)

6.2.1 Subjects

Nine of the 10 subjects reported in Chapter 5 were included in the investigation of IL-6 responses. One subject's data were lost during the IL-6 analysis procedure and were therefore excluded from the analysis. Subject characteristics (n=9) are presented in Table 6.1.

6.2.2 Research design

Similar to that reported in Chapter 5. In brief, each subject completed two separate, randomized, single-blind, experimental trials, repeated at least one month apart. Each experimental trial consisted of two consecutive days of testing. Each day of testing commenced after a 10-h overnight fast. On day 1, subjects were either exposed to hypoglycemia (HYPO) or, on the other occasion, euglycemia (EU) by means of a similar hyperinsulinemic glucose clamp procedure. On day 2, subjects performed 90 minutes of cycling exercise at 70% of $VO_{2\max}$ followed by a cycling time-trial (TT) consisting of time to complete 200 kJ of work. No blood glucose manipulations were done during day 2. The day prior to each round of testing, subjects were required to abstain from exercise, and to keep a detailed food diary, which were to be repeated the day prior to the second experimental trial. During the course of the study subjects were instructed to maintain their habitual training schedule and weight-maintaining diet.

6.2.3 Preliminary testing

As reported in detail in Chapter 5.

6.2.4 Experiment proper

6.2.4.1 DAY 1: Blood Glucose Clamping Procedure

As reported in detail in Chapter 5.

6.2.4.2 DAY 2: Exercise experiment

As reported in detail in Chapter 5. In brief, the next morning after the glucose clamp procedure, subjects again reported to the laboratory after a 10-h overnight fast. One cannula (Jelco; Johnson and Johnson, Halfway House, South Africa) was placed in a forearm vein to enable blood sampling. A second cannula was placed in the contralateral arm to enable infusion of the [$3\text{-}^3\text{H}$]glucose tracer. A primed, continuous infusion of [$3\text{-}^3\text{H}$]glucose was started -45 min prior to the start of exercise (whilst subjects remained rested), and continued for 90 min during steady-state exercise in order to measure hepatic glucose output.

The exercise protocol consisted of 90 min of steady-state cycling at 70% $\text{VO}_2\text{ max}$, with sprints performed every 15 min (after measurements of the various parameters (see below) were performed). Each sprint consisted of the time to complete 10 kJ of work ($\sim 15\text{-}20$ sec). At the end of the 90 min of cycling, subjects were given 2 minutes to rest followed by a cycling time trial consisting of time to complete 200 kJ of work. Subjects were allowed to ingest water *ad lib*, and were cooled by using an electric fan. Environmental conditions in the laboratory were controlled ($20\text{ }^\circ\text{C}$).

6.2.4.2.1 Metabolic measurements

As reported in detail in Chapter 5. In brief, venous blood, on-line respiratory gas exchange and RPE determinations were done at baseline, at 15 min intervals during steady-state cycling (before performing the sprint), and at the end of the TT. Blood samples were collected and analyzed (as explained in Chapter 5) for plasma glucose, [$3\text{-}^3\text{H}$]glucose, and lactate; and serum IL-6, FFA, cortisol and insulin concentrations. Serum IL-6 concentrations were measured using high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA).

6.2.5 Statistical Analyses

The statistical software package STATISTICA 7.0 (2004; StatSoft, Inc., Tulsa, OK, USA) was used for the statistical analysis of the data. All results are presented as means \pm SD. Statistical significance ($p < 0.05$) of between-group differences was assessed by a two-way analyses of variance (ANOVA) for repeated measures over time during the blood glucose clamp procedure and exercise protocol. In order to determine which means were significantly different, Tukey's honest significance difference post hoc analysis was used. Paired *t* Tests were performed for comparisons between groups of variables at the end of the time trial.

6.3 RESULTS

Table 6.1: Subject characteristics

n=9	
Age (yrs)	25±5
Weight (kg)	73±8
BMI	23±1
% Body fat	9±3
W_{peak} (Watts)	367±27

Means±SD.

IL-6 concentrations during the euglycemic (EU) trial (Figure 6.1) remained at baseline concentrations (~0.5 pg/ml) up to 160 min, where after it displayed a significant increase (~1.0 pg/ml) towards the end of the trial (240 min; $p=0.05$). During the hypoglycemic (HYPO) clamp, IL-6 concentrations increased progressively over the first 120 min (the end of the first HYPO exposure), then remained at this level (~1.0 pg/ml) throughout the following 40 min (when euglycemia was restored) and throughout the second HYPO exposure up to the end of the trial (240 min). There was a significant ($p<0.05$) interaction (trial x time) effect between the HYPO and EU trial.

IL-6 concentrations were similar at the start of the exercise trial on Day 2 following antecedent HYPO vs. EU exposure on Day 1, and displayed a similar significant increase over time towards the end of exercise ($p<0.05$). IL-6 concentrations at the end of the TT tended to be lower after Day 1 antecedent HYPO exposure, yet insignificantly so. This result is interesting in the light that the blood glucose concentration was higher at the end of the TT after antecedent HYPO exposure.

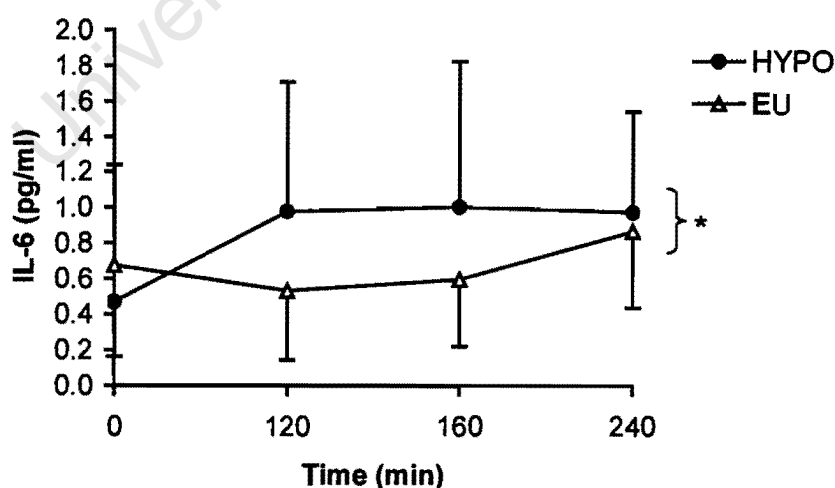


Figure 6.1: Serum IL-6 concentrations from arterialized venous blood during the hyperinsulinemic blood glucose clamp (Day 1). n=9. * Significant trial x time effect ($p=0.03$).

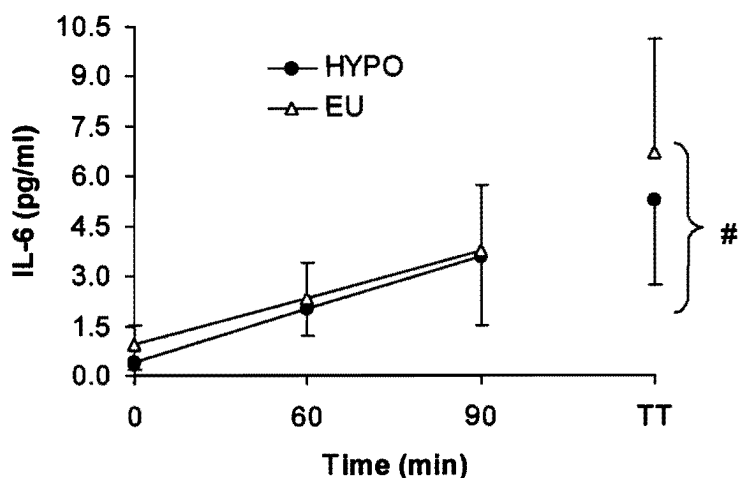


Figure 6.2: IL-6 concentrations during steady-state exercise and at the end of the time-trial (Day 2). n=9. # Significant increase (from baseline) over time in both trials ($p < 0.05$).

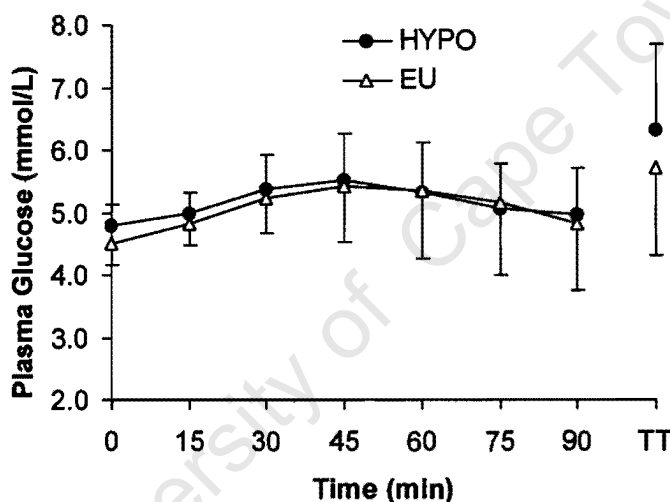


Figure 6.3: Plasma glucose concentrations during exercise (Day 2). n=9.

Blood glucose concentrations were similar between trials and remained within the euglycemic range throughout the exercise protocol. Blood glucose concentrations at the end of the TT were higher compared to any point during the 90 min steady-state cycle protocol, most likely due to the known effect of increased glucose production during/immediately after a high-intensity exercise bout. Though the general trend was for the blood glucose concentration at the end of the TT to be lower following antecedent EU exposure, it was not significantly different compared to antecedent HYPO exposure.

As a whole (mean data from group), the altered IL-6 responses during antecedent HYPO vs. EU exposure on Day 1 had no effect on the rate of hepatic glucose output, cortisol, insulin, FFA or lactate concentrations or overall rates of CHO and fat oxidation (determined from RER) during the Day 2 exercise experiment. Furthermore, RPE, sprint and TT performance were unaffected by antecedent HYPO vs. EU exposure (data presented in Chapter 5). IL-6 concentrations at the end of the TT were significantly correlated to cortisol concentrations at the end of the TT ($r=0.77$, $p<0.05$).

Interestingly, in all parameters presented (IL-6, plasma glucose), the individual range in response was large. When looking at individual IL-6 and blood glucose responses, the one subject (subject I) with the highest IL-6 response during exercise also displayed the highest plasma glucose concentrations, showing increases of above 6 mmol/L beyond 60 min of exercise towards 12 mmol/L at the end of exercise (Figure 6.4 A & B; Figure 6.5 A & B).

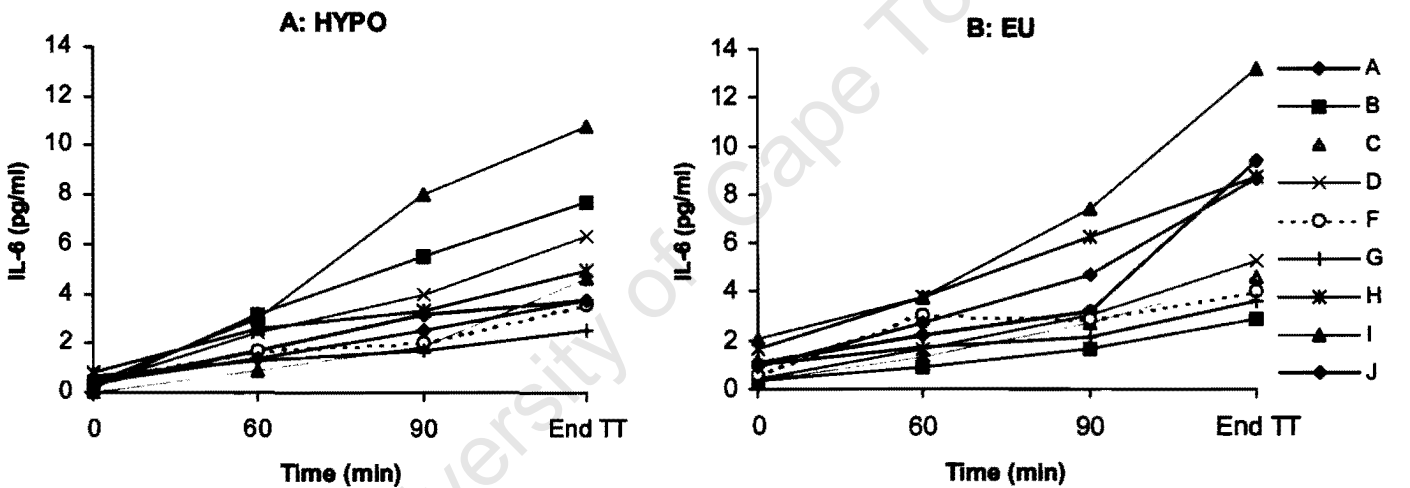


Figure 6.4 A & B: Individual IL-6 response during exercise (Day 2) after the antecedent HYPO (A) and EU (B) exposure.

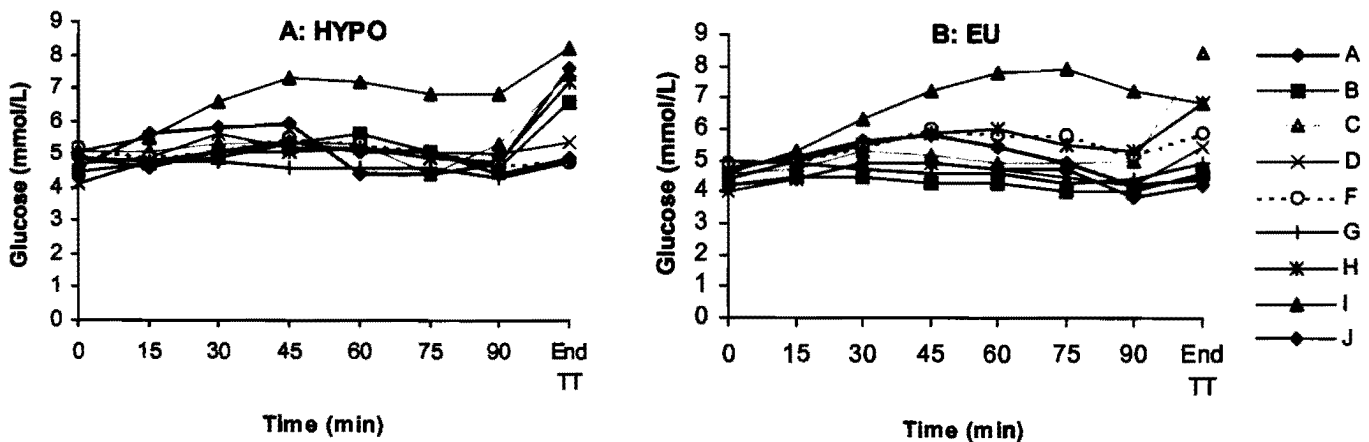


Figure 6.5 A & B: Individual plasma glucose response during exercise (Day 2) after the antecedent HYPO (A) and after EU (B) exposure.

6.4 DISCUSSION

Exposure to hypoglycemia elicited a significant increase in IL-6 concentrations compared to euglycemic exposure. However, the IL-6 response during moderate- to high-intensity exercise appears to be unaltered by recent, antecedent exposure to hypoglycemia.

Exposure to any stressful event provides a “challenge” to the HPA-axis with a resultant increase in stress hormones such as cortisol and catecholamines. With respect to hypoglycemia, these hormones act to help restore blood glucose concentrations by increasing hepatic glucose output (e.g. increase gluconeogenesis). IL-6 has also been shown to be a powerful activator of the HPA-axis³³⁵, and to play a role in glucose homeostasis^{143,337,424}. To our knowledge, the present study is the first to document the IL-6 response during two consecutive bouts of hypoglycemia at rest in healthy well-trained men. In accordance with this literature, the present study demonstrated that the first 80-min bout of hypoglycemia produced a significant cortisol response during exposure to hypoglycemia (HYPO trial; data presented in Chapter 5). This is in contrast to findings by Davis et al.¹⁰⁹ who demonstrated that one episode of 2-h hypoglycemia (2.9 mmol/L) in the morning produced substantial blunting of the cortisol and other associated neuroendocrine responses to a subsequent 2-h bout of hypoglycemia elicited 2 h later in healthy men. In the present study, the cortisol response was accompanied by a significant systemic IL-6 response over the initial 120 min, which then reached a plateau at ~1 pg/ml, and were maintained at this level throughout the second 80-min bout of hypoglycemia.

Recent antecedent exposure to raised glucocorticoids, such as elicited by prior stress such as hypoglycemia^{102,106,109} or exercise¹⁶⁰ have been shown to alter (blunt) the symptomatic and glucose counterregulatory hormone response (e.g. blunted cortisol release) to next-day exposure to a similar stress^{102,160,205}. In fact, it has recently been demonstrated that any stress that can cause prior elevations of cortisol and activation of the HPA-axis can produce blunted responses to subsequent hypoglycemia in healthy men^{102,106,106,107,160,205,300}. In contrast to these findings, our results demonstrated that antecedent exposure to hypoglycemia (compared to euglycemia) and the concomitant elevation in cortisol and IL-6 had no effect on subsequent metabolic, hormonal or IL-6 responses during next-day exercise (~2 h at 70% $\text{VO}_2 \text{max}$) in healthy, well-trained men.

In the present study, serum IL-6 concentrations increased progressively during the 90 min exercise bout, with a further increase at the end of the TT. This is in agreement with the findings from Helge et al.²⁰¹ and Febbraio et al.¹⁴⁰ where it was shown that IL-6 release from the human muscle is positively related to exercise intensity. In the study of Helge et al.²⁰¹ seven untrained healthy males performed knee extension exercise, kicking with both legs, each at 25% of

maximal power output for 45 min and then simultaneously with one leg at 65% and the other leg at 85% maximal power output for 35 min. Blood was sampled from a femoral artery and both femoral veins, and blood flow was determined by thermodilution. Thigh IL-6 release was positively related to exercise intensity, plasma epinephrine concentration and muscle glucose uptake, whilst IL-6 release during the final 35 min of exercise was inversely related to the postexercise glycogen concentrations. Though muscle glycogen and catecholamine concentrations were not measured in the present study, it is likely that the 90 min of exercise, combined with ~20 sec sprints every 15 min, followed by the TT resulted in some degree of muscle glycogen depletion, which may have, in part, contributed to the increased IL-6 concentrations towards the end of the exercise trial.

Furthermore, the increase in IL-6 concentrations in the present study may have contributed to the maintenance of euglycemia seen throughout the exercise experiment. IL-6 have been suggested to contribute to glucose homeostasis by directly acting on hepatocytes to increase hepatic glucose output³⁵⁷, and/or to stimulate the HPA-axis and increase glucoregulatory hormones^{291,335,398}. These findings are supported by the strong relationship found in the present study between the progressive increase in cortisol and IL-6 concentrations towards the end of the exercise experiment.

The findings from the present study indicated that recent antecedent exposure to antecedent hypoglycemia and stimulation of the IL-6 response and HPA-axis did not alter subsequent IL-6 and cortisol responses, nor the tracer-determined rate of hepatic glucose output during subsequent exercise. Though the glucose rate of appearance appeared to be higher during the last 45 min of exercise in the antecedent HYPO trial, it was not significantly increased above the starting value nor was it significantly different compared to the antecedent EU trial. An interesting observation was that the glucose rate of disappearance (Rd), on the other hand, increased significantly over time from 45 min onwards in the antecedent HYPO trial. Though Rd showed a similar increase in the antecedent EU trial over the 90 min exercise period, it appeared that there was more of a delay in increase compared to the antecedent HYPO trial, and a significant increase compared to the 15 min exercise value was only established towards the end of the 90 min exercise period. Hence, the increase in Rd showed a co-incident increase with IL-6 concentrations during the exercise period, however, this relationship was not significant. This co-incident relationship between glucose Rd and circulating IL-6 concentrations is in agreement with the findings from Helge et al.²⁰¹ who demonstrated a strong relationship between IL-6 release by the thigh and glucose uptake. It is also in agreement with recent findings by Febbraio et al.¹⁴⁰ where IL-6 infusion (matching circulating IL-6 concentrations seen with exercise at 70% $VO_{2\text{ max}}$) during 2 h of cycling at 40% $VO_{2\text{ max}}$ increased whole-body glucose disposal compared to exercise at 40% $VO_{2\text{ max}}$ without IL-6 infusion. The authors suggested that an increase in glucose

disposal may indeed be the primary effect of IL-6, and that the increase in endogenous glucose production may be secondary to the increase whole-body glucose disposal¹⁴⁰.

The progressive increase in circulating IL-6 in the present study did not result in a significant increase (over time) in hepatic glucose production during 90 min of exercise. In the recent study of Febbraio et al.¹⁴⁰ increase in glucose production and clearance were shown to be unrelated to changes in the hormonal milieu, hence, concentrations of insulin, glucagon, catecholamines and cortisol were identical with and without IL-6 infusion at 40% $\text{VO}_2 \text{ max}$. However, the concentrations of these hormones were significantly higher during exercise at 70% $\text{VO}_2 \text{ max}$ (without IL-6 infusion), indicating that exercise *per se* elicits the release of glucoregulatory hormones unrelated to IL-6 responses. This again highlights the fact that glucose homeostasis during exercise encompass a complex interplay of several regulatory responses, dictated to a large degree by the exercise intensity and duration.

In conclusion, the results from the present study indicate that prior exposure to hypoglycemia and stimulation of the IL-6 and cortisol response in healthy, well-trained men has no effect on metabolic and neuroendocrine responses to a subsequent bout of hypoglycemia, nor to exercise performed within 24 h, and that the ability to regulate blood glucose homeostasis is effectively maintained.

CHAPTER 7

SUMMARY AND CONCLUSIONS

University of Cape Town

The main aims of this thesis were to investigate the effect of altered endogenous glycogen availability, mainly achieved by pre-exercise dietary manipulation and antecedent exercise exposure, on inter-individual variability in metabolic and neuro-humoral responses to dynamic, steady-state exercise. Further, this thesis examined the impact of altered glucose availability and neurohormonal responses on fatigue development during prolonged exercise. We hypothesized that endogenous CHO availability and the associated metabolic sequelae would impact on effort perception during exercise and fatigue development. We further hypothesized that prior fatiguing exercise and local muscle glycogen depletion would provide some neural and/or humoral signal, thereby altering resistance to fatigue. Finally we proposed that antecedent exposure to hypoglycemia will reduce neuroendocrine and metabolic responses during subsequent (next-day) prolonged exercise, and that this would translate into reduced effort perception and improved exercise performance.

In the first study we examined the effect of varying the glycemic index (GI) of the pre-exercise carbohydrate-rich meal, combined with carbohydrate (CHO) ingestion during exercise on metabolism and endurance exercise performance. Varying the GI of the preexercise CHO-rich meal is known to alter substrate availability and hormonal responses (insulin in particular), and has been proposed to be of potential benefit to athletes in manipulation of their exercise metabolism and performance. We demonstrated that despite differences in preexercise glucose, insulin and free fatty acid (FFA) concentrations between trials, as soon as exercise commenced, these metabolic and hormonal effects were negated. Furthermore, varying the GI of the pre-exercise meal had no distinguishable effect on tracer-determined rate of exogenous CHO oxidation, nor respiratory exchange ratio (RER), nor was exercise performance or ratings of perceived exertion (RPE) altered. It was concluded that when CHO is ingested during endurance exercise in amounts recommended by sports nutrition guidelines, then varying the GI of the preexercise CHO meal and the concomitant metabolic and hormonal responses it elicits have no effect on endurance (~2.5 h) exercise metabolism or performance.

The third study aimed to examine the role of local muscle glycogen depletion on fatigue development. A single limb exercise model was used to explore whether fatigue development, combined with muscle glycogen depletion and the concomitant metabolic and neurohumoral responses elicited during exercise with the first limb but circulating throughout the whole body, affect exercise capacity of the second limb. Exercise time to fatigue was significantly shorter with Leg 2 compared to Leg 1, whilst the muscle glycogen content at exhaustion in Leg 2 was significantly higher than in Leg 1. Plasma glucose concentrations at the point of fatigue were variable and there was no clear pattern to suggest that fatigue development was related to hypoglycemia. Catecholamine concentrations were higher at the start of exercise with Leg 2 compared to Leg 1. It was concluded that the difference in exercise capacity between legs was

unrelated to muscle glycogen depletion or development of hypoglycemia, but may be related to increased sympathetic nervous system activation at the onset of exercise, and a faster absolute rate of increase in RPE.

The next study we aimed to further examine the role of glycogen depletion, as achieved by glycogen-depleting exercise followed by a low CHO diet, on exercise metabolism and performance when combined with, or without exogenous CHO supplementation. This experimental design allowed the opportunity to examine the effect of maintenance of glucose (fuel) supply compared to declining blood glucose concentrations on metabolism and exercise capacity in persons starting exercise in a glycogen-depleted state. The results demonstrated that though glucose infusion increased endurance capacity by $28 \pm 26\%$, the inter-individual variability for improvement in endurance capacity was large. Furthermore, there was an inverse relationship between exercise duration and blood glucose concentrations at exhaustion in the placebo trial. This study showed that the maintenance of glucose availability in the CHO depleted state may have an ergogenic effect, however, the effect is highly variable between subjects and independent of changes in the rate of total CHO oxidation. Further, despite maintenance of euglycemia (constant fuel supply) in a glycogen depleted state, the majority of these endurance-trained subjects still could not complete the 2.5 h exercise task, suggesting that some other aspect, most likely related to intra-hepatic or intra-muscular glycogen depletion and unrelated to fuel availability *per se*, could have contributed to fatigue development. Interestingly, the results indicated a differential sensitivity to declining blood glucose concentrations and that performance in some subjects might be affected with even a small drop in blood glucose whilst others are able to maintain exercise in the face of hypoglycemia (plasma glucose <3.5 mmol/L). This suggests, but do not establish, that those subjects with superior endurance capacity under glycogen-depleted conditions, are somewhat resistant to the potential ergolytic effects of declining blood glucose concentrations, and have a superior ability to utilize alternative fuel sources such as FFA's.

To explore the hypothesis of an altered sensitivity to hypoglycemia, the final study aimed to determine the effect of an antecedent exposure to two 80-min bouts of hypoglycemia (separated by 40 min of euglycemia), on the concomitant gluco-counterregulatory, symptomatic and cognitive function responses during each bout of hypoglycemia in healthy, well-trained men. Furthermore, we wanted to assess the impact of antecedent exposure to hypoglycemia, on next-day metabolic and neuroendocrine and IL-6 responses during prolonged, steady-state exercise in well-trained men, and how these responses relate to exercise performance, perceived exertion, and cognitive function. This study indicated that in a well-trained, healthy study population cortisol, IL and symptomatic responses were significantly higher during the day 1 HYPO compared to EU trial, indicative of normal counterregulation and symptomatic awareness.

However, there was a strong positive relation between individual cortisol response and symptomatic awareness i.e. those subjects with a low symptom score rating (“hypoglycemic unaware”) also had a reduced cortisol response during Day 1 HYPO. During the day 2 exercise trial, plasma glucose concentrations were effectively maintained (>4.5 mmol/L), and isotope-determined hepatic glucose output, RER, insulin, cortisol, and IL-6 responses were comparable between antecedent HYPO and EU exposure. Individual inspection of the data revealed that 7 (of 10) subjects' average sprint times and 6 (of 10) subjects' TT performance were improved after antecedent HYPO vs. EU exposure, however, these differences were not statistically significant.

These results demonstrated that prior exposure to hypoglycemia (and stimulation of the HPA-axis and gluco-counterregulatory responses) in healthy, well-trained men had no effect on metabolic and neuroendocrine responses to a subsequent bout of hypoglycemia, nor to exercise performed within 24 h, and that the ability to regulate blood glucose homeostasis is effectively maintained. Furthermore, antecedent exposure to hypoglycemia has no impact on next-day exercise performance, RPE or cognitive performance.

In conclusion, in accordance with our original hypothesis this thesis demonstrated that when commencing exercise with normal glycogen stores combined with CHO ingestion during exercise, the altered metabolic (glucose, FFA) and hormonal (insulin) milieu elicited by preexercise CHO meals with differing GI is rapidly negated and has no impact on exercise metabolism and performance. However, it was demonstrated that there is a large variability in postprandial glucose responses between and within individuals. This variability may explain the inconsistent findings noted in the literature on the metabolic and performance effects of preexercise CHO intake, and questions the validity and clinical “usefulness” of the GI, particularly in sports nutrition education.

This thesis also demonstrated an individual range in sensitivity to declining blood glucose concentrations, which in turn differentially affects fatigue development. In addition, though this thesis supports evidence on the ergogenic potential of CHO supplementation, it indicates that the ergogenic potential is highly variable between subjects, specifically when exercising in a CHO-depleted state. In contrast to our original hypothesis, CHO oxidation was similar in the placebo and glucose infusion trial, and maintenance of glucose availability with glucose infusion did not enable the majority of well-trained subjects to complete 2.5 h of exercise in a glycogen depleted state. Hence, in contrast to what is commonly been suggested in the literature, these results indicate that ergogenic effect of CHO cannot simply be explained by the maintenance of euglycemia and CHO oxidation at a time when muscle glycogen concentrations are low. These data are in support of a different mechanism whereby glycogen content *per se* may affect fatigue development.

Lastly, this thesis demonstrated that exposure to acute bouts of hypoglycemia in well-trained athletes at rest mounts a significant cortisol and IL-6 response, and that individual symptomatic responsiveness and aspects of cognitive performance were significantly correlated to the cortisol response. Furthermore, prior exposure to hypoglycemia in well-trained athletes did not alter their ability to mount gluco-regulatory metabolic and neuroendocrine responses needed for effective maintenance of glucose homeostasis during subsequent (next-day) prolonged exercise, neither did it alter perceived exertion and exercise performance during or cognitive function immediately after prolonged exercise.

ADDENDUM A

**INTRA- AND INTER SUBJECT VARIABILITY IN GLUCOSE RESPONSE
DURING REST AND EXERCISE AFTER INGESTION OF A
CARBOHYDRATE MEAL:
VALIDATION OF THE GLYCEMIC INDEX AS A TOOL IN NUTRITION
EDUCATION.**

University of Cape Town

A.1 INTRODUCTION

In 1981, the glycemic index (GI) was introduced by Jenkins et al.²²⁶ as a classification system that could provide a comparison of the blood glucose response to different carbohydrate (CHO) containing foods. The GI is calculated as a percentage value based on the incremental area under the blood glucose curve following the ingestion of a food containing 50 g of available CHO (the test food), divided by the incremental area under the blood glucose curve in response to 50 g CHO in a reference food (glucose or white bread), multiplied by 100²²⁶. The test food hence receives a numeric value and is then classified as having a high, moderate or low GI⁴⁵¹. The test food and reference food are given on separate days after an overnight fast and, typically, GI values are calculated from the group mean of a sample size of about 10 subjects⁴¹³. Tables of the measured GI of a large number of CHO-rich foods have now been published internationally¹⁵¹.

Furthermore, the GI has been proposed as a clinical nutritional tool to manipulate meals or diets to produce a desired metabolic outcome²²⁵. In this regard, dietary advice to limit the intake of high GI, and favor the intake of low GI CHO-rich foods have been proposed to be of benefit in the treatment of disorders of metabolism such as diabetes, hyperlipidemia, and overweight (for review see Jenkins et al.²²⁵). However, there is not consensus regarding the utility of the GI to human health and nutrition and many clinicians and researchers have questioned the relevance and practicality of the GI³³⁹. Some feel that there is insufficient valid scientific data to launch recommendations to the general public that foods with high GI should be avoided (for review see³³⁹), and that proof of the clinical value of low GI diets in the prevention and treatment of relevant diseases awaits prospective, long-term clinical trials^{164,274}.

In recent years, the GI has also been proposed as a nutritional tool in sports nutrition to manipulate exercise metabolism and ultimately performance³⁸⁴. It is especially the area of CHO intake prior to endurance exercise where manipulation of GI has been proposed as a means of altering CHO and fat oxidation during exercise in order to delay the onset of fatigue and hence increase performance. Studies in this regard have yielded inconsistent results. Some found that the ingestion of a low compared to high GI pre-exercise meal reduced CHO oxidation^{141,389,408,409,439} whilst others found no difference in CHO oxidation^{145,210,251} or rate of muscle glycogenolysis^{141,145,251}. Whilst some studies reported an improvement in exercise performance (time to exhaustion) with a low GI pre-exercise meal^{117,249,251,408}, the majority found no effect on exercise performance (time to complete a set amount of work)^{141,145,389,409,439}.

One of the potential confounding factors for the discrepancy in these results is the large intra- and inter-individual variability in postprandial glycemic responses and GI values noted^{65,347,348,451}.

An inherent day-to-day variation in glycemic-insulinemic response exists within the same subject and between different subjects ^{273,452}. Furthermore, work from several laboratories around the world have highlighted the fact that the metabolic responses to pre-exercise CHO ingestion is complex and responses are largely individual ^{141,145,251,266,389,409,439}. The extent to which this variability impacts on the potential benefits of manipulating the GI of the diet for sports performance has not been documented.

Other factors that can contribute to the variability of the GI include food-related factors that may affect the rate of digestion and absorption such as gelatinization, amylose-to-amylopectin ratio, the fat and fiber contents of the food ^{18,323}. Methodologically-related factors such as choice of reference food (glucose or white bread), differences in fasting blood glucose concentrations, timing and frequency of blood sampling, time of day of testing, the use of venous or capillary blood, method of calculating the area under the blood glucose curve (for review see ⁴⁵¹) can further affect the variability in GI values. Clearly, the interaction of these factors within in a food makes it difficult to predict the final rate of its digestion and absorption, and therefore its effect on blood glucose levels and ultimately the GI of that particular food.

Though food and methodologically-related factors can be controlled for, the inter- and intra-individual variability remain a major concern as one can not predict how one person would respond on a particular day, nor how one person would differ from another in glycemic response. Factors that may affect intra- and inter-individual variability include age, race, gender ⁴⁵¹, however, these factors have not been studied comprehensively. Furthermore, physical exercise and training status may also contribute to intra-and inter-individual variability in postprandial glucose responses, yet this area has received little consideration in terms of GI research. For example, one might expect a difference in response between trained athletes and sedentary individuals due to increased sympathetic drive, increased insulin sensitivity (for review see Borghouts & Keizer ³⁴) and enhanced glucose disposal in the former ²⁶⁵. Additionally, athletes typically ingest a CHO-rich meal followed by an exercise bout, which may alter the glycemic and subsequent metabolic and hormonal responses. To our knowledge, the effect of the trained vs. untrained state and exercise *per se* on the variability of the GI in healthy subjects have not been studied.

For the GI to be used with confidence as an effective nutritional tool in clinical dietary therapy and as a supplement on food labels, a greater understanding of the extent of the variation in GI need to be established. Repeated testing of the same meal in each subject is required to determine whether the variation in glycemic responses is due to consistent differences between subjects or due to day-to-day variation within the subjects themselves.

Therefore the aim of this study is to investigate the effect of untrained vs. trained state of healthy males on intra- and inter-individual variability in glycemc response after ingestion of the GI reference food (white bread), when food- and methodologically-related factors are stringently controlled for. Furthermore, we wanted to investigate the effect of a moderate-intensity exercise bout performed 1 h postprandially on the glycemc response and determination of the GI. Such an investigation will allow determination of the variation existing solely due to day-to-day related factors within and between trained and untrained individuals. The hypothesis was that the trained athletes would display a less pronounced postprandial glucose response (due to increased insulin sensitivity and glucose disposal), and that the exercise bout in the second h would further attenuate the postprandial glucose response. It was further hypothesized that these attenuations in the postprandial glucose responses would reduce the intra- and inter-individual variation in postprandial glucose responses compared to that seen in untrained individuals.

A.2 METHODS

A.2.1 Subjects

8 Healthy, untrained males volunteered to participate in part 1 (Study A), and 8 healthy, well-trained male endurance cyclists volunteered to participate in part 2 (Study B) of this study. Subject characteristics are given in Table 1. Subjects were recruited based on the following criteria: (a) Untrained, healthy males were not to have participated in regular exercise training of any sort, nor to have participated in any organized or recreational sports, and were matched to trained males according to age and body mass index (BMI); (b) Trained males were to engage in regular cycling training consisting of at least 2 h of cycling, 4 sessions per week; and to have completed a local 105 km cycle race in less than 3 h 30 min. All study procedures were carefully explained to the subjects, and their informed, written consent was obtained before the study commenced. The study was approved by the Research and Ethics Committee of the University of Cape Town, South Africa.

A.2.2 Preliminary testing

A.2.2.1 Oral glucose tolerance test (OGTT):

Before subjects could participate in the study, they were required to undergo an oral glucose tolerance test (OGTT) in order to ensure normal glucose tolerance by all the study participants. Blood were sampled via venous forearm catheter at baseline and at 15 min intervals for a period of 2 h after ingestion of 75 g of glucose dissolved in 250 ml water. Glucose response-curves

were calculated and subjects displaying signs of glucose intolerance or diabetes were excluded from the study.

A.2.2.2 Anthropometry:

Mass and stature were predetermined and body mass index (BMI) was calculated for subjects participating in both the studies. These results were used to match study participants for Study A_{sedentary} and Study B_{exercise}. Subjects participating in Study B_{exercise} also had an anthropometric assessment done. Body fat was calculated using the equation of Durnin & Womersley¹²⁷ and from the sum of seven skinfolds (biceps, triceps, subscapularis, abdominal, thigh and calf).

A.2.2.3 Peak sustained power output (W_{peak}) test:

The 8 trained cyclists (Study B) were requested to perform an incremental cycle test to exhaustion in order to determine each subject's individual working capacity (peak sustained power output (W_{peak}) test). On arrival at the cycle laboratory, a full anthropometric analysis was performed (as mentioned above), followed by a 10 min warm-up exercise bout on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands). After the warm-up, the W_{peak} test was started at a workload equivalent to 3.3 Watts/kg body mass and increased first by 50 Watts (W) after 150 sec and then by 25 W every 150 sec until the pedaling frequency dropped below 50 revolutions/min. This information was used to adjust the work rate in the subsequent phases of the trial so that each subject (in Study B) exercised at an intensity corresponding to 63% of W_{peak} (corresponding to 70% of $VO_{2\ max}$ ¹⁹⁷).

A.2.3 Research Design

The glycemic response to white bread (GI standard) was tested on 3 separate occasions, one week apart. In Study A, subjects remained rested for 2 h postprandially, in Study B subjects remained rested for 1 h postprandially, followed by 1 h of cycling at 70% of their predetermined maximal oxygen consumption ($VO_{2\ max}$; corresponding to 63% of their predetermined W_{peak} ¹⁹⁷). A single batch of white bread was used in order to minimize food-related variability. All testing and data analysis procedures were stringently controlled for and standardized in order to minimize methodologically-related variability.

Habitual dietary intake and training regime were recorded and subjects were instructed to maintain their current dietary and training regime for the duration of the study. Food intake and training were standardized for the 24 h period prior to each trial. Subjects were asked to refrain from doing exercise, and avoid the intake of alcohol and caffeine-containing beverages.

A.2.4 Experimental procedure

To allow for standardized, controlled conditions, subjects were required to sleep over at the Research Unit on the evening prior to each of the 3 trials. The day before the initial trial, subjects were instructed to record their dietary intake and to repeat this diet the day before subsequent trials. Subjects reported to the Research Unit by 8 pm the evening prior to the trial. Upon arrival they received a standardized snack (final meal for the day) consisting of two slices of white bread (high glycemic CHO) and two slices of cheddar cheese. This was provided to ensure that the initial plasma substrate concentrations and any metabolic differences between trials were standardized and not a consequence of the final meal³⁵⁸. After ingestion of the snack subjects were allowed to drink water only.

A.2.4.1 Blood sampling and analysis

The following morning, the trial was started after a 10 h overnight fast. An 18-gauge Teflon cannula (Jelco; Johnson and Johnson, Halfway House, South Africa) was placed into the subject's right forearm vein and connected to a three-way stopcock (Uniflex; Mallinckrodt Medical, Hennef-Sieg, Germany). A baseline blood sample was obtained immediately prior to the start of the trial, and then repeated at 15 min intervals during the subsequent 2-h period (~12 ml of blood was drawn at each time-point). After each blood sample was taken, the cannula was kept patent by flushing it with sterile saline. Aliquots of the blood sample were placed into tubes containing potassium oxalate and sodium fluoride (Midran; Novo Nordisk, Johannesburg, South Africa) for subsequent analysis of plasma glucose concentration. The tubes were immediately placed on ice and, after 20 min, centrifuged at 3 500 rpm for 12 min at 4 °C, and the supernatants were then stored at -20 °C for later analysis. Plasma glucose concentrations were determined by the glucose oxidase method using a glucose analyzer (Glucose analyzer 2; Beckman, Fullerton, CA).

After collection of the baseline blood sample, a standardized test meal was ingested consisting of a 106 g portion of white bread, providing 50 g of available CHO. This amount was chosen according to the standardized protocol for determination of the GI as outlined by Wolever et al.⁴⁵¹. A local bakery was asked to bake a single batch of white bread, of which the crusts were removed, then portioned and frozen prior to the study. This was done in order to minimize food-related variability. Before each trial, each individually packed bread portion was defrosted overnight at room temperature. Subjects were asked to consume the test meal within 15 min, and along with 300 ml of water (room temperature). After 15 min had lapsed, another blood sample was taken and the clock was started at 0 min. The 8 untrained subjects were then requested to remain rested (in seated position) for the next 2 h, whilst the 8 trained cyclists were

requested to remain rested for 1 h, followed by 1 h of steady-state exercise at 70% $\text{VO}_{2\text{ max}}$ (or 63% W_{peak}).

Before exercise was started, the cycle ergometer, fitted with racing saddle and handle bars, was set-up to fit the cyclist's normal riding position. Exercise was started exactly 1 h after the meal was consumed. Each cyclist was given 6 min to warm up (3 min at 100 W followed by 3 min at 150 W). After 6 min, the workload was set at 63% of each subject's individual W_{peak} and 1 h of steady-state cycling followed. During exercise, subjects were cooled by an electric fan, and could consume water ad libitum.

A.2.4.2 Respiratory exchange measurements

At baseline (fasting) and at 20 min intervals during exercise, VO_2 and VCO_2 were determined on-line using a computerized system (Oxycon Alpha, Jaeger-Mijnhart, The Netherlands). Prior to each test, the flow meter of the Oxycon Alpha analyzer was calibrated using a Hans Rudolph 3 liter syringe, and the gas analyzer was calibrated using a two-point calibration of fresh air and a 4% CO_2 , 96% N_2 gas mixture as per manufacturer specifications. The reliability of the Oxycon Alpha analyzer was tested on a weekly basis using the combustion of absolute ethanol (99% Analytical Report, Associated Chemical Enterprises (Pty.) Ltd., Glenvista, South Africa) and its concomitant respiratory exchange ratio (RER) as a reference.

A.2.5 Statistical analysis

The statistical software package STATISTICA 7.0 (2004; StatSoft, Inc., Tulsa, OK, USA) was used for the statistical analysis of the data. Area under the plasma glucose curve ($\text{AUC}_{\text{glucose}}$) was determined by using the GraphPad PRISM software package, version 3.0 for Windows (GraphPad Software, San Diego, CA, USA). The baseline plasma glucose concentration was used as the start of the curve, and only the area above the baseline plasma glucose concentration was considered i.e. incremental AUC ($\text{IAUC}_{\text{glucose}}$). Statistical significance ($p < 0.05$) of between-group differences for plasma glucose responses was assessed by a three-way analyses of variance (ANOVA) for repeated measures over time. In order to determine which means were significantly different, Tukey's honest significance difference post-hoc analysis was used. In order to determine intra-individual variability, the coefficient of variation (CV) for each subject was determined across the three trials (individual SD \div individual mean); and to determine inter-individual variability, the CV for each trial was determined (trial SD \div trial mean). For comparison (between studies) of the glycemic responses of the first and second h postprandially, the $\text{IAUC}_{\text{glucose}}$ of the first h was determined using the baseline plasma glucose sample as the start of the curve and the 15 min sample points up to the 60 min timepoint. For

the calculation of $IAUC_{\text{glucose}}$ for the second hour, the $IAUC_{\text{glucose}}$ of the first hour was subtracted from the total (120 min) $IAUC_{\text{glucose}}$. For comparisons between studies of AUC, Students' t-test was used. Data are presented as means \pm SD.

A.3 RESULTS

Subject characteristics are presented in table 3.1. Subjects from Study A and B were matched according to age and BMI.

Table A.1: Subject characteristics

	Untrained males (n=8) Study A	Trained males (n=8) Study B
Age (yrs)	21 \pm 1	23 \pm 5
Weight (kg)	69 \pm 8	71 \pm 8
Height (m)	1.8 \pm 0.1	1.8 \pm 0.1
BMI	22 \pm 2	22 \pm 2
Body Fat (%)	-	13 \pm 3
W_{peak} (W)	-	376 \pm 29
$VO_{2\text{max}}$ (ml/kg/min)	-	6.6 \pm 0.6

Total incremental area under the plasma glucose curve ($IAUC_{\text{glucose}}$) over 120 min was significantly higher in the untrained study population (Study A) compared to the trained subject population (Study B) during all three the experimental trials ($p < 0.05$) (Figure 1). When breaking the analysis down into the first and second hour postprandially, untrained subjects revealed a significantly higher glycemic response within the first 60 min postprandially in all three the trials compared to trained subjects. There was no difference between glycemic responses between Study A and B in the second hour postprandially. This is somewhat surprising seeing that in Study B the trained subjects performed 1 h of steady-state cycling (70% $VO_{2\text{max}}$) during the second hour. Hence, most of the difference seen in the overall 120 min glycemic response between the two study populations can be attributed to a significantly higher glycemic response in untrained individuals within the first hour postprandially, with little difference in the second hour.

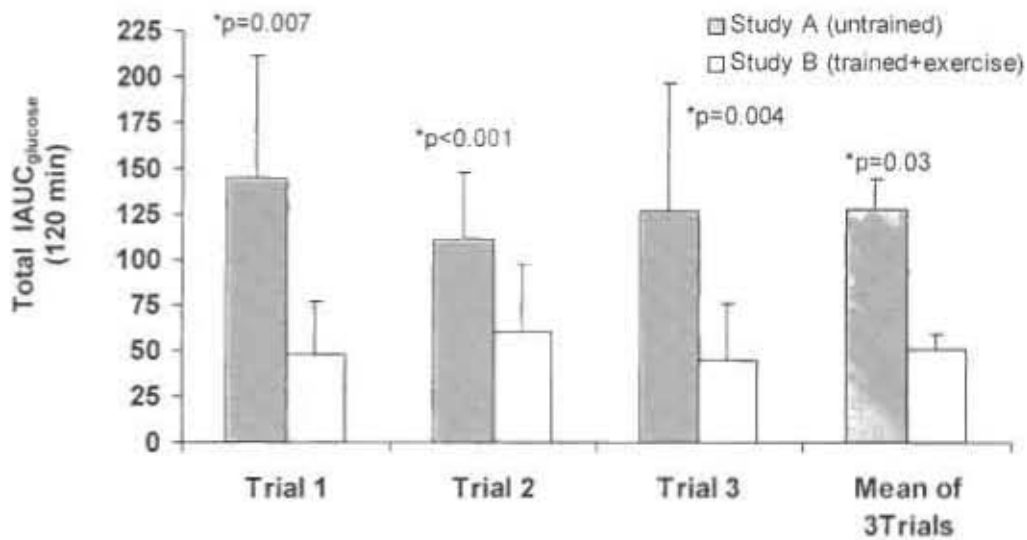


Figure A.1: Total IAUC_{glucose} (120 min) for each of the 3 trials and the mean of the 3 trials, respectively. n=8 for Study A and B, respectively. *Significant difference (p<0.05) between Study A and B.

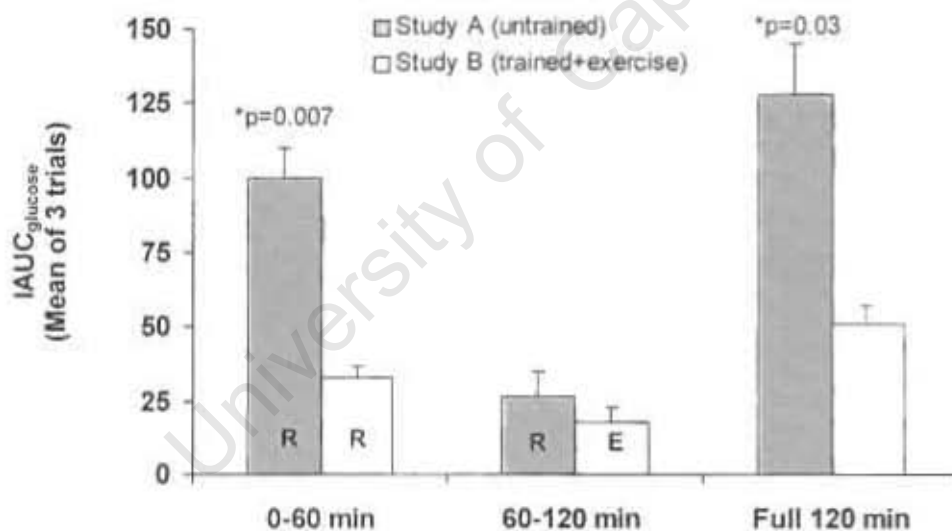


Figure A.2: Comparison of IAUC_{glucose} (mean of 3 trials) of first vs. second h postprandially. R = Rest; E = Steady-state exercise (70% VO_{2max}). *Significant difference between Study A and B, p<0.05).

Intra-individual coefficient of variation (CV) in IAUC_{glucose} (120 min) over the three trials was 34 ± 18 compared to $48 \pm 43\%$ for Study A_{untrained} and Study B_{trained+exercise}, respectively (NS). The mean inter-individual CV over the 3 trials was 45 ± 11 vs. 65 ± 3 for Study A_{untrained} and Study B_{trained+exercise}, respectively (p=0.05).

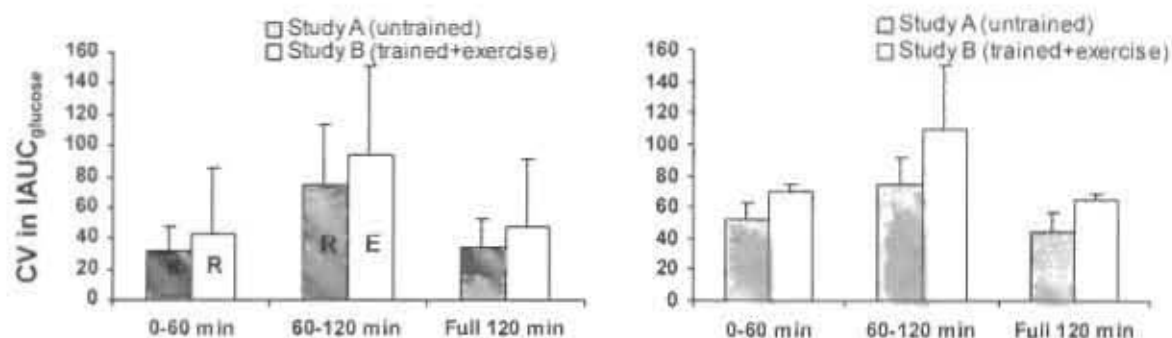


Figure A.3: Intra-individual (A) and inter-individual (B) coefficient of variation (CV) in IAUC_{glucose}. R = Rest; E = Steady-state exercise (70% VO_{2max}). (NS)

When the whole AUC_{glucose} was considered, i.e. the area above and below the baseline plasma glucose concentration, the inter and intra-individual CV in the trained study population was dramatically reduced. The intra-individual CV for the whole AUC_{glucose} (120 min) over the three trials was 29±16 in Study A_{Untrained} compared to 24±15% in Study B_{trained+exercise} (NS). The mean inter-individual CV for the whole AUC_{glucose} (120 min) over the 3 trials was 44±10 vs. 43±2 for Study A_{untrained} and Study B_{trained+exercise}, respectively (NS).

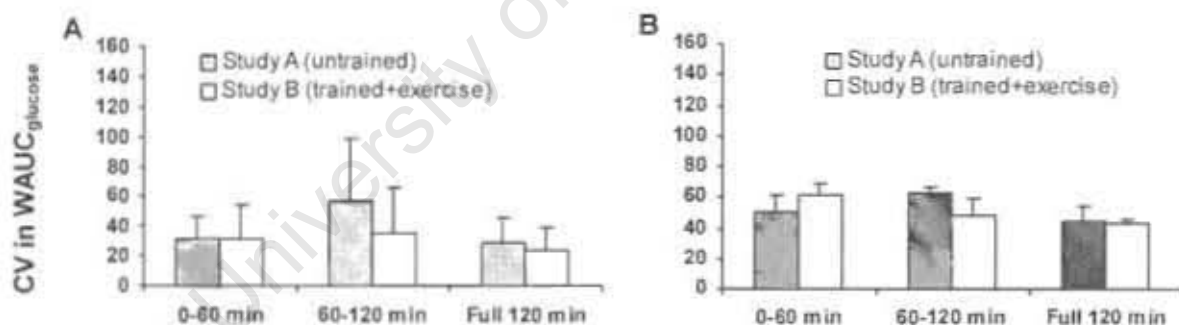


Figure A.4: Intra-individual (A) and inter-individual (B) coefficient of variation (CV) for whole AUC_{glucose} (WAUC_{glucose}; area above and below baseline plasma [glucose]). R = Rest; E = Steady-state exercise (70% VO_{2max}). (NS)

In Study B, RER responses during the 1 h of steady-state cycling remained similar between all three trials.

A.4 DISCUSSION

The glycemic response following repeated ingestion of the GI reference food (white bread) showed a large intra- and inter-individual variability. Training status (and exercise) has little impact on the inter- and intra-individual variability under stringently controlled conditions, compared to that found in untrained persons under resting conditions.

It has been demonstrated that there is considerable variation between individual subjects with regard to the *absolute* glycemic response to a CHO-rich food, and variability also exist within subjects due to day-to-day fluctuations^{65,347,348,413}. Factors that may affect intra- and inter-individual variability include age, race, gender, presence of diabetes, the type and treatment of diabetes⁴⁵¹. Regular exercise and training status may further affect glycemic responses as they can alter, amongst other, insulin sensitivity and rate of glucose disposal^{34,200}. The impact of many of these factors has not been adequately studied, especially with regards to its impact on the GI, and needs further investigation. The present study indicates that despite stringent standardization of methodological and food-related factors, the intra- and inter-individual variability in glycemic response in trained and untrained subjects remain large (CV of 30-40%). In practical terms, this wide range in glycemic response variability can cause a CHO-rich food to produce a GI that varies by 20-30%. For example, if a GI is calculated for the same portion of white bread ingested during each of the three trials by using the average of the individual IAUC's over the 3 trials as the standard, then the GI from the untrained subject population varies from 87 to 114 (~20%) and from 88 to 118 (~30%) in the trained subject population. The 95% confidence intervals calculated from these GI values were 87 to 113 in the untrained and 89 to 111 in the trained subject population. This large variability may cause the food to shift GI categories (low, moderate or high GI) when tested repeatedly (on different occasions) or when compared between individuals. In the present study, despite the 20-30% variability in the GI response, the food (white bread) will still remain in the high GI category. This is in agreement with others showing that there is good agreement between the *relative* magnitude of responses to various CHO-rich foods between individuals⁴⁵¹, hence though the absolute numbers may differ, the food will still remain in high GI category (GI >70) or moderate GI category (GI 55-69) or low GI category (GI <54).

However, in the recent study by Chlup et al.⁶⁵ the GI determination for white bread (providing 50 g available CHO, tested on 5 occasions) resulted in a GI of 70, with a similar range in variability to the present study of 38%, with 95% confidence intervals of 44 to 96. This would result in the GI classification for white bread to completely shift categories from a low to a high GI classification, from one test to the next. It seems likely that if the glycemic responses and calculation of the GI to a single batch of white bread (tested under controlled and standardized

conditions) is this variable, then one can expect the variability in response to be even larger and hence less predictable when GI determinations are made on generally-available CHO-rich foods where variability in food-related factors (e.g. plant variety and degree of ripeness, processing, cooking, fat, fiber and protein content) will lead to further variability in rates of digestion and absorption and ultimately glycemic responses. This belies the concept that a food has a definitive GI and/or that the glycemic response can be accurately predicted. Furthermore, it raises concern over the validity of labeling CHO-rich food items with absolute numbers and basing dietary advice there-upon.

In the present study, healthy subjects were matched for age, race and gender, but differed in exercise training status. It is well documented that individuals who engage in regular exercise training can be expected to have a lower body fat content, increased insulin sensitivity, increased sympathetic drive and enhanced glucose disposal compared to habitually sedentary individuals^{34,200}. Indeed, in the present investigation the trained subjects (Study B) had a significantly reduced glycemic response ($IAUC_{\text{glucose}}$) compared to that of untrained subjects (Study A). When the 2-h postprandial glucose response is divided into the first and second hour, it becomes apparent that most of the difference in response is due to a significantly reduced glycemic response in the trained subject group within the first hour postprandially. The glycemic response between groups in the second hour were comparable, and this despite performing exercise within the second hour in Study B. These results can be substantiated by the recent findings of Schenk et al.³⁶⁹.

Schenk et al.³⁶⁹ was the first and only study to our knowledge that has to date measured the actual glycemic effect of a CHO food by directly measuring the plasma glucose kinetics, which is a function of both glucose appearance (Ra_{glucose}) into, and glucose disappearance (Rd_{glucose}) from the systemic circulation. A fundamental assumption regarding the GI concept, is that a low GI food, for example, means that the food produces a low glycemic response merely as a result of a slower rate of digestion, resulting in a slower rate of absorption into the circulation (i.e. Ra_{glucose})^{229,226,451}. However, the plasma glucose concentration is a function of both Ra_{glucose} and Rd_{glucose} , and the impact of a CHO food on plasma glucose concentration can only be inferred by directly measuring glucose kinetics (Ra and Rd), and not from the GI³⁶⁹. Though certain intrinsic properties of the CHO food can affect rate of absorption and influence Ra_{glucose} , the Rd_{glucose} (glucose uptake) is primarily influenced by insulin secretion and action on tissue¹¹⁵. A food may, for example, demonstrate a low GI as a result of having either a low Ra_{glucose} or relatively high Rd_{glucose} ³⁶⁹. Direct measurement of glucose kinetics can further explain the underlying factors responsible for differences in the GI of foods and will provide a better understanding and substantiation for its potential therapeutic applicability than only considering glucose and insulin concentrations.

Schenk et al.³⁶⁹ investigated the glucose kinetics in response to ingestion of a low GI bran cereal compared to high GI corn flakes in healthy men. Data on the fitness level of the subjects are not reported, but subjects performed 60 min of exercise (at ~60% of maximal heart rate) the day before each trial, perhaps indicating that it was not an untrained / sedentary study population. Though the GI of the corn flakes (132 ± 33) was almost twice that of bran cereal (55 ± 7), the Ra_{glucose} was similar for both cereals over a 180 min postprandial period. Instead, the lower GI of the bran cereal was attributed to an earlier and marked insulin response during the initial 20-min postprandial period combined with an earlier increase in Rd_{glucose} (especially over the 30-60 min period), which attenuated the plasma glucose concentration. This study demonstrated that a low GI food does not necessarily have a lower Ra_{glucose} ³⁶⁹,

The higher plasma insulin concentrations for bran cereal in the 0-30 min postprandial period was attributed to the ~3.5 times more protein that it contains (15 g protein) compared to corn flakes (4 g protein)³⁶⁹. It has previously been demonstrated that the addition of protein to CHO attenuates the glycemic responses due to augmented insulin secretion (for review see²²⁷), indicating that the presence of macronutrients other than CHO can also impact and alter glucose kinetics and hence the GI. The earlier rise in insulin concentration seen in the bran cereal experiment appears functionally important for increasing Rd_{glucose} early in the postprandial period, thereby reducing postprandial hyperglycemia³⁶⁹. This is further supported by the strong correlation ($r=0.7$, $p<0.009$) found between the 30-60 min glucose clearance rate (an index of insulin-mediated glucose uptake) and the initial (0-30 min) insulin response in attenuating postprandial hypoglycemia³⁶⁹. In the present study, though insulin concentrations were not measured, the lower glycemic response within the first hour postprandially in the trained compared to untrained subject population is likely due to increased insulin sensitivity and Rd_{glucose} (uptake by the muscle) in the trained group^{34,200}. This suggestion is supported by studies demonstrating that individuals who are more insulin-sensitive show a more rapid rise in postprandial insulin secretion compared to individuals with impaired glucose tolerance^{49,418}.

Based on the findings of Schenk et al.,³⁶⁹ it seems that the glycemic potential of a food is largely determined by the insulin response it elicits within the 0-30 min postprandially, and that factors influencing insulin release (such as inherent food-related factors) and the responsiveness to insulin (such as degree of insulin sensitivity) play an important role in the ultimate glucose response. The findings of this and other studies^{49,418} suggest that the glycemic potential of a CHO-rich food vary between study populations with variable insulin sensitivity. In addition, it also indicates that the major impact of a CHO-rich food is elicited over the first 60 min postprandially, but when only the AUC over the full 2 h period (or longer) is considered, the impact and importance of the potential underlying factors may be overlooked. This provides justification for

more frequent blood sampling (e.g. every 10 min) over the initial 60 min period, which allows for better assessment of insulin concentrations specifically ³⁶⁹. It is the first-phase insulin response in particular, and not so much the total AUC that seems important in altering postprandial hyperglycemia and hence the GI ³⁶⁹.

The choice to codify the glucose response using the 2 h postprandial period was based mainly because of employing this time period in the diagnostic classification for identifying impaired glucose tolerance or type 2 diabetes. However, as can be seen from the present investigation, postprandial glucose disposal can be more rapid (as apparent in healthy, trained individuals), or more prolonged (e.g. in the presence of diabetes ^{49,339,418}). The time-period of measuring the postprandial glucose response can again have an impact on the final GI as shown by Gannon and Nuttall ¹⁶⁴ who demonstrated that differences in GI between foods is reduced as longer postprandial time frames are used. Furthermore, seeing that GI determinations are primarily calculated from only considering the area above the baseline plasma glucose curve, a large proportion of the glycemic response may be discounted, particularly in a trained or insulin-sensitive subject population where glucose concentrations may decline below baseline concentrations. Though IAUC has been chosen as the preferred method of calculating the glucose response and GI, some experts favor using the whole AUC as the real measure of glucose availability instead ^{164,339}. Pi-Sunyer ³³⁹ noted that differences in the GI between foods would be greatly attenuated if the whole AUC are used and provided an example that if a person with a fasting blood glucose of 4.2 mmol/l ingest 2 foods and the IAUC is used, then the GI would be 100 and the other 72. If the whole AUC is used, then the difference would be 100 and 92, respectively ³³⁹. Interestingly, when the whole AUC_{glucose} is used in the calculation of the GI in the present study, the variability in GI increases in the untrained subject population from ~20 units (GI 87 to 114; IAUC_{glucose}) to 40 units (GI 84 to 120). It has the opposite effect in the trained subject population in that the variability in GI was reduced from a ~30 unit difference between the 3 trials (88 to 118; IAUC_{glucose}) to a difference of ~20 units (90 to 118) when whole AUC_{glucose} was used.

Apart from an increase in insulin sensitivity, an enhanced capacity for glucose uptake (Rd_{glucose}) in the trained subject population may also be achieved by other mechanisms, such as higher skeletal muscle GLUT-4 protein expression, increased activity of hexokinase and other oxidative enzymes, a higher proportion of type I skeletal muscle fibers, and greater capillary density that may all account for a greater ability for glucose uptake ^{130,184,265}.

When plasma glucose responses of individual subjects are evaluated, one subject in the trained population showed a reduction in plasma glucose below 3.5 mmol/L in all three trials at the 90 min timepoint i.e. 30 min after the onset of exercise (also referred to as rebound hypoglycemia),

with a return to >4.0 mmol/L at 120 min. A further one subject showed a similar pattern of rebound hypoglycemia (plasma glucose <3.5 mmol/L), but only in two of the three trials (with plasma glucose >4.0 mmol/L at 90 min in the other trial), and another subject displayed rebound hypoglycemia (plasma glucose <3.5 mmol/L) in only one of the three trials (with plasma glucose of 3.8 mmol/L in each of the other two trials). Only two of the trained subjects did not have a blood glucose concentrations of below 4.0 mmol/L at the 90 min timepoint (or any other timepoint) in any of the 3 trials, the other subjects varying from 3.8 to 4.7 mmol/L between trials. This is consistent with findings from other studies where CHO ingestion within the hour prior to exercise resulted in rebound hypoglycemia in a subset of subjects^{152,187,260,266,305,426}, but in contrast to studies where it was not observed^{69,114,147,171,186,279,288,294,346,374}. It should further be noted that when only the incremental area above the baseline glucose concentration is used in the calculation of the glycemic response curve, then an important part of the glucose response (below baseline) would be discounted, particularly in those individuals who develop rebound hypoglycemia. This may further impact on the variability in GI calculations (as noted previously).

Rebound hypoglycemia is most likely the result of the combined effects of insulin and muscle contraction on increasing muscle glucose uptake at the onset of exercise^{145,288}. However, one of the untrained subjects in Study A also had a blood glucose below 3.5 mmol/L in one of the three trials, and coincidentally, at the 90 and 120 min timepoints (despite remaining in the rested state). It is not yet known why some individuals are predisposed to the development of rebound hypoglycemia following CHO ingestion and others not, especially when ingested within the 60-min period prior to performing physical exercise.

Higher insulin sensitivity, as may result from differences in training status^{34,200}, has been suggested as the reason why some individuals (especially in the trained subject population) develop rebound hypoglycemia and others not²⁶⁶. However, one study recently discounted this theory when a similar range in insulin sensitivity was demonstrated between subjects who varied in training status and developed rebound hypoglycemia, and those who did not develop rebound hypoglycemia²²⁹. In the present study we do not have data to support or refute any of these suggestions. Our main aim was a descriptive characterization of the postprandial glycemic responses in trained and untrained individuals and measurements of insulin and insulin sensitivity were not done. The present data does indicate that there is also an intra-individual variability in the development of rebound hypoglycemia i.e., the same amount and source of high glycemic CHO (50 g of white bread), ingested 60 min prior to exercise of moderate-intensity ($70\% \text{VO}_{2 \text{max}}$) in well-trained subjects does not consistently cause rebound hypoglycemia in the same individual. This again demonstrates that regulation of blood glucose concentrations are complex and cannot be accurately predicted, especially not when many of the key mechanisms and regulating factors are not fully elucidated.

In conclusion, this study demonstrates that despite stringent standardization of food and methodologically related factors (known to affect variation in the GI), the comparison of intra- and inter-individual glycemic response is highly variable both in untrained and trained individuals (CV of 30-40%; estimated GI varying by 20-30 units). The variation in response between individuals seems to be higher than the variation within individuals, and both the inter- and intra-individual responses are more variable in the trained compared to untrained subject population. The glycemic response curve in the trained subject population is significantly reduced compared to that of the untrained subject population, largely due to a significantly reduced glucose response within the 0-60 min postprandial period, with less of a difference in the 60-120 min postprandial period, and likely due to the known effect of differences in insulin sensitivity between the untrained and trained study populations. The exercise performed from 60-120 min postprandially by the trained study population had no marked effect on the $IAUC_{\text{glucose}}$ compared to that of the untrained study population who remained rested, suggesting that the 0-60 min period after ingestion of a high glycemic CHO is when glucose kinetics are mostly affected. It remains to be determined whether a CHO-food with a lower glycemic response would show a similar pattern, as slower insulinemia may "delay" glucose kinetics into the 60-120 min period. This study further suggests that ingestion of the same amount of high glycemic CHO 60 min prior to exercise of moderate-intensity ($70\% \text{VO}_{2 \text{max}}$) in well-trained subjects does not consistently cause rebound hypoglycemia in the same individual i.e. that there is also an intra-individual variability in the proneness to develop rebound hypoglycemia.

This study, albeit mostly descriptive in nature, highlights the need for further investigation surrounding the use of the GI as a credible, reproducible clinical tool for use in nutrition intervention, especially with applicability to different study populations with potentially different glycemic-insulinemic responses. Seeing that the actual underlying glucose kinetics may be variably affected by CHO ingestion in different study populations, it remains to be determined whether the GI, currently determined by using either healthy sedentary or diabetic study populations can be extrapolated for use in, for example trained athletes. For the GI to have relevance as a clinical tool, it must be proven to be reliable and to be capable of altering metabolic responses to food intake to a degree that is clinically or physiologically relevant. Furthermore, for the GI to be used with confidence as an effective nutritional tool in clinical dietary therapy and as a supplement on food labels, a greater understanding of the extent of the variation in GI need to be established.

Acknowledgements

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ADDENDUM B

DIFFERENTIAL EFFECTS OF ACUTE HYPOGLYCEMIA ON COGNITIVE
RESPONSE SPEED AND VARIABILITY.

University of Cape Town

Manuscript in review in Neuropsychologia, 2005
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B.1 INTRODUCTION

Optimal management of insulin-treated diabetes is often compromised by the increased risk of hypoglycemia associated with tight glycemic control²⁰⁷. Severe hypoglycemia occurs commonly among type 1 diabetic patients, with one study reporting an incidence of 150 episodes per 100 patient years⁴⁰⁷. There is now substantial evidence that hypoglycemia can have both acute and lasting effects on cognition^{6,156,176,183,324,364}. Acute hypoglycemia induces cognitive dysfunction as a consequence of neuroglycopenia, including changes in psychomotor function, attention and concentration, learning and memory^{135,295,387}. A pattern of cognitive dysfunction during acute hypoglycemia is emerging from recent research utilizing the hyperinsulinemic clamp procedure in both healthy and diabetic subjects^{135,158,202,295,368,387,435}. While some authors have observed global changes in cognition³⁸⁷, several studies have reported performance declines on tests of simple cognitive function (for example simple reaction time, decision making, attention) while more complex or 'higher order' cognition remains unimpaired^{135,295}.

McAulay and colleagues²⁹⁵ observed changes in attention and speed of information processing during hypoglycemia while scores on a non-verbal intelligence test (Raven's Progressive Matrices) remained unchanged. Another study observed that impairments on an attentional task (4-choice reaction time) remained impaired during recovery from hypoglycemia while performance on more complex language-based tasks returned to normal¹⁵⁸. Furthermore, impairments on tests of attention and information processing tasks can be identified at blood glucose levels of 2.9 – 3.1 mmol/L, whereas impairments in other aspects of cognition require a greater blood glucose decline (< 2.5 mmol/L)⁸. These findings suggest that acute changes in blood glucose may differentially affect simple over more complex cognitive functions.

Clinical observation of severely hypoglycemic individuals suggests a slowing of motor responses associated with a diminution of attentional capacity, which may be associated with the syndrome of 'hypoglycemia unawareness'¹⁶⁹. Recent exposure to hypoglycemia can attenuate counterregulatory responses and may impair the symptom awareness at the physiological threshold of ~3.5 mmol/L during subsequent hypoglycemic exposures²⁰⁷. While there is agreement in the literature that neuroendocrine responses are blunted by prior hypoglycemic exposure^{7,98,207}, what is less clear is whether this change is coupled with symptomatic response failure and cognitive impairment. Prior hypoglycemia has been reported to have either no effect on thresholds for hypoglycemic symptoms^{71,443}, or to significantly reduce the capacity to recognize hypoglycemic symptoms during subsequent exposures in both diabetic¹⁸⁹ and healthy individuals^{102,102,160}.

A common finding from studies of individuals with transiently impaired attention (e.g. due to fatigue or alcohol impairment) is that slowing of responses on motor and attention tasks is accompanied by significant increases in the variability of responses¹³⁷. It has been demonstrated that changes in mean estimates of response speed between groups or between assessments in healthy individuals arise as a consequence of an increased proportion of slow responses (or increased 'trial-to-trial variability') rather than any change in the maximum speed of responses^{123,137,343}. The contribution of alterations in response variability to the commonly observed slowing of motor and attentional speed during hypoglycemia is yet to be investigated.

This study sought to examine performance on motor and attention tasks in healthy individuals during two consecutive bouts of hypoglycemia (2.9 mmol/L) performed within the same day. It was hypothesized that slowing of performance on these tasks would be accompanied by an increase in the proportion of slow responses in the reaction time distribution (i.e. increased skewness). Furthermore, we sought to determine the impact of a second bout of hypoglycemia performed within short succession to the first one, on symptomatic awareness and cognitive function.

B.2 METHODS

B.2.1 Subjects

Ten healthy, physically active males aged between 19 and 35 years were recruited to participate in this study (age 24 ± 4 years; BMI 23 ± 1 kg/m², body fat 9 ± 3 %). Eight participants were right-handed. None had any chronic diseases, intercurrent illnesses, were taking any medication or had a personal or family history of diabetes. Each volunteer gave their written, informed consent before participation in the study, which was approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town (South Africa).

B.2.2 Materials

CogState™ is a software program comprising five non-verbal computerized neuropsychological tests assessing reaction time, decision making, sustained and divided attention, working memory and new learning⁸². For the purposes of this study, tests of simple and choice reaction time were reported. This test battery requires the participant to respond manually (via the keyboard) to playing cards presented on the computer screen. Although the physical response remains consistent between tasks (the same 2 keys are used), each of the tasks is set in a different context and requires use of different cognitive processes. For example, the simple reaction time (SRT) task requires participants to press a key as soon as the presented card turns face-up. The

choice reaction time (ChRT) task requires participants to press one of two keys depending upon the color of the presented card. Thirty accurate responses are required to complete each task. The SRT and ChRT tasks are highly reliable when administered serially to healthy adults and children⁸³, and have documented sensitivity to the effects of fatigue or sustained wakefulness¹³⁷, alcohol intoxication¹³⁷, mild cognitive impairment or the early stages of Alzheimer's disease⁹⁹, sports-related head injury²⁰⁴, stimulant medication³¹⁰, and cardiovascular disease³⁸¹.

B.2.3 Procedure

Each subject was studied on two separate occasions (in random order, counter-balanced), separated by at least one month. On one occasion, subjects were either exposed to two 80-min bouts of hypoglycemia (HYPO trial) separated by 40 min of euglycemia, on the other occasion, euglycemia was maintained throughout (EU trial). Studies were identical other than the plasma glucose profiles (detailed below). Subjects were blinded to the order of the studies as well as their plasma glucose concentration at all times. On the day prior to each test day, subjects were required to abstain from alcohol and exercise, and were asked to keep a detailed food diary, which were to be repeated the day prior to the second experimental trial. During the course of the study subjects were instructed to maintain their habitual exercise schedule and diet.

B.2.3.1 Blood Glucose Clamping Procedure

On the morning of each trial (8:00 A.M.), subjects reported to the testing laboratory after a 10-hour overnight fast. Two intravenous catheters were inserted into the non-dominant arm. One cannula was placed in retrograde fashion distally in the forearm for blood sampling and kept patent with a slow infusion of normal saline. The other cannula was placed in the antecubital fossa to enable administration of insulin and glucose. The forearm was then placed in a heated box (55-60 °C) to arterialize the venous blood samples. The heated box was exchanged for a heating blanket during the 5-min it took to perform the computerized cognitive test. A primed, continuous intravenous infusion of regular insulin (Human Actrapid, Novo Nordisk), which made up to 55 ml in 0.9% sodium chloride to which 2 ml of the subject's blood had been added, was infused at a maintenance rate of 1.5 mU/kg BM/min. The clock was started as soon as 0.5 ml of the insulin solution had been delivered. Plasma glucose (measured at the bedside every 5 minutes (Glucose analyzer 2; Beckman Instruments, Fullerton, CA)) was maintained at the required concentration during HYPO and EU using a variable-rate intravenous infusion of 20% dextrose⁹⁴. Plasma glucose was maintained at euglycemia (5 mmol/L) for the first 40 minutes of each of the glucose clamping trials (HYPO and EU). In the HYPO trial plasma glucose was reduced (by reducing the glucose infusion rate) to 2.9 mmol/L and maintained at this level for 80 min. This was followed by 40 min of euglycemia (5 mmol/L), and a subsequent second 80-min

bout of hypoglycemia (2.9 mmol/L). The clamp lasted a total of 240 min. In the EU trial, euglycemia (5 mmol/L) was maintained throughout the 240 min.

On completion of the trials (at 240 min), the insulin infusion was stopped. The plasma glucose was restored to euglycemia if necessary, and subjects were given a meal to consume. Plasma glucose monitoring continued until euglycemia was maintained spontaneously, after which all lines were withdrawn and subjects could return home.

B.2.3.2 Cognitive testing

All participants completed a baseline cognitive test consisting of a practice trial and a baseline trial (performed 1-2 weeks before the start of the experimental trial). Baseline testing was conducted in a quiet, well-lit environment and was supervised by one of the study investigators. Cognitive testing was conducted towards the end of the first 80 min bout of hypoglycemia (at time 115 min), and again at the end of the second bout of hypoglycemia (at time 230 min), before euglycemia was restored. Cognitive testing was conducted at equivalent time points in the EU trial. Eight of ten participants completed a second baseline test, performed 2 weeks after completion of the experimental trial. In addition to the cognitive testing, symptoms were assessed by the use of a questionnaire (administered every 20 min), asking subjects to rank sweating, warmth, palpitations, tingling, anxiety, trembling, hunger, blurred vision, drowsiness, confusion, weakness, headache, difficulty speaking, dizziness, and irritability individually on a linear analog scale. Autonomic symptom scores were derived from the first 7 symptoms listed, and neuroglycopenic scores from the latter 8 symptoms with symptoms being ranked from 0 (not at all) to 6 (very severe/maximal) throughout each of the trials²⁰⁷.

B.2.4 Statistical analysis

The statistical software package STATISTICA 7.0 (2004; StatSoft, Inc., Tulsa, OK, USA) was used for the statistical analysis of the data. To ensure that data met the assumptions of normality for parametric significance testing, all reaction time (RT) data were transformed using a logarithmic base 10 (log₁₀) transformation prior to analysis. Although all statistical analysis was conducted on transformed data, raw data is shown in Table B.1 for ease of interpretation. For each individual participant, change from baseline was calculated by subtracting performance during each experimental condition from performance at the first baseline test. Data was then submitted to a 2 (condition: HYPO, EU) by 2 (test: first, second), repeated measures analysis of variance (ANOVA) for both SRT and ChRT tasks. As the main hypothesis concerned the difference between hypoglycemia and euglycemia conditions, post-hoc *t*-tests were conducted when either a significant main effect of condition or a significant interaction were observed.

To examine the magnitude of change in RT between baseline and experimental conditions a change statistic was calculated for each experimental condition. For each individual, performance in the baseline condition was subtracted from performance in the EU and HYPO conditions. This difference was expressed as a ratio of the within subjects standard deviation (WSD; ²¹) calculated between the two baseline tests. The resulting statistic is interpreted as a z-score, with values greater than 1.64 ($p < 0.05$ one-tailed) considered a significant decline. For each task, the group mean change score is reported here, as well as the number of individuals showing decline in each experimental condition.

Finally, we sought to test the hypothesis that RT slowing in hypoglycemia is caused by an increase in the number of slow responses (i.e. skewness) in the RT distribution. The skewness of the RT distributions for each individual was calculated for both tasks under all conditions. In this case, a large positive value for skewness would indicate a long right tail to the distribution caused by outlying slow responses. Change from baseline was calculated by subtracting skewness during each experimental condition from baseline. This data was then submitted to a 2 (condition: HYPO, EU) by 2 (test: first, second) repeated measures ANOVA.

B.3 RESULTS

B.3.1 Cognitive data

Table B.1 presents the group mean (\pm SD) performance on SRT and ChRT tasks at baseline and in all experimental conditions. No significant differences were observed on the SRT task. For the ChRT task, ANOVA revealed a significant main effect of condition [$F(1,9)=12.54$, $p=0.006$]. Post-hoc *t*-tests revealed significant slowing of ChRT during the first bout of hypoglycemia compared to baseline [$t(1,9)=3.38$, $p=0.008$], and both tests undertaken during euglycemia [$t(1,9)=2.24$, $p=0.05$ for bout 1, and $t(1,9)=3.04$, $p=0.014$ for bout 2]. Significant ChRT slowing was also observed during the second bout of hypoglycemia compared to baseline [$t(1,9)=3.17$, $p=0.011$] and both bouts of euglycemia [$t(1,9)=2.59$, $p=0.029$ for bout 1, and $t(1,9)=2.74$, $p=0.023$ for bout 2]. Performance between the baseline and euglycemic conditions was not different. Further, performance did not differ significantly between the two bouts of hypoglycemia, nor under conditions of euglycemia.

Table B.1: Performance on simple and choice RT tasks during baseline, euglycemia and hypoglycemia conditions.

Task	Baseline	EU trial		HYPO trial		p
		120 min	240 min	Bout 1 (120 min)	Bout 2 (240 min)	
<i>Simple RT task</i>						
Mean±SD	293.6±67.3	295.8±38.2	293.9±22.8	294.5±35.4	309.5±46.4	NS
Skewness	1.8 ± 1.4	1.3 ± 1.1	1.1 ± 0.7	1.0 ± 1.1	1.0 ± 0.9	NS
<i>Choice RT task</i>						
Mean±SD	481.5±52.5	502.2±65.9	492.1±46.0	545.0±79.2	569.9±102.8	a,b,c,d
Skewness	0.6 ± 0.4	0.7 ± 0.6	0.5 ± 0.7	0.2 ± 0.6	0.4 ± 0.5	NS

RT = reaction time; a = $p < 0.05$ between baseline and HYPO bout 1 (120 min); b = $p < 0.05$ between baseline and HYPO bout 2 (240 min); c = $p < 0.05$ between both EU conditions and HYPO bout 1; d = $p < 0.05$ between both EU conditions and HYPO bout 2.

Figure B.1 displays the group mean change scores for each experimental condition. No substantial changes from baseline were observed during the euglycemic conditions for either SRT or ChRT tasks. Performance on the ChRT task slowed substantially during both hypoglycemic exposures. The group mean z-scores recorded were 1.28 during the first bout of hypoglycemia and 1.77 ($p < 0.05$) during the second bout of hypoglycemia. These changes represent a large mean change in performance according to conventional statistical criteria. Performance also slowed during the SRT task, with group mean z-scores recorded as 0.63 and 0.70 during the first and second bout of hypoglycemia, respectively. However these changes were not sufficient to reach statistical significance, as indicated above.

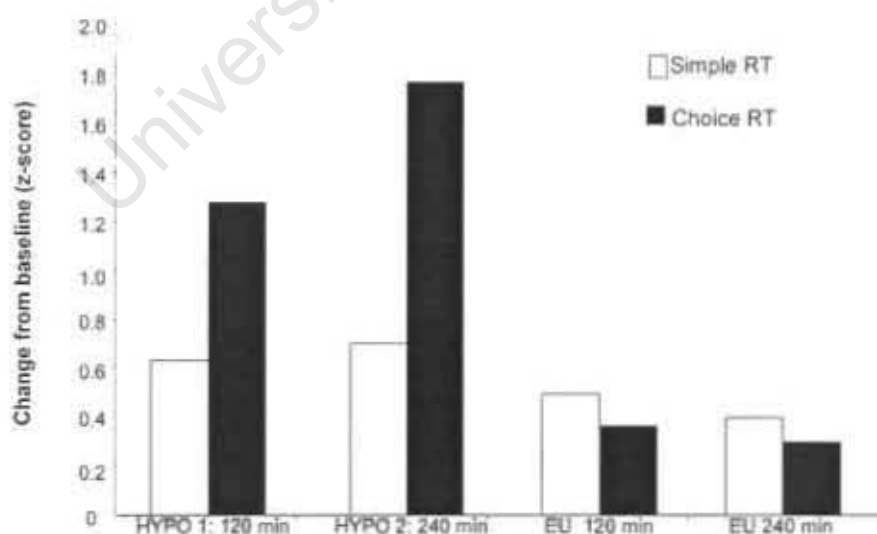


Figure B.1: Group mean z-score (change from baseline) on simple and choice reaction time tasks for all four experimental conditions. Note that positive values indicate a slowing in reaction time. White bars = Simple reaction time; Black bars = Choice reaction time.

Analysis of individual z-scores indicated that 4 of 10 participants displayed a significant change in performance (>1.64) on the SRT task under both hypoglycemia conditions. Further, 3 of 10 participants displayed significant changes on the ChRT task during bout 1, and 4 of 10 during bout 2 of hypoglycemia. On the ChRT task, a further 2 participants approached statistical significance during the second bout of hypoglycemia with z-scores between 1.20 and 1.64. In the euglycemia condition fewer individuals displayed significant impairment during hypoglycemia (Table B.2).

Skewness statistics for both tasks are presented in Table B.1. Both the SRT and ChRT tasks display positively skewed distributions. Such skewness is common in RT distributions and is a consequence of a small proportion of slow responses²⁸³. However, ANOVA indicated that there were no significant changes in skewness between experimental conditions for either the SRT or ChRT task.

Table B.2: Number of people meeting statistical criteria for significant decline in each experimental condition for both simple and choice reaction time tasks.

Condition	Simple RT	Choice RT
<i>Hypoglycemia (HYPO):</i>		
HYPO bout 1 (120 min)	4	3
HYPO bout 2 (240 min)	4	4
<i>Euglycemia (EU):</i>		
120 min	1	1
240 min	2	0

B.3.2 Symptoms

Symptom ratings remained unchanged throughout the EU trial. Autonomic symptoms ratings taken at equivalent timepoints as the cognitive function tests (120 and 240 min), were significantly higher at the end of both bouts of HYPO compared to that obtained in the EU trial (Bout 1: 8 ± 7 vs. 2 ± 1 ($p=0.04$); Bout 2: 12 ± 6 vs. 2 ± 2 ($p=0.0005$), for HYPO vs. EU respectively). Neuroglycopenic symptom ratings at the end of bout 1 of HYPO (7 ± 9) were not significantly different to that of the EU trial (3 ± 2), but were significantly higher at the end of bout 2 of HYPO compared to that of the EU trial (9 ± 8 vs. 2 ± 3 for HYPO vs. EU, respectively, $p=0.01$). The second bout of HYPO elicited significantly higher ratings of autonomic symptoms compared to the first bout of HYPO ($p=0.04$). The neuroglycopenic symptom ratings, however, did not differ significantly between the first and second HYPO bout.

Autonomic symptom ratings at the end of the second bout of hypoglycemia were positively correlated to SRT performance at the same timepoint ($r=0.70$; $p=0.03$). However, no significant relationship between neuroglycopenic symptoms and cognitive function test results were found.

B.4 DISCUSSION

Performance on an attention test (choice RT) completed at the end of 2 consecutive 80-min bouts of hypoglycemia, was significantly slower than that observed during both baseline and euglycemic conditions. In contrast, no differences were observed on a psychomotor task (simple RT), suggesting that hypoglycemia affects attention, but not motor speed, in healthy young individuals. These findings are consistent with previous research suggesting that choice RT tasks are more sensitive to exposure to moderate hypoglycemia than simple RT tasks (for discussion see ref. ⁶). However, impaired motor function has previously been noted during hypoglycemia ^{41,42}, unlike our findings. One explanation for this discrepancy is that the depth of hypoglycemia induced during the two bouts of hypoglycemia in the present study (2.9 mmol/L) was insufficiently low to induce motor impairments. Evans and colleagues ¹³³ observed that the glucose threshold for motor speed impairment (using the Finger tapping task) was below 2.2 - 2.4 mmol/L during acute hypoglycemia, while others have observed that the blood glucose threshold for dysfunction on choice reaction time tasks was 2.8-3.1 mmol/L ^{133,285}.

Fatigue or drowsiness is one of the most commonly reported symptoms of hypoglycemia, and along with cognitive dysfunction, is considered to occur as a consequence of neuroglycopenia ²⁹⁶. Furthermore, fatigue has been previously associated with an increased variability in cognitive performance ^{137,236} and thus it would be reasonable to expect that hypoglycemic individuals, presenting with fatigue, would display similar performance variability. In the present study, ChRT test results were significantly impaired whilst autonomic symptom awareness was increased at the end of both bouts of hypoglycemia compared to the euglycemic trial. However, symptoms indicative of neuroglycopenia were significantly increased above that observed in the euglycemic trial only at the end of the second bout of hypoglycemia. Overall, cognitive test results were statistically unrelated to either autonomic or neuroglycopenic symptom responses. Additionally, the response slowing observed on an attention task was not accompanied by a change in the skewness of the RT distribution. This suggests that attentional slowing during hypoglycemia is associated with a general increase in the time to complete the task, rather than an increase in the proportion of slow responses. We are unaware of previous studies examining cognitive performance variability during acute hypoglycemia.

The magnitude of the impairments in attentional slowing was large according to conventional statistical standards. Interestingly, despite these large group mean changes only a minority of participants displayed statistically significant slowing of attention (Table B.2). Thus suggesting that the cognitive response to hypoglycemia may vary widely between individuals. This finding is further supported by the large inter-individual variability in symptom ratings reported during the hypoglycemic exposures. It is also consistent with prior research suggesting that the blood

glucose threshold for cognitive impairment during hypoglycemia may vary between individuals¹³⁹, and that prior exposure to hypoglycemia may result in cognitive adaptation¹⁵⁸. However, a larger sample size than that recruited in the present study is required to adequately investigate this hypothesis.

We were also interested to see whether a second exposure (bout) of hypoglycemia in short succession to the first exposure would alter cognitive function and/or symptomatic responses compared to the first exposure. To our knowledge, though other studies have employed similar protocols where two bouts of hypoglycemia (of various durations) were elicited within one day, the symptomatic and cognitive responses thereof have traditionally only been compared to responses during next-day exposure to hypoglycemia^{71,96,103,205,443}. These studies reported that prior (previous-day) hypoglycemia had either no effect on thresholds for hypoglycemic symptoms^{71,443} or produced a significant blunting of symptomatic awareness during subsequent exposure (for review see ref.⁹⁰). Additionally, blunting of neuroendocrine responses have been observed whilst cognitive function was preserved^{98,219}. In the present study, each bout of hypoglycemia produced autonomic symptoms that were significantly higher compared to that in the euglycemic trial. Neuroglycopenic symptom awareness, on the other hand, were significantly increased above that in the euglycemic trial only after the second 80-min bout of hypoglycemia was induced. This is in agreement with the results by Towler et al.⁴¹⁰ which demonstrated that the awareness of hypoglycemia is largely, perhaps exclusively, the result of perception of autonomic rather than neuroglycopenic symptoms, mediated to a large extent by muscarinic cholinergic mechanisms.

The present study further indicates that a second 80-min exposure to moderate hypoglycemia, elicited within short (40-min) succession to an identical prior exposure, results in a significant increase in autonomic (but not neuroglycopenic) symptom awareness compared to the first exposure. Neuroglycopenic symptom awareness as well as attentional slowing (indicated by the ChRT cognitive test) were statistically equivalent at the end of the two 80-min exposures to hypoglycemia. These findings are interesting in the light of other studies where prior (previous-day) exposure to hypoglycemia resulted in a blunting of subsequent (next-day) autonomic symptom awareness. The role played by the duration of prior hypoglycemia, the time period between exposures and the differential effects on the magnitude of autonomic and neuroglycopenic symptomatic awareness or 'unawareness' thereof needs further investigation.

In summary, the response slowing observed commonly on choice reaction time tasks in hypoglycemic individuals is not associated with an increase in the number of slow responses. Rather, such dysfunction appears to result from a general slowing of responses. These findings were apparent on tasks that require both attentional and motor processes, but not on tasks

requiring simple motor responses alone. Furthermore, a second 80-min exposure to moderate hypoglycemia, elicited within short succession to the first exposure, results in a significant increase in autonomic (but not neuroglycopenic) symptom awareness, with no relation between symptomatic awareness and choice RT results.

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CHAPTER 8

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