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**NUTRITIONAL STRATEGIES FOR ENDURANCE AND
ULTRA-ENDURANCE CYCLING**

By

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Dedicated to:

My Mother – for her *love, strenght* and *heart of gold*

My Father – who helped *shape me*

My Brother – for his *courage* to persist

And my Sister – through whose eyes life is *an adventure*.

University of Cape Town

TABLE OF CONTENTS

| | |
|--|-----------|
| DECLARATION..... | viii |
| ACKNOWLEDGEMENTS | ix |
| PUBLICATIONS ASSOCIATED WITH THE THESIS..... | xi |
| LIST OF ABBREVIATIONS..... | xiii |
| THESIS ABSTRACT | xv |
| | |
| CHAPTER 1: Literature Review | 1 |
| 1.1 INTRODUCTION | 2 |
| 1.2 REGULATION OF SUBSTRATE UTILIZATION DURING EXERCISE.... | 6 |
| 1.2.1 CHO utilization during exercise..... | 6 |
| 1.2.2 Fat utilization during exercise | 12 |
| 1.2.3 The interaction between CHO and fat utilization during exercise..... | 20 |
| 1.3 NUTRITIONAL STRATEGIES AIMED TO IMPROVE ENDURANCE PERFORMANCE | 22 |
| 1.3.1 Carbohydrate-loading..... | 22 |
| 1.3.2 Fat-loading..... | 34 |
| 1.3.2.1 Mechanisms underlying the adaptations to a high fat diet.. | 35 |
| 1.3.2.2 The effect of a long-term (>4 days) high fat diet on exercise performance..... | 40 |
| 1.3.2.3 Ingestion of a high-fat diet followed by CHO-loading | 50 |
| 1.4 RESPIRATORY EXCHANGE RATIO (RER) PHENOTYPES | 56 |

| | |
|--|-----------|
| CHAPTER 2: Nutritional practices of male cyclists before and during an ultra-endurance event | 61 |
| 2.1 INTRODUCTION..... | 62 |
| 2.2 METHODOLOGY..... | 64 |
| 2.2.1 Subjects and study design | 64 |
| 2.2.2 Data collection | 64 |
| 2.2.3 Statistical analysis | 66 |
| 2.3 RESULTS | 67 |
| 2.3.1 Subject characteristics | 67 |
| 2.3.2. Habitual dietary intake 3 days prior to the race..... | 68 |
| 2.3.3 Pre-event breakfast/meal | 72 |
| 2.3.4 Dietary and supplement intake during the race..... | 73 |
| 2.3.5 Reported symptoms during the race | 76 |
| 2.4 DISCUSSION..... | 76 |
| | |
| CHAPTER 3: Comparison of a high carbohydrate diet vs. a moderate carbohydrate diet on substrate utilization and performance in athletes with different RER phenotypes | 85 |
| 3.1 INTRODUCTION..... | 86 |
| 3.2 METHODOLOGY..... | 88 |
| 3.2.1 Subjects and study design | 88 |
| 3.2.2 Preliminary testing | 89 |
| 3.2.3 Dietary manipulations | 93 |
| 3.2.4 Exercise trial | 93 |
| 3.2.5 Total CHO and fat oxidation | 94 |

TABLE OF CONTENTS

| | |
|---|------------|
| 3.2.6 Blood sampling and analysis..... | 95 |
| 3.2.7 Analysis of muscle biopsies..... | 96 |
| 3.2.8 Statistical analysis..... | 96 |
| 3.3 RESULTS..... | 97 |
| 3.3.1 Subject characteristics..... | 97 |
| 3.3.2 Dietary control..... | 97 |
| 3.3.3 Respiratory exchange ratio (RER) during exercise..... | 99 |
| 3.3.4 Total CHO and fat oxidation during exercise..... | 99 |
| 3.3.5 Circulating hormone and substrate concentrations..... | 103 |
| 3.3.6 Muscle glycogen content..... | 106 |
| 3.3.7 Time-trial performance..... | 107 |
| 3.4 DISCUSSION..... | 109 |
| | |
| CHAPTER 4: The effect of fat-adaptation followed by carbohydrate-loading on 100-km time-trial performance..... | 115 |
| 4.1 INTRODUCTION..... | 116 |
| 4.2 METHODOLOGY..... | 118 |
| 4.2.1 Subjects and study design..... | 118 |
| 4.2.2 Preliminary testing..... | 119 |
| 4.2.3 Dietary manipulations..... | 120 |
| 4.2.4 Exercise training sessions..... | 120 |
| 4.2.5 Experimental trials..... | 122 |
| 4.2.6 Blood sampling and analysis..... | 123 |
| 4.2.7 Statistical analysis..... | 123 |
| 4.3 RESULTS..... | 124 |

| | |
|---|------------|
| 4.3.1 Subject characteristics | 124 |
| 4.3.2 Training and dietary control | 124 |
| 4.3.3 Resting variables | 125 |
| 4.3.4 RER, HR and RPE during constant-load training rides | 127 |
| 4.3.5 Experimental constant-load cycle on day 7 | 129 |
| 4.3.6 Metabolic and performance data during the 100-km time-trial. | 130 |
| 4.4 DISCUSSION | 136 |
| | |
| CHAPTER 5: The effect of fat-adaptation followed by carbohydrate-loading on simulated ultra-endurance race performance | 143 |
| 5.1 INTRODUCTION | 144 |
| 5.2 METHODOLOGY | 146 |
| 5.2.1 Subjects and study design | 146 |
| 5.2.2 Preliminary testing | 147 |
| 5.2.3 Dietary manipulations | 149 |
| 5.2.4 Exercise training sessions | 149 |
| 5.2.5 Experimental trial | 151 |
| 5.2.6 Blood sampling and analysis | 154 |
| 5.2.7 Statistical analysis | 156 |
| 5.3 RESULTS | 156 |
| 5.3.1 Combined results (n=9) | 156 |
| 5.3.1.1 Subject characteristics | 156 |
| 5.3.1.2 Training and dietary intake | 157 |
| 5.3.1.3 Resting variables | 158 |

| | |
|--|-----|
| 5.3.1.4 Physiological measurements during the constant-load cycle rides..... | 160 |
| 5.3.1.5 Metabolic and performance data during 200-km time-trial | 161 |
| 5.3.2 Responsiveness of the high-RER and low-RER groups to the HFD-CHO and HCD-CHO dietary interventions | 167 |
| 5.3.2.1 Subject characteristics and dietary intake | 168 |
| 5.3.2.2 Resting respiratory exchange ratio | 170 |
| 5.3.2.3 RER, HR and RPE during constant-load training rides | 171 |
| 5.3.2.4 Metabolic and performance data during 200-km time-trial | 172 |
| 5.4 DISCUSSION | 179 |
| | |
| CHAPTER 6: Summary and conclusions | 187 |
| | |
| CHAPTER 7: References | 197 |

DECLARATION

I, **Lize Havemann**, do hereby declare that the work on which this dissertation is based is my original work (except where otherwise indicated) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in the University of Cape Town or any other University.

This thesis is presented in fulfillment of the requirements for the degree of PhD.

I hereby empower the University of Cape Town to reproduce this thesis in part or whole, for the purpose of research.

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Abstract Publications

Havemann L, Goedecke JH, Harwood L, Barlow G, St Clair Gibson A, Noakes TD, and Lambert EV. RER Phenotypes in athletes: Response to CHO-loading and effect on performance. *SAJCN* 49:(1) S212, 2005.

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Local Conference Presentations

The 33rd meeting of the Physiology Society of South Africa in Cape Town, South Africa, September 2005 – Free communication: “RER phenotypes in athletes: Response to CHO-loading and effect on performance.”

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LIST OF ABBREVIATIONS

- 3-HAD – 3-hydroxyacyl coenzyme A dehydrogenase
ACC – acetyl-CoA carboxylase
ADP – adenosine diphosphate
AMP – adenosine monophosphate
ANOVA – analysis of variance
AMPK – AMP-activated protein kinase
ATP – adenosine triphosphate
ATGL - adipose triglyceride lipase
BM – body mass
BMR – basal metabolic rate
Ca⁺⁺ – calcium
CaMK – calcium/calmodulin-dependent protein kinase
CHO – carbohydrate
CPT1 – carnite palmitoyltransferase 1
CPT2 – carnitine palmitoyltransferase 2
CoA – coenzyme A
CO₂ – carbon dioxide
CS – citrate synthase
CV – coefficient of variation
DG - diglycerides
Ei – energy intake
FFA – free fatty acid
G1P – glucose-1-phosphate
G3P – glyceraldehyde-3-phosphate
G6P – glucose-6-phosphate
HCD – high carbohydrate diet
HCD-CHO – 6 days high fat diet followed by 1 day carbohydrate-loading
HF – high frequency
HFD – high fat diet
HFD-CHO – 6 days high carbohydrate diet followed by 1 day carbohydrate-loading
HK – hexokinase

HR – heart rate
HRV – heart rate variability
HSL – hormone sensitive lipase
IMTG – intramuscular triglyceride stores
LF – low frequency
LPL – lipoprotein lipase
MCD – moderate carbohydrate diet
MAPK – mitogen-activated protein kinase
METS – metabolic equivalents
NAD – nicotinamide adenine dinucleotide
PAL – physical activity level
PDH – pyruvate dehydrogenase
PDK – pyruvate dehydrogenase kinase
PDP – pyruvate dehydrogenase phosphate
PFK – phosphofructokinase
RER – respiratory exchange ratio
SD – standard deviation
SNA – sympathetic activation
TCA – tricarboxylic acid
TG – triglyceride
TT – time-trial
VE – ventilation volume
VO₂ – oxygen consumption
VCO₂ – carbon dioxide production
VLDL – very low density lipoprotein
VO_{2peak} – peak oxygen consumption
W_{peak} – peak power output
w.w – wet weight

THESIS ABSTRACT

Ingestion of a high carbohydrate (CHO) diet (7-10 g CHO/kg body mass) for 3 days, typically referred to as 'CHO-loading', is a commonly recommended dietary practice for endurance sporting events lasting >90 minutes. CHO-loading effectively maximizes muscle glycogen stores and has been shown to enhance prolonged exercise performance. However, the body's glycogen stores are limited, therefore a dietary strategy that would not only increase CHO availability but also 'spare' muscle glycogen during exercise may be more beneficial during prolonged exercise compared to a standard CHO-loading diet. Preliminary studies in which athletes ingested a high fat diet (4-4.6 g fat/kg body mass) for 5-6 days followed by 1 day of CHO-loading have been shown to increase fat oxidation and 'spare' muscle glycogen during prolonged exercise compared to a high CHO diet. However, the effectiveness of a high fat diet followed by CHO-loading has not been tested in self-paced endurance and ultra-endurance events. Further, there is little available evidence concerning the pre-event habitual dietary practices of ultra-endurance athletes. It is possible that athletes and cyclists competing in endurance and ultra-endurance events have diets which may differ in macronutrient content compared to that typically recommended for endurance events. As a result, athletes may not respond in a similar way to diets typically recommended for endurance and ultra-endurance events, such as CHO-loading.

The aims of this thesis were therefore: (1) to characterize the habitual dietary intakes of sub-elite male cyclists before and during an ultra-endurance event;

(2) to investigate the effects of different dietary strategies aimed at increasing carbohydrate availability and 'sparing' muscle glycogen (e.g. CHO-loading and fat-adaptation), on substrate utilization and exercise performance during simulated endurance and ultra-endurance exercise; and (3) to investigate the individual responsiveness of athletes to these dietary strategies.

The first study characterized the habitual pre- and during race nutritional intakes of cyclists competing in a 210-km 1-day ultra-endurance cycle race. Forty five endurance-trained male cyclists participated in this dietary survey and completed a 3-day dietary record prior to the 210-km cycle race. Mean reported CHO intake over the 3 days prior to the race was 5.6 ± 1.7 g/kg body mass, well below the recommended guidelines of 7-10 g/kg body mass for athletes participating in endurance events. Although 57% of cyclists indicated that they CHO-loaded 1-3 days before the race, only 23% of these cyclists achieved CHO intakes of ≥ 7 g/kg body mass over the 3-day period prior to the race, demonstrating a discrepancy between perceived and actual intakes of CHO. The majority of subjects indicated the use of CHO supplements 1-3 days prior to (84.5%) and during (98%) the race and achieved a CHO intake of 63 ± 23 g/hour during the race. Although a high CHO diet (≥ 7 g CHO/kg body mass) is generally recommended 1-3 days prior to an endurance event, the majority (62%) of subjects in the present study consumed a moderate CHO (4-6 g CHO/kg) diet. When covarying for training status, there was no link between pre-race CHO intake and race time performance. The data suggests that endurance cyclists have diets which vary widely in CHO content. Less than 1 in 4 cyclists are meeting the recommended CHO intake prior to prolonged

exercise, and CHO intake seems unrelated to performance. The question remains as to whether the self-selection of moderate CHO diets and the variability in macronutrient content of the diet may explain differences in performance in endurance athletes.

The second study therefore investigated the effects of a high CHO diet compared to a moderate CHO diet on substrate utilization and performance in athletes with a high or low fasting respiratory exchange ratio (RER) phenotype. This was based on a previous study that demonstrated a large variability in fasting RER. Consequently, high and low RER groups were defined and shown to persist during short-term exercise of varying intensity, as well as during prolonged exercise in the fasted state and when CHO was ingested prior to and during exercise. However, the robustness of phenotypes and the effect on performance have not been tested in response to CHO-loading. Twenty endurance-trained cyclists with different metabolic profiles based on fasting resting RER, 9 with a RER of ≥ 0.84 (high-RER) and 11 with a RER of ≤ 0.80 (low-RER) participated in this single-blind crossover study, consisting of two 4-day trials separated by two weeks. Subjects ingested in random order, either a high CHO diet (8-10 g CHO/kg) or an iso-caloric moderate CHO diet (4-6 g CHO/kg) for 3 days. On day 4, after ingesting a standardized breakfast, subjects completed a 2.5-hour constant-load cycle at 55% W_{peak} , followed by a 250 kJ time-trial. During the constant-load cycle, CO_2 production (VCO_2) and oxygen uptake (VO_2) were measured to determine exercising RER and calculate total CHO and fat oxidation. In addition, blood samples were drawn for the determination of circulating glucose, lactate, free fatty acid and insulin

concentrations. The high CHO diet did not alter the RER phenotypes as demonstrated by higher rates of total CHO oxidation during the 2.5-hour constant-load trial in the high-RER vs. the low-RER group ($p < 0.05$). Mean exercising lactate concentrations were also higher in the high-RER vs. the low-RER group on both diets, but there was a group x diet interaction with the highest lactate concentrations after CHO-loading in the high-RER group ($p < 0.05$ group x time). The high CHO diet tended to improve overall mean time-trial performance compared to the moderate CHO diet ($p = 0.096$), however there were no differences in performance between the RER groups in response to the 2 diets. The data in this chapter suggests that RER phenotypes are maintained during prolonged exercise despite CHO-loading. However, it is unclear as to how this effect may impact more prolonged exercise when rates of fuel utilization may determine performance. Future research should consider the RER phenotype when assessing the efficacy of dietary interventions.

The third study examined the potential of an alternative dietary strategy that not only increases CHO availability but also 'saves' muscle glycogen during exercise. Previous studies have demonstrated that 5-6 days of high fat intake followed by 1 day of CHO-loading, is sufficient to alter substrate utilization at rest and during exercise, increasing the reliance of fat, without compromising glycogen stores. However, the effect of this dietary strategy on exercise performance that simulates 'real-life' race situations is not known. We hypothesized that this fat-loading strategy followed by CHO-loading would sufficiently spare endogenous glycogen stores, such that performance of 100-km time-trial, interspersed with alternating 1-km and 4-km sprints would be

enhanced. Therefore, the aim of the third study of this thesis was to examine the effect of 6 days of high-fat intake followed by 1 day of CHO-loading compared to 7 days of high CHO intake on substrate utilization, effort perception and performance during a 100-km cycling time-trial, interspersed with 1-km and 4-km sprints that simulates a 'real-life' race situation. We further hypothesized that the high fat diet may alternatively impair exercise training performance and alter effort perception, by increasing sympathetic activation (SNA). Therefore, heart rate variability (HRV) as a proxy of SNA was measured during the period of dietary intervention. In this randomized single-blind cross over study, 8 well-trained cyclists completed two trials, ingesting either a high CHO diet (68% CHO energy) or an iso-caloric high fat diet (68% fat energy) for 6 days, followed by 1 day of CHO-loading (8-10 g CHO/kg). Subjects completed a 100-km time-trial on day 1 and a 1-hour constant-load cycle at 63% W_{peak} on days 3, 5 and 7, prior to which resting HRV and resting RER were measured. On day 8, subjects completed a 100-km performance time-trial, during which effort perception and power output were recorded and blood samples were drawn. Compared to the high CHO diet, ingestion of the high fat diet reduced RER at rest ($p < 0.005$) and during constant-load exercise ($p < 0.01$) and increased serum FFA levels ($p < 0.01$), indicating increased fat utilization. There was a tendency for the low frequency power component of heart rate variability to be greater for the fat-loading diet compared to the high CHO diet ($p = 0.056$), suggestive of increased SNA. Overall 100-km time-trial performance was not different between diets, however 1-km sprint power output following 6 days of fat-loading followed by 1 day of CHO-loading was lower compared to the high CHO diet ($p < 0.05$). Despite a reduced power output with the high-fat diet, effort

perception and heart rate were not different between trials. In conclusion, 6 days of high fat intake followed by 1 day of CHO-loading increased fat oxidation, but compromised high intensity sprint performance, possibly due to the increase in SNA and effort perception associated with high fat intake.

Since we demonstrated that 6 days of fat-adaptation followed by 1 day of CHO-loading compromised high-intensity sprint performance, the final study of this thesis tested the hypothesis that this dietary strategy may potentially enhance ultra-endurance exercise (>4-5 hours) that is typically undertaken at sub-maximal exercise intensities where very high rates of CHO oxidation are not necessarily required, and where high rates of fat oxidation and/or a muscle glycogen 'sparing-effect' may also be beneficial to performance. The aim of the final study was therefore to investigate the effects of a high fat diet for 6 days followed by 1 day of CHO-loading (HFD-CHO) compared to a 7-day high CHO diet (HCD-CHO) on substrate utilization, effort perception and performance during a simulated self-paced 200-km cycling time-trial. This study is also based, in part, on the findings from the first study, in which cyclists competing in a similar "double century" event demonstrated widely varying, and lower than the expected CHO intakes in their habitual pre-race diet. Further, we attempted to group subjects based on their resting RER as in study 2, with an aim to explore the responsiveness of athletes with different RER phenotypes to a HFD-CHO and HCD-CHO dietary strategy. In this randomized single-blind crossover study, 9 well-trained cyclists completed two trials, ingesting either a high CHO diet (~67% CHO energy) or an iso-caloric high fat diet (~67% fat energy) for 6 days, followed by 1 day of CHO-loading (8-10 g CHO/kg).

Subjects completed a 100-km time-trial on day 1 to deplete muscle glycogen stores, and then performed a 1-hour constant-load cycle at 55% W_{peak} on days 3 and 5 during which heart rate, RER and effort perception were measured. On day 7, subjects completed a 45 minute constant-load cycle at 40% W_{peak} followed by a 200-km performance time-trial on day 8, during which blood samples were collected and effort perception, heart rate, RER and power output were recorded. Ingestion of the HFD-CHO reduced RER at rest ($p < 0.001$), during exercise training ($p < 0.005$) and during the 200-km time-trial ($p < 0.05$) compared to the HCD-CHO. Despite an increase in fat utilization during exercise in response to the HFD-CHO compared to the HCD-CHO, mean 200-km time-trial performance was not different between the two dietary interventions, with 5 of the 9 subjects improving performance on the HFD-CHO compared to the HCD-CHO. When the subjects were divided into high-RER and low-RER groups based on their fasting resting RER, rates of fat oxidation during the 200-km time-trial tended to be lower in the low-RER group compared to the high-RER group ($p = 0.084$) in response to the HFD-CHO. Although overall 200-km time-trial performance was not different between phenotypes or diets, the low-RER group completed the time-trial ~6 minutes faster following the HFD-CHO compared to the HCD-CHO. In contrast, the high-RER group completed the time-trial ~10.5 minutes faster following the HCD-CHO compared to the HFD-CHO. Furthermore, climb performance was significantly improved in the low-RER group but not in the high-RER group, following the HFD-CHO compared to the HCD-CHO ($p < 0.05$). In conclusion, the HFD-CHO increased fat oxidation without demonstrating a clear overall mean performance benefit during a simulated ultra-endurance cycle race. However, the preliminary

evidence suggests that the HFD-CHO strategy might be beneficial for athletes with a low-RER phenotype participating in ultra-endurance events where an increase ability to utilize fat may enable the athlete to meet the increased energy demands of exercise (i.e. during the climbs).

In summary, this thesis demonstrated that the majority of sub-elite cyclists that participated in an ultra-endurance cycle race failed to meet recommended pre-race CHO intakes, including the majority of those who attempted to CHO-load prior to an ultra-endurance event. This thesis further demonstrated that 6 days of fat-adaptation followed by 1 day of CHO-loading induced metabolic adaptations that increased fat oxidation during exercise without significantly affecting overall 100-km or 200-km performance. However, the fat-adaptation strategy compromised high-intensity sprint performance during a 100-km time-trial in all subjects, but improved prolonged climb performance during the 200-km time-trial in the majority (89%) of subjects. In addition, this thesis demonstrated individual differences in 100-km and 200-km performance in response to the different diets, suggesting individuality in response to the dietary strategies. Indeed, this thesis demonstrated that RER phenotypes persisted during exercise despite 3 days of CHO-loading and 6 days of fat-adaptation. Although not significant, ultra-endurance exercise performance in response to the dietary strategies tended to be dependent on the RER phenotype. However, future research is required to confirm whether the RER phenotype affects the efficacy of dietary interventions and should therefore be considered when prescribing a specific pre-race dietary strategy for athletes prior to endurance and ultra-endurance exercise.

CHAPTER 1

Literature Review

University of Cape Town

1.1 INTRODUCTION

Carbohydrate (CHO) and fat serve as the two main substrates for the production of energy during prolonged muscle contraction. Although CHO and fat are oxidized simultaneously, the relative contribution of these substrates to oxidative metabolism during exercise varies, and is dependent on a variety of factors including exercise intensity and duration, substrate availability (69) and training status (9), amongst other factors (3; 103).

The effects of exercise intensity on substrate oxidation are presented in Figure 1.1 (151). Romijn et al. (151) demonstrated that plasma free fatty acids (FFA) derived from adipose tissue lipolysis were the major fuel source utilized at a low exercise intensity (25% VO_{2peak}).

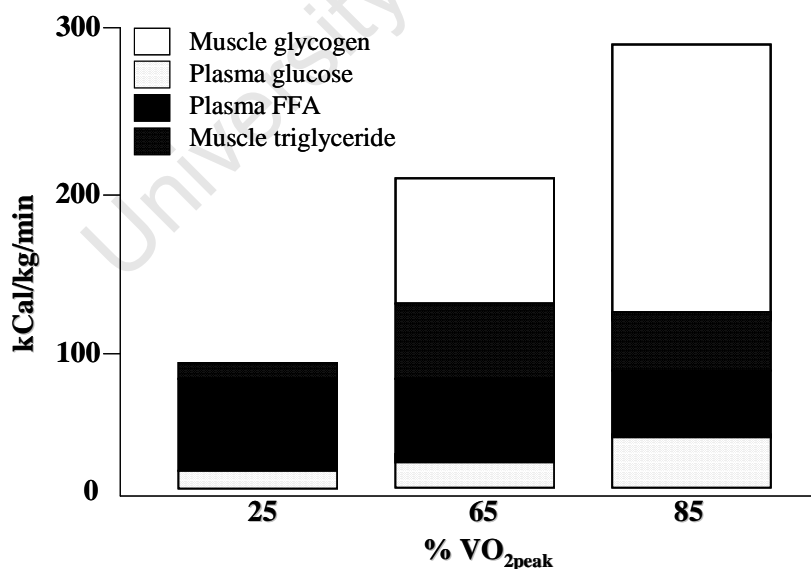


Figure 1.1: Substrate utilization during low, moderate and high exercise intensities (151).

At a moderate exercise intensity (65% VO_{2peak}), energy requirements increased more than 2 fold and, for the most part, were met by an increase in muscle glycogen utilization. Although increases in catecholamine concentrations at the higher exercise intensity stimulated an increase in lipolysis, Romijn et al. (151) demonstrated that the increase in fat oxidation was the result of an increase in estimated intramuscular triglyceride (IMTG) oxidation, and not an increase in adipose tissue lipolysis (Figure 1.1) (151). At 65% VO_{2peak} the relative contribution of fat and CHO to total energy were very similar. When the exercise intensity was further increased to 85% VO_{2peak} , muscle glycogen oxidation increased significantly and became the predominant fuel source for the working muscle, contributing more than 60% to total energy expenditure. In contrast the relative contribution of IMTG and plasma FFA decreased significantly at 85% VO_{2peak} (Figure 1.1) (151). Therefore, the absolute contribution of fat to the total energy production during exercise increased from low intensity exercise and peaked at a moderate intensity (~45-65% VO_{2peak}), after which a decline in the rate of fat oxidation and a concomitant increase in the rate of CHO oxidation was observed as the exercise intensity increased (3; 151).

Coyle (41) examined the effect of exercise duration on the shift in substrate utilization during prolonged exercise at moderate intensity (65-75% VO_{2peak}). As the exercise duration increased, an increase reliance on plasma FFA and glucose occurred (Figure 1.2). At the onset of exercise, fat and CHO contributed equally to total energy expenditure. As the exercise duration increased the relative contribution of fat, mostly derived from plasma FFA, increased to ~60% of total energy expenditure. In contrast, the relative contribution of total CHO to

energy utilization decreased to ~40%. Muscle glycogen concentrations decreased significantly over the 4 hours of exercise, resulting in an increased reliance on plasma glucose (Figure 1.2).

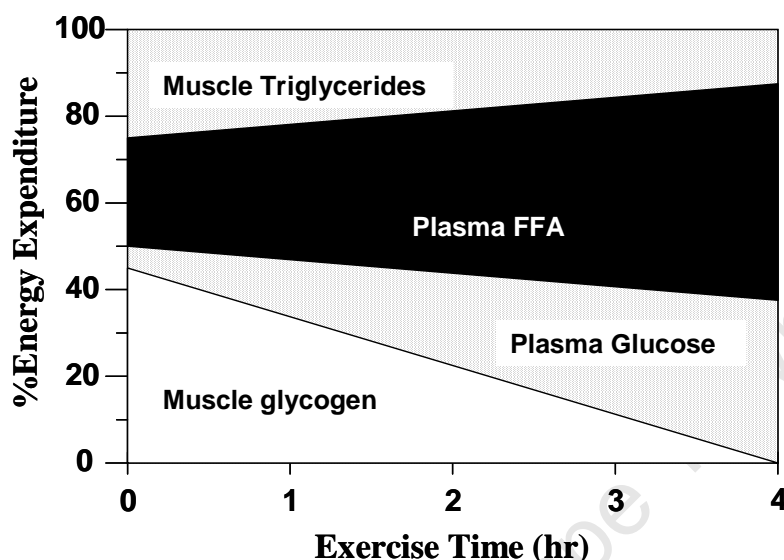


Figure 1.2: Percentage of energy derived from the major substrates during prolonged exercise at 65-75% VO_{2peak} (151).

Endurance (>90 minutes) and ultra-endurance exercise (>4-5 hours) are typically undertaken at a moderate-to-high (65-85% VO_{2peak}) exercise intensity during which muscle glycogen is the predominant fuel (Figure 1.1), especially during the earlier stages of exercise (Figure 1.2) (41; 151). Studies have shown that the ingestion of a high CHO diet (7-10 g CHO/kg body mass) 3 days prior to an endurance event can maximize pre-exercise muscle glycogen stores and improve prolonged endurance time to fatigue (5; 12; 59; 116). As a result, endurance athletes are often advised to ingest a high CHO diet or “carbohydrate-load” (CHO-load) during the few days prior to an endurance event. However, the body’s endogenous glycogen stores are restricted to a maximum of approximately 350-500 g (58) and are significantly depleted after

2-3 hours of moderate intensity (65-75% VO_{2peak}) exercise in the fasted state (5; 12; 116). The potential of endogenous carbohydrate stores alone to fuel endurance events lasting longer than 2-3 hours, are therefore limited.

It has been suggested that an increased ability to utilize fat during exercise may be of particular benefit to performance during longer duration endurance and ultra-endurance events. Indeed, more recent nutritional strategies have not only focused on optimizing pre-exercise muscle glycogen stores, but also on increasing fat oxidation during exercise, in an attempt to improve endurance exercise performance (26; 28; 33). A dietary strategy that has been associated with an increase in fat oxidation during exercise and a concomitant 'sparing' of muscle glycogen is 5-6 days of "fat-loading" followed by 1 day of CHO-loading (26; 28; 33).

This review will provide an overview of CHO and fat metabolism during exercise, and explore the mechanisms underlying the shift in substrate utilization in response to dietary interventions, in particular a high fat diet. Further, this review will summarize the performance outcomes of studies that have investigated the effect of different pre-race nutritional strategies aimed at increasing CHO availability (e.g. CHO-loading) and/or 'sparing' endogenous glycogen stores during exercise (e.g. fat-loading) on exercise performance. In addition, this review will introduce the concept of individuality of response to dietary interventions.

1.2 REGULATION OF SUBSTRATE UTILIZATION DURING EXERCISE

Both endogenous CHO and fat are utilized during exercise for energy production. They enter distinct metabolic pathways producing one common metabolite, acetyl-CoA, which enters the tricarboxylic acid (TCA) cycle and electron transport chain, to produce a high energy adenosine triphosphate (ATP). Regulation of skeletal muscle CHO and fat metabolism is multi-factorial, and different mechanisms may dominate in different conditions (i.e. rest vs. exercise or in the fed or fasted states). A detailed discussion of the regulation of CHO and fat metabolism is documented elsewhere (49; 91; 101; 109; 171), however this review will provide a brief overview of CHO and fat utilization during exercise in human skeletal muscle, and will focus on the potential sites of regulation.

1.2.1 CHO utilization during exercise

Exercise stimulates an increase in glycogenolysis and skeletal muscle glucose uptake (94) that results from a coordinated increase in rates of glucose delivery, increase membrane glucose transport and an increase intracellular substrate flux through glycolysis (153). The rate of glycogenolysis and glucose uptake is mainly determined by exercise intensity, with a greater rate of glycogenolysis and muscle glucose uptake at higher intensities (41; 151). As mentioned in section 1.1, muscle glycogen is the major source of energy during moderate-to-high exercise intensities (Figure 1.1). However, as the exercise duration increases, muscle glycogen decreases and oxidation from plasma glucose increases (Figure 1.2). The major metabolic fate of plasma glucose entering the

skeletal muscle cell during exercise is glycolysis (195) and subsequent oxidation (105).

CHO metabolism during exercise in the skeletal muscle: A brief overview.

Glycolysis is the anaerobic process in the muscle cytoplasm whereby glucose and Glucose-6-Phosphate (G6P) (from glycogenolysis) are converted to pyruvate by a number of steps that are outlined in Figure 1.3.

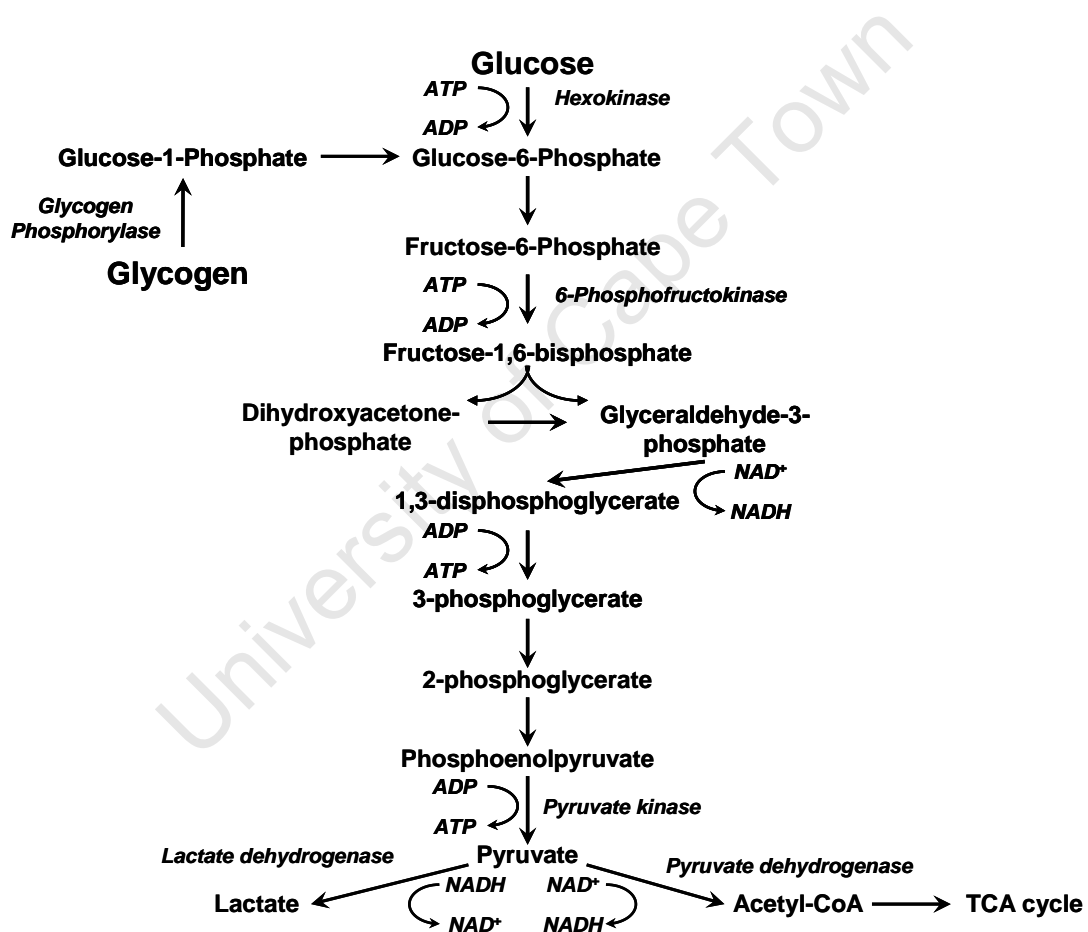


Figure 1.3. Overview of glycolysis and glycogenolysis (101)

After glucose has been transported across the muscle membrane by a specific transporter protein GLUT4, the glucose molecule is irreversibly phosphorylated to G6P by the enzyme hexokinase (HK) (153). The HK reaction is an energy-consuming reaction, requiring one molecule of ATP for each molecule of glucose, and is inhibited by an accumulation of G6P. If muscle glycogen, rather than blood glucose, is the substrate for glycolysis, a single glucose molecule is split off by the enzyme glycogen phosphorylase and converted to glucose-1-phosphate (G1P) and subsequently to G6P through the process of glycogenolysis (Figure 1.3) (101). After further phosphorylation, the glucose/G6P molecule is cleaved to form 2 molecules of the 3-carbon sugar glyceraldehyde-3-phosphate (G3P). The second stage of glycolysis involves the conversion of G3P to pyruvate, accompanied by the formation of ATP and the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH. The net effect of glycolysis is the conversion of 1 molecule of glucose to 2 molecules of pyruvate, with the formation of 2 molecules of ATP and the conversion of 2 molecules of NAD^+ to NADH. Glycolysis is primarily regulated by phosphofructokinase (PFK), which is allosterically modified by activators such as inorganic phosphate and AMP, and inhibited by high levels of adenosine 5'-triphosphate (101; 109).

Pyruvate either undergoes oxidative metabolism to carbon dioxide (CO_2) and water in the mitochondria, or is reduced to lactate to reproduce NAD^+ with an increase in glycolytic flux (172; 180). During oxidative metabolism, pyruvate enters the mitochondria and is converted to Acetyl-CoA in a reaction catalyzed

by pyruvate dehydrogenase (PDH). Acetyl-CoA is then oxidized to CO_2 in the tricarboxylic acid cycle (TCA cycle).

The conversion of pyruvate to acetyl-CoA is the first irreversible step in the oxidation of CHO-derived carbon, and regulates the entry of CHO into the TCA cycle. PDH activity is tightly regulated by reversible phosphorylation and dephosphorylation reactions catalyzed by an intrinsic PDH kinase (PDK) and phosphatase (PDP) (180). The amount of PDH in its active form (PDHa) determines its activity and is regulated through the action of PDP which dephosphorylates and activates PDH and PDK. The intrinsic PDK in turn phosphorylates and inhibits PDH (inactive form PDHb) (Figure 1.4) (132).

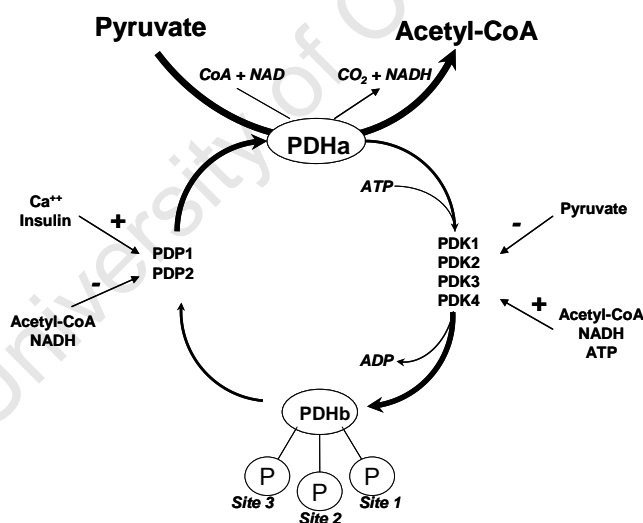


Figure 1.4. Allosteric regulation of the pyruvate dehydrogenase (PDH) enzyme complex (135). PDHa, activated pyruvate dehydrogenase; PDHb, inactivated pyruvate dehydrogenase; PDP, PDH phosphate isoforms; PDK, PDH kinase isoforms.

Phosphorylation and deactivation of the complex is catalyzed by a category of 4 isoforms (PDK1-4) which differ in their responsiveness to allosteric inhibition by

pyruvate, or activation by energy charge (ATP/ADP), redox (NADH/NAD⁺ ratio) and acetyl-CoA-to-free CoA ratio (180).

Regulation of muscle glucose uptake during exercise

Glucose transport across the muscle cell membrane during exercise is facilitated mainly by the glucose transporter GLUT4 (78). During exercise, an increase in glucose transport is due to an increase in the number of GLUT4 transporter proteins in the plasma membrane, as a result of GLUT4 translocation from the intracellular storage site (70; 71; 100). GLUT4 translocation during exercise is stimulated by muscle contraction and is independent of insulin (22; 72; 96). Although relatively little is known about how exercise regulates skeletal muscle GLUT4 translocation, calcium (Ca⁺⁺) has been recognized as a stimulator of glucose transport in the working muscle (88; 100) and may act via signaling pathways sensitive to Ca⁺⁺ such as protein kinase C (PKC) and the Ca⁺⁺/calmodulin-dependent protein kinase (CaMK) (Figure 1.5).

Other signals that stimulate GLUT4 translocation during exercise include AMP-activated protein kinase (AMPK) and mitogen-activated protein kinase (MAPK) (77; 157). During muscle contraction, AMPK is activated by a decrease in phosphocreatine and adenosine triphosphate (ATP) and an increase in adenosine monophosphate (AMP) (197) (Figure 1.5). Although MAPK has been implicated in glucose transport (157), more studies are required to elucidate the importance of MAPK pathways in activating GLUT4 translocation.

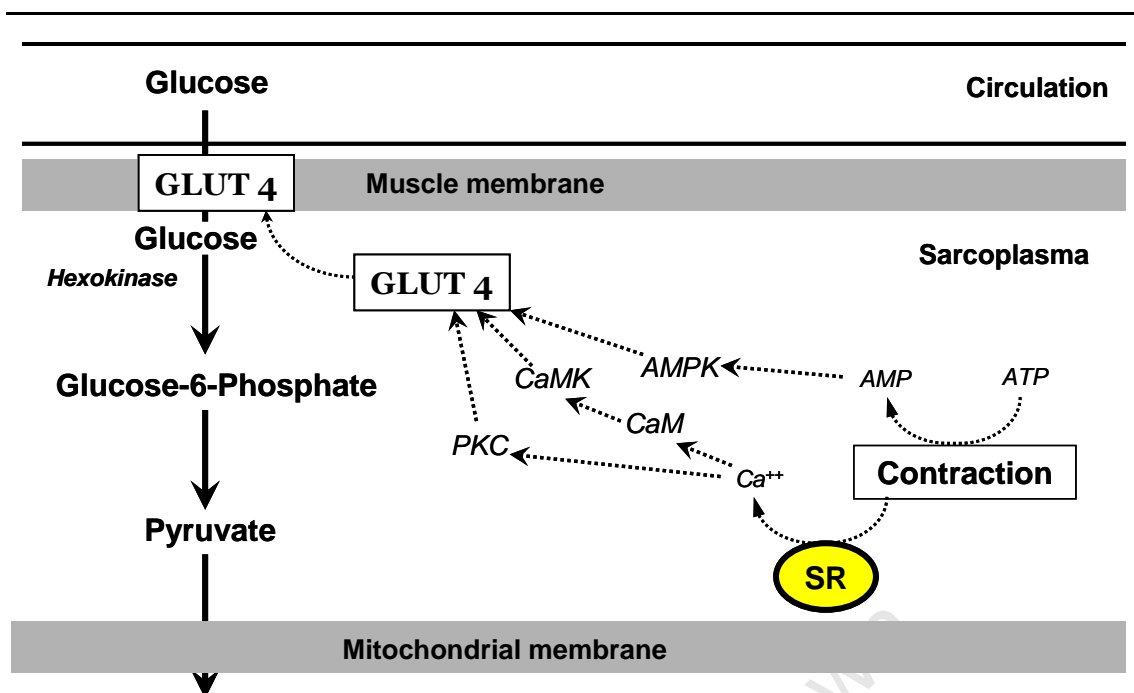


Figure 1.5: A schematic overview of GLUT4 regulation in the working muscle. ATP, adenosine triphosphate; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; Ca⁺⁺, calcium; CaM, calmodulin; CaMK, calcium/calmodulin-dependent kinase; PKC, protein kinase C; SR, sarcoplasmic reticulum.

The effects of glycogen and glucose availability on CHO oxidation during exercise

Several studies have reported that the rate of glycogen utilization during exercise is directly related to starting glycogen concentration (18; 74; 147; 193; 7; 69; 170). Therefore, an increase in pre-exercise muscle glycogen results in an increase in muscle glycogen utilization during exercise. Although pre-exercise muscle glycogen determines subsequent glycogen utilization rates during exercise, rates of plasma glucose oxidation were not affected by pre-exercise glycogen content (7; 18; 74; 192). In contrast, ingestion of exogenous CHO during exercise increased rates of plasma glucose oxidation (7; 19). Furthermore, the oxidation rates of ingested CHO tended to increase when

larger amounts of CHO were consumed during exercise, and was found to have a liver glycogen sparing effect (19).

1.2.2 Fat utilization during exercise

Although fat is potentially an excellent energy source for prolonged exercise, the capacity to oxidize fat is limited, especially during higher intensity exercise. The contribution of fat to the total energy production during exercise increases from low intensity exercise and peaks at a moderate intensity (~45-65% VO_{2peak}), after which a decline in the rate of fat oxidation is observed as the exercise intensity increases (3; 151). The mean exercise intensity that elicited a maximal fat oxidation rate (FATmax) in well-trained athletes is ~62-63% VO_{2peak} (1). Plasma FFAs contribute to 40-60% of total fat oxidation, while circulating triglycerides in very low density lipoproteins (VLDL) and IMTG provide the remainder (151; 187). The mechanisms that down-regulate fat metabolism in the transition from moderate-to-high exercise intensities are not completely understood.

Fat metabolism during exercise in the skeletal muscle: A brief overview.

The oxidation of FFA in the working muscle involves a number of steps that are outlined in Figure 1.6 (171). FFA from the adipose tissue are transported via the circulation, bound predominantly to albumin, and delivered to the working muscle. Circulating FFA are transported into the muscle cytoplasm by fatty acid binding proteins located in the plasma membrane (FABPpm, FAT/CD36 and FATP) and transported to the surface of the outer mitochondrial membrane by a fatty acid binding protein present in the cytosol (FABPc) (2; 61).

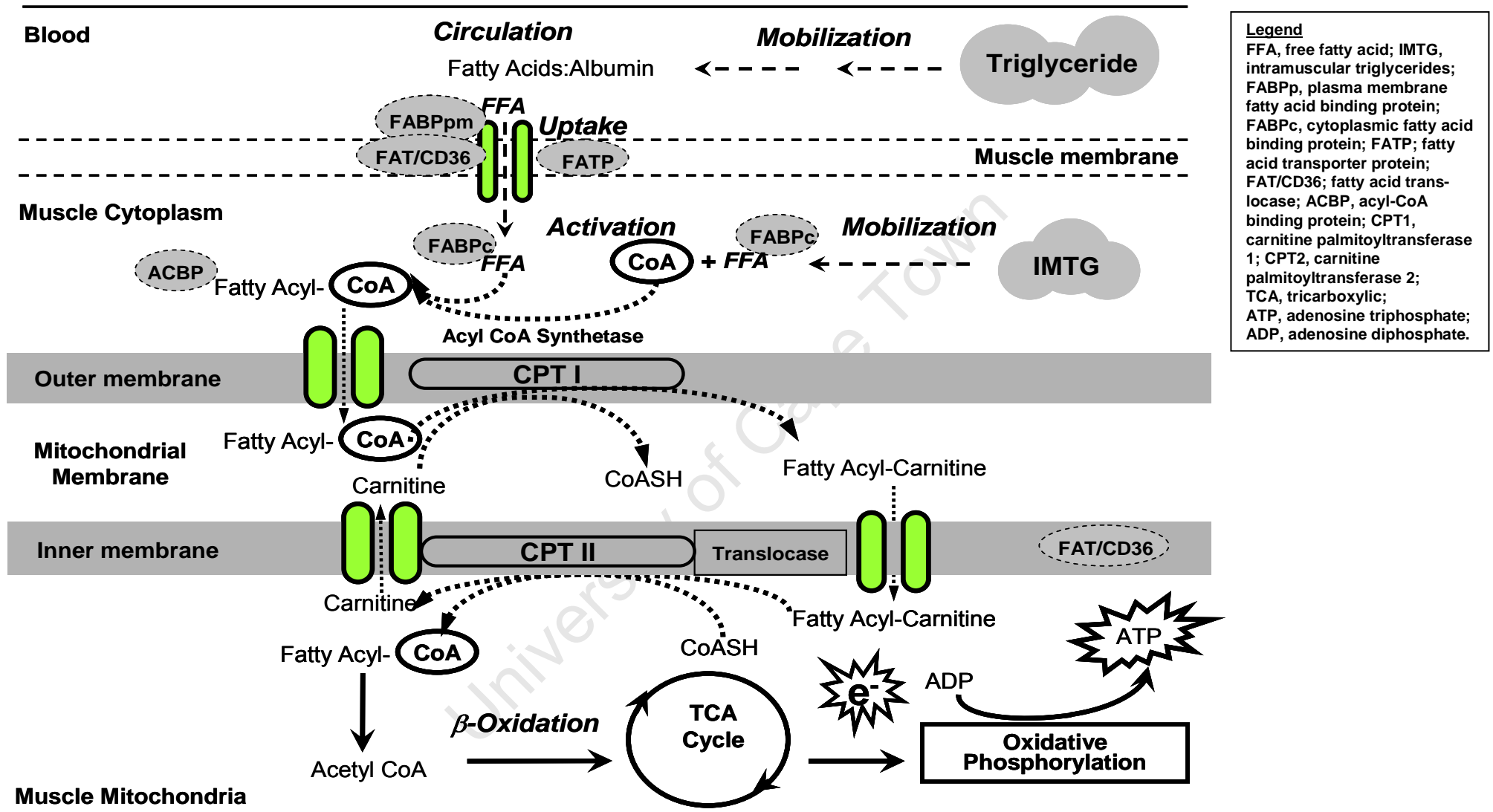


Figure 1.6 A schematic overview of fat oxidation in the working muscle.

FFA released by the hydrolysis of intramuscular triglycerides (IMTG), are also transported to the surface of the outer mitochondrial membrane by FATPc. The FFA are then activated via binding to coenzyme A (CoA) by the action of acyl-CoA synthetase and converted to fatty acyl-carnitine by the action of carnitine palmitoyltransferase 1 (CPT-1) located on the outer mitochondrial membrane (124).

Fatty acyl-carnitine is then transported through the inner mitochondrial membrane via translocase in exchange for free carnitine. Inside the mitochondria the carnitine is removed by the action of carnitine palmitoyltransferase 2 (CPT-2), an enzyme located on the inner-mitochondrial membrane (124). The fatty acyl-carnitine is reconverted to acyl-CoA that is made available for β -oxidation, from which acetyl-CoA then enters the TCA cycle for complete oxidation (171) (Figure 1.6).

The potential mechanisms that regulate skeletal muscle fat oxidation during exercise include:

- 1) Adipose tissue lipolysis and FFA delivery to the muscle;
- 2) Transport of FFA across the muscle membrane;
- 3) Lipolysis of intra-muscular triglycerides (IMTG) by triacylglycerol lipase (TG lipase) activity;
- 4) FFA transport across the mitochondrial membrane (CPT-1 activity);

Adipose tissue lipolysis and FFA delivery to the muscle

During exercise, an increase in circulating catecholamines and β -adrenoreceptor stimulation increase adipose tissue lipolysis by activation of hormone sensitive lipase (HSL), via a cascade of cellular signals (91). β -adrenergic stimulation activates adenylate cyclase that catalyzes the conversion of ATP to cyclic AMP (cAMP), which acts as a 2nd messenger to activate cAMP-dependent protein kinase, which then phosphorylates HSL and perilipins. Phosphorylated HSL moves from the cytosol of the adipocyte to the surface of the lipid droplet within the cell (91). Phosphorylation of perilipins enables HSL to gain access to intracellular triglycerides, possibly by modifying the surface of the lipid droplet and providing a docking site for phosphorylated HSL (21). The net result is the degradation of TG to glycerol and FFA, which is released into the blood and delivered to the working muscle.

HSL has long been considered the only lipase hydrolyzing TG, however more recent evidence have shown that a second lipase know as adipose triglyceride lipase (ATGL), also plays an important role in hydrolyzing TG (166). Unlike HSL, ATGL activation is not dependent on phosphorylation and translocation to the lipid substrate. ATGL is localized on the lipid droplet in the basal and hormone-stimulated state of the cell and is regulated by an activator protein. ATGL predominantly hydrolyzes TG to form diglycerides (DG) that is then further hydrolyzed by HSL. (166). HSL hydrolyzes both TG and DG however the affinity for DG is 10-fold higher (156).

The delivery of FFA to the working muscle is a function of FFA concentration and blood flow to the muscle (151). Blood flow increases as a function of power

output, therefore, at a higher power output or exercise intensity, blood flow and FFA delivery to the muscle is higher. Romijn et al. (151) demonstrated that FFA delivery to the muscle increases as a function of power output at 25% and 65% VO_{2peak} , without a further increase at 85% VO_{2peak} . At 85% VO_{2peak} , the concentration of FFA decreased by ~50%. This was however, not due to a decrease in adipose tissue lipolysis, as demonstrated by an increase in plasma glycerol concentrations. The authors speculated that this was due to decreased adipose tissue blood flow during very high exercise intensities that resulted in decreased FFA delivery from the adipose tissue (FFA entrapment in the adipocyte) (151). However, when FFA delivery was artificially increased with lipid-heparin infusion, FFA uptake and oxidation increased by 27% at 85% VO_{2peak} but was still lower than the rate measured at 65% VO_{2peak} , suggesting that factors other than only FFA availability regulate lipid oxidation at high exercise intensities (152).

Transport of FFA across the muscle membrane

Until recently, it was believed that FFA simply diffused through the lipid layer of the muscle membrane. However there is now strong evidence that the majority of FFA enter muscle cells via protein-mediated mechanisms (122). A number of fat transport proteins have been identified and include: the fatty acid binding protein in the plasma membrane (FABPpm) (122), the fatty acid transport protein (FATP), also situated at the plasma membrane, and the fatty acid translocase (FAT/CD36), a transport protein with a high affinity for FFA located at the plasma membrane, and at the mitochondria membrane (15; 89). While FFA transport across the muscle membrane is facilitated by FATP, FAT/CD36

and FABPpm, intracellular transport of FFA is mediated by a cytoplasmic fatty acid binding protein (FABPc) (61-63). In addition to the fatty acid binding proteins, there is evidence from cell lines HEP2G and endothelial capillary cells to suggest that caveolin, an important protein for the formation of caveolae (flask-shaped invaginations of the plasma membrane) is also implicated in FFA uptake and intracellular trafficking of FFA (139; 148). The acyl-CoA compound is also transported by an acyl-CoA binding protein (ACBP) (144).

Earlier studies suggested a positive correlation between plasma FFA concentration and FFA uptake into the muscle cell during exercise (151). However, more recent studies, in which higher concentrations of FFA have been reached during prolonged submaximal exercise, revealed a leveling off in net uptake of FFA despite increasing concentrations (110; 184). This relationship is altered by endurance training, as Kiens et al. (110) have found that FFA uptake and oxidation during exercise was greater in the trained muscle compared to the untrained muscle. This increase may be mediated by an increase in muscle FATPpm content associated with exercise training (110; 112). Although there is evidence that the fatty acid binding proteins facilitate FFA transport across the muscle membrane and possibly across the mitochondrial membrane, the transport process is not necessarily a rate limiting step for FFA utilization during exercise. Indeed, Roepstorff et al. (150) demonstrated that FFA uptake following a high fat vs. high CHO diet was not significantly different, however the fraction FFA uptake that was oxidized was significantly higher in the high fat compared to the high CHO diet. The authors concluded that transport across the muscle membrane is not limiting plasma

FFA oxidation, rather oxidation rates seems to be limited by intracellular regulatory mechanisms (150).

Lipolysis of IMTG by muscle triacylglycerol (TG) lipase activity

The importance of IMTG contribution to total fat oxidation during exercise is not yet fully understood, however, the majority of studies that have examined IMTG oxidation during prolonged exercise, have demonstrated a 20-40% decrease in IMTG stores (182; 185). The hydrolysis of IMTG during exercise is also less clear, but similarities have been found between IMTG and adipose tissue triglyceride lipolysis. IMTG are also catalyzed by the muscle version of hormone sensitive lipase in response to β 2-adrenergic stimulation (114; 121). An exercise-induced increase in epinephrine increases HSL activity through phosphorylation of extracellular-regulated kinase (190) (Figure 1.7). Muscle HSL has a natural pH optimum and is covalently activated by the action of a kinase that adds a phosphate group and deactivated by a phosphatase that removes a phosphate group similar to the activation of HSL.

Muscle HSL activity can also increase independently of adrenergic stimulation and involves HSL phosphorylation, possibly mediated by Ca^{++} release during muscle contractions (119; 120).

FFA transport across the mitochondrial membrane

FFA transport across the mitochondrial membrane for subsequent β -oxidation is regulated by the carnitine palmitoyltransferase (CPT) complex, consisting of carnitine palmitoyltransferase 1 (CPT-1) and carnitine palmitoyltransferase 2

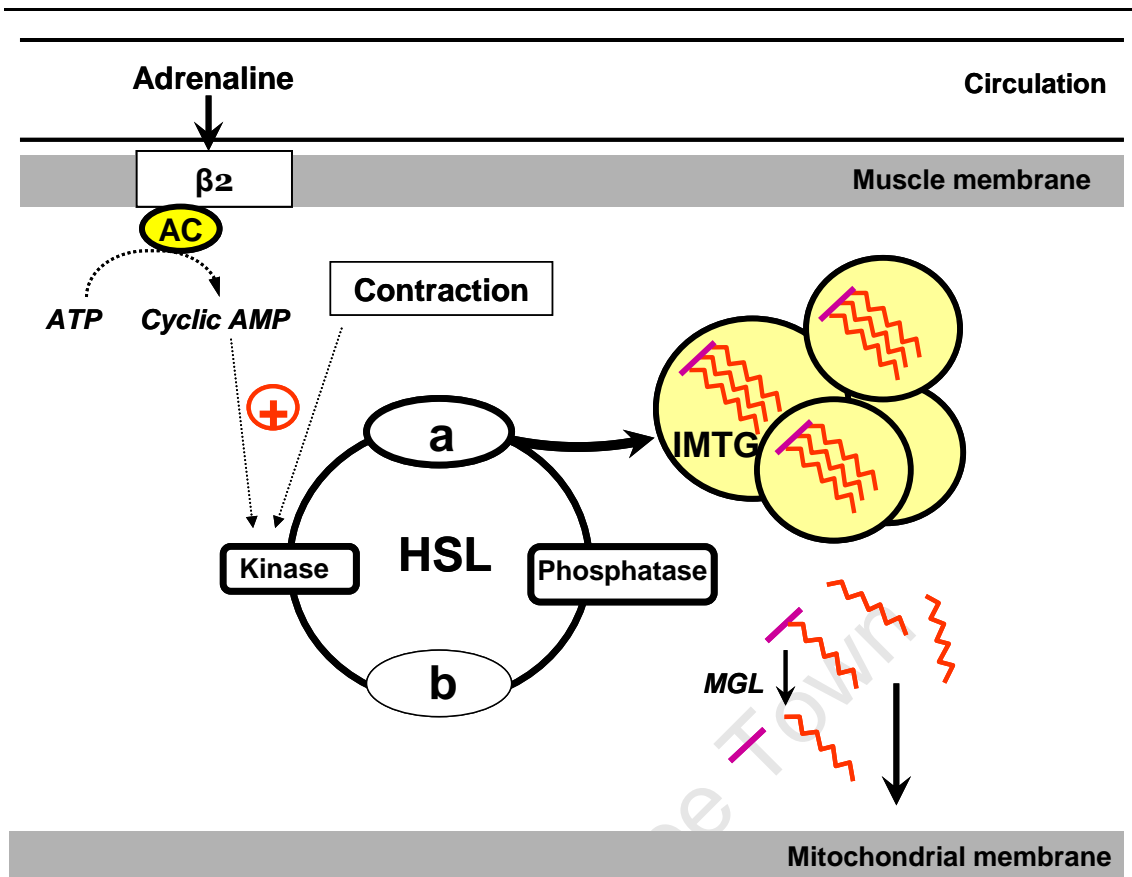


Figure 1.7. Schematic diagram outlining the regulation of intramuscular lipolysis during exercise. AC, Acetyl coxylase; ATP, adenosine triphosphate; HSL, hormone sensitive lipase; a, activated form of HSL; b, unactivated form of HSL; IMTG, intramuscular triglyceride; MGL, monoglyceride lipase.

(CPT-2) (124). CPT-1, the enzyme located on the outer mitochondrial membrane catalyzes the transfer of the fatty acyl group from acyl-CoA to acyl-carnitine (171) (Figure 1.4). CPT-1 is considered the major rate limiting step in the transport of FFA into the mitochondria for subsequent oxidation (14; 124) and a couple of mechanisms, including malonyl-CoA, the cytosolic concentrations of carnitine, and pH have been proposed for the intracellular regulation of CPT-1.

McGarry et al. (125) first described the 'Reverse Randle' and hypothesized that high rates of glycolytic flux during high intensity exercise could result in the

accumulation of acetyl-CoA in the muscle cell, which via the subsequent production of malonyl-CoA, the first intermediate in FFA synthesis via acetyl-CoA carboxylase (ACC), could lead to inhibition of CPT-1 activity (176). However, muscle malonyl-CoA concentrations have been found not to increase during high-intensity exercise (127).

The second mechanism that has been proposed to down-regulate CPT-1 activity during high-intensity exercise included a decrease in intracellular free carnitine availability (187). In the skeletal muscle, carnitine plays an essential role in the translocation of FFA into the mitochondrial matrix for subsequent β -oxidation, therefore decreased levels of free carnitine may limit FFA uptake into the mitochondria. Carnitine can be acetylated by acetyl-CoA when the rate of acetyl-CoA formation from pyruvate exceeds the rate of acetyl-CoA utilization by the TCA cycle (22). Therefore, during high intensity exercise, the increased the rate of glycogenolysis enhances mitochondrial acetyl-CoA production and acetyl-carnitine formation, decreasing the availability of free carnitine (86; 187) thereby reducing CPT-1 activity. Finally, reductions in intracellular pH that may occur during high-intensity exercise may also inhibit CPT-1 and, hence, FFA transport into the muscle (174).

1.2.3 The interaction between CHO and fat utilization during exercise.

Exercise intensity and duration determine, for the most part, the relative contribution of CHO and fat to total energy production. The relative contribution of CHO increases with intensity whereas the relative contribution of fat increases with duration (151). It was postulated that an increase in acetyl-CoA,

due to increased glycolytic flux during higher exercise intensity, would increase the production of malonyl-CoA via ACC, and therefore reduces FFA uptake and oxidation due to reduced CPT-1 activity (176). However, muscle malonyl-CoA concentrations have been found not to increase during high-intensity exercise (127). It was further proposed that an increase in PDH flux during higher intensities, lowers the the available free carnitine and thereby limiting the ability of CPT-1 to transport long-chain acyl-CoA into the mitochondria for subsequent oxidation (177; 187).

As the duration of exercise increases, muscle glycogen levels decreases and as a result the relative contribution of fat oxidation to total energy increases. In fact, fat utilization during exercise has been shown to be largely regulated by the state of endogenous CHO stores (192). Low muscle glycogen stores significantly increased fat oxidation during exercise, even when euglycemia was maintained with glucose infusion (193). In contrast, when muscle glycogen was normal or supercompensated, CHO oxidation was improved and fat oxidation was attenuated. The ingestion of CHO prior to exercise has also been shown to regulate fat utilization, mainly via increased insulin concentrations that attenuate lipolysis (93). CHO ingestion during exercise in the fasted state slightly attenuated lipolysis, but did not suppress fat oxidation (92).

Effects of endurance training on CHO and fat utilization during exercise

Endurance training results in a shift in substrate metabolism with a greater reliance on fat and sparing of limited glycogen stores at the same absolute exercise intensity (87; 95; 123). Adaptive responses to endurance training

include a number of structural and metabolic adaptations in the skeletal muscle. Training enhances the capillarization of the skeletal muscle, thereby increasing the lipolytic capacity of muscle LPL (113). Endurance training also increases the ability to store and utilize a greater amount of IMTG during prolonged exercise (95). Furthermore, endurance training increases FABPpm expression, resulting in increased FFA uptake and oxidation (112) and it also increases 3-HAD (110), citrate synthase and malate dehydrogenase activity (60; 184) as well as CPT-1.

In contrast, endurance training has shown to decrease CHO utilization at the same *absolute* exercise intensity (34) due to a reduced uptake of glucose by the skeletal muscle (10). Paradoxically, skeletal muscle GLUT4 protein content increased during exercise following endurance training (136), suggesting that factors other than skeletal muscle GLUT4 content are involved in the regulation of glucose uptake during exercise following exercise training.

Various strategies nutritional and training strategies have aimed to enhance fat utilization during exercise by manipulating the regulatory steps involved in fat metabolism. This review will focus on investigations that have manipulated pre-exercise muscle glycogen concentration using chronic diet and or diet/exercise interventions.

1.3 NUTRITIONAL STRATEGIES AIMED TO IMPROVE ENDURANCE PERFORMANCE

1.3.1 Carbohydrate-loading

Christensen and Hansen (36) were the first to demonstrate the importance of carbohydrate for exercise capacity in the 1930's when they demonstrated an improved individual capacity for prolonged exercise in 3 trained subjects after a 3-day high CHO diet vs. a 3-day low CHO diet. The reintroduction of the muscle biopsy technique in the 1960s enabled scientists to directly measure muscle glycogen utilization in response to exercise and demonstrated a relationship between muscle glycogen and endurance exercise performance. Alhborg and colleagues (4) were the first to report a strong positive correlation ($r=0.68$) between pre-exercise muscle glycogen content and exercise time to fatigue in nine untrained volunteers who exercised to exhaustion at a workload corresponding to 62% of the load that elicited a heart rate of 170bpm. Shortly thereafter, Hermansen et al. (85) investigated the relationship between muscle glycogen and endurance capacity during a moderate intensity (77% VO_{2peak}) cycle to fatigue in 10 untrained and 10 trained subjects. Mean exercise time to fatigue was correlated with initial muscle glycogen content and was near depletion for all subjects at exhaustion (85). As a result, subsequent studies focused on increasing pre-exercise muscle glycogen through various diet and exercise protocols with the aim to further improve prolonged exercise time to fatigue.

The relationship between CHO-loading and endurance exercise capacity is presented in Table 1.1. Bergstrom and Hultman (13) demonstrated that muscle glycogen stores could be increased to a greater degree if they were depleted prior to the loading phase. Subsequently, Bergstrom et al. (12) introduced the 'classic super-compensation protocol', which consisted of a glycogen-depleting

exercise bout on day 1 followed by 3-days of a high fat/protein diet and another exhausting bout of exercise at 75% VO_{2peak} on day 4, followed by 3-days of a high CHO intake (12). Bergstrom et al. (12) reported higher muscle glycogen values (192% vs. 182% of starting value) and increased sub-maximal exercise time to fatigue at 75% VO_{2peak} ($\pm 38\%$) with this classic depletion-loading protocol compared to 3 days of CHO-loading only. In a companion article, Alhborg et al. (5) demonstrated a similar increase in muscle glycogen (192% vs. 164% of starting value) and an increased sub-maximal exercise time to fatigue ($\pm 57\%$) when employing a 3-day high fat/protein diet followed by 3 days of CHO-loading compared to a 3-day habitual diet followed by 3 days of CHO-loading. The authors of both studies concluded that endurance time to fatigue during sub-maximal exercise is related to pre-exercise muscle glycogen levels and that fatigue occurs as a consequence of muscle glycogen depletion. On the basis of this conclusion, the common practice of CHO-loading has been widely accepted as the nutritional strategy of choice for endurance exercise. Subsequently, many studies have examined the effectiveness of these glycogen-loading strategies to improve performance, using different exercise and dietary protocols.

Galbo et al. (59) demonstrated a 150% increase in pre-exercise muscle glycogen levels and a 66% improvement in running endurance capacity following 4 days of high CHO intake (without the depletion phase) compared to 4 days of low CHO intake (Table 1.1). Subjects in this study (59) started the performance test with very low pre-exercise glycogen levels following the low

Table 1.1: Effect of Carbohydrate-loading on moderate intensity exercise (60-75% VO_{2peak}) capacity (time to fatigue)

| Reference | Subjects | Dietary and training protocol | Performance protocol and dietary intake pre- and during exercise | Muscle glycogen (mmol/kg wet weight) | | Time to fatigue |
|-----------------------------------|----------------------------|---|--|--|---|---|
| | | | | Pre-exerc | Post-exerc | |
| Christensen and Hansen. 1939 (36) | 3 T ♂ | A: 3-day high-fat, low-CHO (4% CHO) diet B: 3-day high-CHO (83% CHO) diet | Cycle to exhaustion at 60-65% VO _{2peak} . Overnight fasted? | - | - | A: 80 min B: 210 min ↑ |
| Ahlborg et al. 1967 (5) | 7 T ♂ Diets in sequence | A: Habitual diet prior to start of trial B: Cycle to exhaustion* followed by 3-day high-fat/protein, low-CHO (<1% CHO) diet. C: Cycle to exhaustion on day 4 followed by 3 days high-CHO (>90% CHO). | *Cycle to exhaustion at 85% of a workload that elicited a heart rate of 170 bpm on days 1, 4 and 7. Overnight fasted. Only water during exercise.. | A: 85 B: 35 C: 152 | A: 20 | A: 64 min B: 42 min ↓ vs. A C: 100 min ↑ vs. A |
| Bergstrom et al. 1967 (9) | 9 T ♂ Diets in sequence | A: Habitual diet prior to start of trial B: Cycle to exhaustion** followed by 3 days high-fat/protein, low-CHO (5% CHO) diet. C: Cycle to exhaustion on day 4 followed by 3 days high-CHO (82% CHO). | **Cycle to exhaustion at 75% VO _{2peak} on days 1, 4 and 7. Overnight fasted. Only water during exercise. | A: 100 B: 33 C: 183 | A: 11 B: 5.5 C: 22 | A: 114 min B: 57 min ↓ vs. A C: 167 min ↑ vs. A, B |
| Galbo et al. 1979 (59) | 7 T ♂ cross-over | A: 45 min exercise at 80% VO _{2peak} followed by 4 days high-fat, low CHO (10% CHO) B: 45 min exercise at 80% VO _{2peak} followed 4 days high-CHO (77% CHO). | Treadmill run to exhaustion at 70% VO _{2peak} . Overnight fasted. Only water during exercise. | A: 45 B: 112 | A: 35 B: 75 | A: 64 min B: 106 min ↑ |

CHO, Carbohydrate; VO_{2peak}, peak oxygen uptake; UT, untrained; T, trained. Arrows represent significant ↑, increase; ↓ decrease; ↔, no change between treatments.

Table 1.1 (Continue)

| Reference | Subjects | Dietary and training protocol | Performance protocol and dietary intake pre- and during exercise | Muscle glycogen (mmol/kg wet weight) | | Time to fatigue |
|-------------------------|------------------------------|--|--|--|------------|--|
| | | | | Pre-exerc | Post-exerc | |
| Brewer et al. 1988 (23) | 15 T ♂ 15 T ♀ 3 groups | A1: 3-day habitual mod-CHO* diet vs. A2: 3-day control (mod-CHO, fat and prot) B1: 3-day habitual mod-CHO diet vs. B2: 3-day high-CHO** (complex CHO) diet C1: 3-day habitual mod-CHO diet vs. C2: 3-day high-CHO (simple CHO) diet *mod-CHO: ~4-5 g CHO/kg BM/day **high-CHO: ~7-8 g CHO/kg BM/day | Treadmill run to exhaustion at 70% VO _{2peak} . Overnight fasted. Only water during exercise. | - | - | A1: 119 min A2: 122 min ↔ B1: 106 min B2: 133 min ↑ vs. B1 C1: 114 min C2: 141 min ↑ vs. C1 |
| Lamb et al. 1991 (116) | 14 T ♂ 2 Groups | A1: 3.5-day mod-CHO* diet (pasta) A2: 3-day low-CHO** + depletion exercise followed by 3-day high-CHO*** (pasta). B1: 3.5-day mod-CHO* diet (beverage) B2: 3-day low-CHO** + depletion exercise followed by 3-day high-CHO*** (beverage). *low-CHO: 2.4 g CHO/kg BM/day **mod-CHO: 6.1 g CHO/kg BM/day ***high-CHO: 11.6 g CHO/kg BM/day | Treadmill run to exhaustion at 75% VO _{2peak} during the afternoon of day 4 after 3.5 days of dietary intake. Only water during exercise.. | A1: 103 A2: 130 B1: 107 B2: 150 | | A1: 153 min A2: 169 min ↔ B1: 139 min B2: 168 min ↑ vs. B1 |

CHO, Carbohydrate; VO_{2peak}, peak oxygen uptake; UT, untrained; T, trained. Arrows represent significant ↑, increase; ↓ decrease; ↔, no change between treatments.

CHO diet compared to the high CHO diet (45 vs. 112 mmol/kg w.w., respectively), explaining the large difference in performance time to fatigue.

Lamb et al. (116) also examined the effect of a depletion-loading protocol on sub-maximal treadmill running time to fatigue, using either pasta or a CHO beverage to super-compensate pre-exercise muscle glycogen levels. Similar to the findings from the 1960 studies, the depletion-loading protocol increased pre-exercise muscle glycogen levels and improved exercise capacity to fatigue compared to a moderate CHO diet (116) (Table 1.1). However, running capacity was remarkably similar between the pasta and the beverage trial, suggesting that the quantity of CHO rather than the type of CHO used to super-compensate pre-exercise muscle levels determined exercise time to fatigue. In contrast, Brewer et al. (23) demonstrated a significantly greater increase in running time to fatigue following 3 days of CHO-loading with a simple CHO vs. complex CHO compared to a 3-day moderate CHO diet (Table 1.1).

The classic depletion-loading protocol, which required two exhaustive exercise bouts and 3 days of a high-fat/protein diet, often resulted in hypoglycemia, irritability and discomfort (169). Furthermore, Sherman et al. (169) showed that 3 days of high CHO intake (500-600g per day) resulted in similar pre-exercise muscle glycogen levels compared to the “classic super-compensation” protocol. In addition, Sherman et al. (169) failed to show any additional performance benefit for the depletion-loading protocol compared to 3 days of CHO-loading in well-trained. As a result Sherman et al. (169) proposed an alternative CHO-loading process that consisted of 7 days exercise taper and 3 days of high CHO

intake. More recently, Bussau et al. (31) demonstrated that a CHO intake of ~10 g/kg body mass/day combined with active rest for as little as 1 day can maximize muscle glycogen stores in well trained athletes. The subjects in that study started to CHO-load after their last training session undertaken the day before, therefore the true loading phase was ~36 hours (31). This is of practical relevance for athletes who rest on the final day before the race and do not want to CHO load for a full 3-day period.

Whilst CHO-loading (both the depletion-loading protocol and 3-4 days of loading) has been shown to improve cycling and running capacity (exercise time to fatigue) (5; 12; 23; 36; 59; 85; 116), only a few studies have tested the effects of CHO-loading on exercise performance during prolonged (>90 minutes) time-trial or race situations in which a set distance is covered as fast as possible. This is extremely relevant as the effect of CHO-loading on endurance capacity can not be extrapolated to 'real-life' race or time-trial performances. In addition, time to exhaustion has poor reliability compared with time trial protocols (102). Time-to-exhaustion tests typically have a CV of >10% compared to a CV of less than 5% for time trial protocols (46). Shorter high intensity distances have been shown to be reliable (e.g. CV for 5-km, 20-km and 40 km time-trial is 2.3, 1.1 and 0.9% respectively) as well as longer stochastic protocols such as Schabort's 100-km time-trial interspersed with 1-km and 4-km sprints [between-test CV = 0.93 (95%CI 0.79 to 0.89); within-subject CV = 1.7% (95%CI 1.1 to 2.5%) (160)].

Of the 8 studies documented in this review that have investigated the effects of CHO-loading on prolonged time-trial performance (Table 1.2), only half of these studies have demonstrated improved performance with a 3-7 day high-CHO diet (6.9-10.5 g/kg BM per day) compared to a habitual or moderate-CHO diet (~4-6 g/kg BM per day) in trained athletes (108; 146; 194; 196).

Karlsson and Saltin (108) used the depletion-loading protocol in non-randomized cross-over design and demonstrated higher pre-exercise muscle glycogen levels and a ~6% improvement in running time in response to a high CHO compared to a habitual diet. The higher starting muscle glycogen levels following the CHO-loading diet did not increase initial running speed but allowed the subjects to maintain their initial pace for longer (108). Similarly, Williams et al. (196) demonstrated a ~2% improvement in running performance in response to a 7-day CHO-loading diet compared to a moderate CHO diet. The higher intake of CHO enabled runners to increase their speed over the last 5 km of the 30-km run. The findings from the 2 previous studies are confirmed by Widric et al. (194). Using a randomized cross-over design, Widric et al. (194) investigated the effect of CHO-loading compared to a low CHO diet on 70-km cycle time-trial performance and demonstrated that the higher initial muscle glycogen content following the CHO-loading diet enabled cyclists to maintain their initial pace and increase their speed over the last 14% of the simulated 70-km time-trial (194). Moreover, performance was improved by 3.2-3.4% irrespective of whether or not CHO was ingested during the trial (194). Finally, Rauch et al. (146) also demonstrated an improved performance and a 6% higher mean power output during a 1-hour time-trial ($p < 0.05$) following a 2-hour steady-state ride

interspersed with 60-second sprints, in response to the high-CHO diet compared to the habitual diet (146).

In contrast to these studies, the remaining 4 studies reviewed in this thesis found no difference in simulated self-paced time-trial performance in response to a CHO-loading diet (~9 g CHO/kg) compared to a low (0.6 g CHO/kg) (106) or moderate CHO (4-6 g/kg BM) diet (6; 29; 181) in a group of *trained* athletes (Table 1.2). Two of these studies employed a placebo-controlled design and suggested that the subjects' awareness of high CHO ingestion rather than actual high CHO intakes may affect performance (29; 106). Johnson et al. (106) failed to demonstrate a performance effect during a ~3 hour time-trial with a high CHO compared to a very low CHO diet in a randomized placebo-controlled study.

The failure to demonstrate an overall performance effect with the CHO-loading diet may have been the result of the placebo effect and not the restriction of CHO. A decrease in power output during the last 30% of the time-trial in response to the low CHO diet compared to the high CHO diet (106) was however demonstrated, possibly due to limited glycogen levels (not measured) in response to the very low CHO diet. Burke et al. (29), failed to demonstrate a performance effect between a placebo-controlled high CHO vs. moderate CHO diet. However it is important to note that pre-exercise muscle glycogen content in this study was not significantly different between the two diets. This suggests that higher CHO intake in a high CHO diet compared to a moderate CHO diet does not provide additional benefits especially when CHO is ingested before

Table 1.2: Effect of Carbohydrate-loading on prolonged (>90 minutes) time-trial performance

| Reference | Subjects | Dietary and training protocol | Performance protocol and dietary intake pre- and during exercise | Muscle glycogen (mmol/kg wet weight) | | Performance |
|--------------------------------|--------------------------------|---|--|--|--|--|
| | | | | Pre-exerc | Post-exerc | |
| Karlsson and Saltin 1971 (108) | 10 T ♂ Diets in same order | A: Normal habitual diet B: 3-day high-fat/protein, low-CHO (<1% CHO) followed by 3-day high-CHO (9 g CHO/kg BM/day) | 30-km running race Overnight fasted? Water and 20 g CHO every 4 km. | A: 100 B: 194 | A: 29 B: 105 | A: 143 min B: 135 min ↑ All improved on B (6/10 ↑ by >5-10 min, 5/10 ↑ by only 1- 3 min) |
| Williams et al. 1992 (196) | 12 T ♂ 6 T ♀ 2 groups | A1: Habitual diet (5 g CHO kg BM/day) A2: 7-day habitual + fat and protein B1: Habitual diet (5.1 g CHO kg BM/day) B2: 7-day habitual + extra CHO (confectionary) (6.9-8.6 g CHO kg BM/day) | 30-km treadmill time-trial Overnight fasted? Water and CHO during exercise. | - | - | A1: 135 min A2: 135 min ↔ B1: 137.5 min B2: 134.5 min ↑ vs. B1 |
| Widrick et al. 1993 (194) | 9 T ♂ randomized cross-over | A: High-CHO (z% CHO) diet with a CHO beverage during time-trial. B: High-CHO diet with a non-CHO beverage C: Low-CHO (y% CHO) diet with a CHO beverage during time-trial D: Low-CHO diet with a non-CHO beverage | 70-km cycling time-trial. Last meal ≥6 hours prior to trial. Conditions A and C ingested a 9% CHO beverage at the onset of exercise and at the completion of every 10 km (2.35 ml/kg). Conditions B and D ingested a non-CHO placebo beverage at the onset of exercise and at the completion of every 10 km (2.35 ml/kg). | A: 180 B: 170 C: 100 D: 110 | A: 45 B: 31 C: 21 D: 25 | A: 117.2 min B: 118.7 min ↔ C: 121.2 min ↑ vs. A D: 122.9 min ↑ vs. B |

CHO, Carbohydrate; VO_{2peak}, peak oxygen uptake; UT, untrained; T, trained. Arrows represent significant ↑, increase; ↓ decrease; ↔, no change between treatments.

Table 1.2 (Continue)

| Reference | Subjects | Dietary and training protocol | Performance protocol and dietary intake pre- and during exercise | Muscle glycogen (mmol/kg wet weight) | | Performance |
|-------------------------|--|--|--|--------------------------------------|------------------------------|--|
| | | | | Pre-exerc | Post-exerc | |
| Rauch et al. 1995 (146) | 8 T ♂ randomized cross-over | A: 3-day habitual mod-CHO* diet vs. B: 3-day high-CHO** *mod-CHO: 6.1 g CHO/kg BM/day **high-CHO: 10.5 g CHO/kg BM/day | 2-hour cycle at 75% VO _{2peak} with 5 x 60 seconds sprints at 100% VO _{2peak} every 20-km, followed by a 1-hour time-trial. Standardized breakfast (70-80 g CHO). 600 ml of a 10% glucose polymer during the 2-hour constant-load cycle. | A: 104 B: 153 | A: 18 B: 26 | A: 36.7 km B: 38.0 km ↑ 7/8 improved on B |
| Rauch et al. 2005 (145) | Data captured from study mentioned above (146) was re-analyzed to examine differences in power output during the 1-hour time-trial | | | | | A: 219 W B: 233 W ↑ 7/8 improved on B |
| Sullo et al. 1998 (181) | 12 T ♂ 12 UT ♂ 2 groups 2 diets (2x2 design) | A1 (T): 3-day habitual diet (~4.8g CHO/kg) A2 (T): 7-day high-CHO* B1 (T): 3-day habitual diet (~4.3g CHO/kg) B2 (T): 7-day control (~4.8g CHO/kg) C1 (UT): 3-day habitual diet(~4.7g CHO/kg) C2 (UT): 7-day high-CHO* D1 (UT): 7-day habitual diet(~4.4g CHO/kg) D2 (UT): 7-day control (~4.7g CHO/kg) *~7.8g/kg for d 1-4 + ~6.3g/kg for d 5-7 | 25-km treadmill time-trial. Overnight fasted Only water during exercise. | - | - | A1: 93.4 min A2: 92.6 min ↔ B1: 92.2 min B2: 93.5 min ↔ C1: 101.1 min C2: 95.3 min ↑ D1: 99.8 min D2: 100.2 min ↔ |

CHO, Carbohydrate; VO_{2peak}, peak oxygen uptake; UT, untrained; T, trained. Arrows represent significant ↑, increase; ↓ decrease; ↔, no change between treatments.

Table 1.2 (Continue)

| Reference | Subjects | Dietary and training protocol | Performance protocol and dietary intake pre- and during exercise | Muscle glycogen (mmol/kg wet weight) | | Performance |
|---------------------------|-----------------------------|--|---|--------------------------------------|------------------------------|---|
| | | | | Pre-exerc | Post-exerc | |
| Burke et al. 2000 (29) | 7 T ♂ randomized cross-over | A: 3-day mod-CHO (6 g CHO/kg BM/day) B: 3-day high-CHO (9 g CHO/kg BM/day) Placebo controlled | 100-km cycling time-trial interspersed with 1-km and 4-km sprints. Breakfast (2 g CHO/kg BM) 2 hours prior to the time-trial. CHO during (~1 g CHO/kg BM/hr) the time-trial in both conditions | A: 133 B: 113 | A: 22 B: 13 | A: 147.5 min B: 149.1 min ↔ |
| Andrews et al. 2003 (6) | 8 T ♀ randomized cross-over | A: 3-day habitual (50% CHO) B: 3-day CHO-loading (75% CHO) C: 3-day placebo diet (50% CHO) | 24.2-km treadmill time-trial. 6% CHO drink before (6 ml/kg) and every 20 minutes during exercise for conditions A and B. Placebo for condition C. | - | - | A: 132.5 min B: 134.4 min C: 136.6 min ↔ 6/8 improved on A and B vs. C |
| Johnson et al. 2006 (106) | 8 T ♂ randomized cross-over | A: Bout of strenuous interval cycling to deplete muscle glycogen followed by 2-d low-CHO (0.6 g CHO/kg BM/day) B: Bout of strenuous interval cycling to deplete muscle glycogen followed by 2-d high-CHO (9.3 g CHO/kg BM/day) Placebo controlled | ~2750 kJ time-trial (~3 hours) Overnight fasted A 7% glucose polymer (15 ml/kg BM/hr of cycling) during the time-trial in both conditions | - | - | Self-selected power output during first 70% of time-trial ↔ for A and B Power output ↓ last 30% for A vs. B. |

CHO, Carbohydrate; VO_{2peak}, peak oxygen uptake; UT, untrained; T, trained. Arrows represent significant ↑, increase; ↓ decrease; ↔, no change between treatments.

and during exercise (29). Although Sullo et al. (181) failed to demonstrate a performance effect with a high CHO diet compared to a moderate CHO diet on running performance in trained subjects, they demonstrated a 2% improvement in performance with the CHO-loading strategy in untrained subjects ($p < 0.05$).

In summary, there is evidence to suggest CHO-loading prolongs endurance capacity (5; 12; 36; 59; 85; 108) and enhances exercise performance in trained (108; 146; 194; 196) and untrained athletes (181) compared to a low-moderate CHO diet. However, many of these studies did not use a randomized, placebo-controlled cross-over design required to investigate the effectiveness of a dietary strategy. Indeed, recent evidence suggests that time-trial performance is not improved in studies where a placebo-controlled design is used and subjects are not aware of their absolute CHO intake (29; 106). Moreover, there are no studies of which we are aware of, that have examined the effects of CHO-loading on exercise performance in events lasting longer than 4-5 hours. This needs to be investigated using a randomized placebo-controlled design before definite dietary recommendations can be prescribed. Nonetheless, Burke (25) demonstrated that highly trained male athletes typically consume a high CHO diet (7-10 g CHO/kg body mass) during training and pre-competition. However, there is limited data on the habitual nutritional intakes of recreational and sub-elite cyclists, who are not necessarily training and competing under the supervision of coaches and nutritionists.

1.3.2 Fat-loading

While the ingestion of a high fat, low CHO diet for 1-4 days reduces resting muscle glycogen stores and compromises the capacity to perform prolonged sub-maximal exercise (5; 12), there is evidence to suggest that prolonged (>5 days) high fat intakes induce metabolic and hormonal adaptations that 'retool' the muscle to enhance rates of fat oxidation and reduce rates of CHO oxidation during exercise and, to a large extent, compensate for the reduced CHO availability (26; 33; 66; 84; 118; 137). As such, "fat-loading" or "fat-adaptation" is a nutritional strategy whereby well-trained athletes adapt to a high fat diet in an attempt to increase rates of fat oxidation and 'spare' muscle glycogen during exercise.

1.3.2.1 Mechanisms underlying the adaptations to a high fat diet

Although the precise mechanisms involved in the shift in substrate oxidation from CHO to fat with a prolonged (>4 days) high fat diet are not completely understood, a number of mechanisms have been identified, including:

- 1) low muscle glycogen levels;
- 2) increased FFA availability;
- 3) increased IMTG stores and IMTG oxidation;
- 4) changes in proteins involved in FFA transport and oxidation;
- 5) changes in glucose tolerance and insulin sensitivity/resistance;

Low muscle glycogen levels

The increase in fat oxidation with a high fat diet may be explained, in part, by significantly lower pre-exercise muscle glycogen levels associated with the

ingestion of high fat (low CHO) compared to a high CHO (low fat) diet. Weltan et al. (192) demonstrated that low muscle glycogen levels significantly increased fat oxidation during exercise, even when euglycemia was maintained by glucose infusion at basal (~5 mmol/l) or hyperglycemic (10 mmol/l) levels (193). However, Vogt et al. (188) demonstrated a significant increase in fat oxidation, despite similar pre-exercise muscle glycogen levels following 4 weeks of a high fat (53% fat), moderate CHO (30-35% CHO) diet compared to 4 weeks of a high CHO (60-70% CHO) diet. Furthermore, when muscle glycogen levels were restored following a period of fat-adaptation, fat oxidation remained higher following a high fat compared to a high CHO diet (26; 28; 33; 81). Therefore, the increase in fat oxidation associated with a high fat, low CHO diet can not only be explained by reduced muscle glycogen stores and suggests that other mechanisms are involved.

Increased fatty acid availability

Ingestion of a high fat diet has also been shown to increase plasma FFA availability (82; 137; 164), possibly contributing to the increase in fat oxidation and reduction in CHO oxidation during exercise. In the early 1960's Randle et al. (142; 143) proposed the so-called glucose-fatty acid cycle to explain the reciprocal relationship between an increased plasma FFA utilization and the concomitant decrease in CHO utilization. It was suggested that an increased FFA availability increased muscle acetyl-CoA and citrate, leading to the down regulation of PDH and PFK activities. It was further suggested that the reduced flux through the glycolytic pathway caused an accumulation of G6P, which inhibited HK activity and ultimately decreased the uptake of glucose (142; 143).

However, studies examining the Randle effect in exercise by manipulating FFA availability via various mechanisms (e.g. lipid-heparin infusion) have failed to support its role in regulation of CHO mechanisms during exercise, particularly during moderate to high exercise where glycogen is a predominant fuel source. In addition, a number of studies have found that ingestion of a high fat diet does not consistently increase *plasma* FFA concentrations and FFA oxidation rates during exercise. Rather, these studies have demonstrated that the increase in total fat oxidation in response to a high fat diet was attributed to higher rates of IMTG oxidation (see next section).

Increase IMTG stores and oxidation

A number of studies have consistently demonstrated a substantial increase (37%-130%) in IMTG stores with different fat-adaptation periods ranging from 2 days to 7 weeks and consisting of 41-65% fat (79; 84; 90; 97; 107; 111; 164; 165; 173; 188). The increase in IMTG may be associated with an increase in skeletal muscle LPL activity, which has also also been shown to increase to almost 2-fold in response to a 4-week high fat diet (111). An increase in IMTG following a high fat diet may provide an additional available substrate pool, which could account for the enhanced fat oxidation rates in response to a high fat intake. However, there is controversy regarding the contribution of IMTG oxidation to total energy expenditure (189) during exercise, mainly due to differences in the methodology used to measure IMTG (for review see Kiens (109)). While there is evidence to suggest that a high fat diet significantly increased IMTG oxidation during exercise (164) and that the increase in IMTG oxidation was correlated with an increase in pre-exercise IMTG levels (107;

198), some studies failed to demonstrate an increase in IMTG oxidation despite significantly higher pre-exercise IMTG levels (165).

Changes in skeletal muscle proteins involved in FFA transport and oxidation

Ingestion of a high fat diet has been shown to increase FAT/CD36 mRNA levels, with a concomitant increase in the cellular content of the transcribed protein (32; 150), possibly facilitating an increase FFA uptake into the muscle cell. However, Roepstorff et al. (150) demonstrated an increase in fat oxidation in response to a high fat compared to a high CHO diet, despite similar rates of FFA uptake between the two diets, suggesting that trans-sarcolemmal transport is not limiting FFA oxidation. In fact, Roepstorff et al. (150) demonstrated that the high expression of FAT/CD36 coincides with an increase in IMTG rather than an increase in FFA oxidation in response to a high fat diet.

The shift in substrate metabolism has also been attributed, in part, to changes in skeletal muscle enzyme activities that increase the uptake and oxidation of fatty acids in the mitochondria, and reduce CHO oxidation (54; 65; 80). Chronic high-fat diets (4-7 weeks) have been shown to result in increases in 3-HAD and CPT-1 content in the sarcolemma and decreases in hexokinase (HK) and PDH activity. Helge and Kiens (80) demonstrated a 25% increase in muscle 3-HAD activity in cyclists after 7 weeks of HFD. Fisher et al. (54) found that 4 weeks of a ketogenic HFD in well-trained cyclists resulted in a 35% increase in muscle CPT1 activity. Similarly, Goedecke et al. (65) demonstrated an increased in CPT activity in well-trained cyclists after as little as 10 days of high fat intake compared to high CHO intake.

Conversely, ingestion of a high fat diet has been shown to decrease enzymes involved in CHO oxidation. Fisher et al. (54) demonstrated a 46% reduction in muscle hexokinase activity in response to a 4-week ketogenic diet. Moreover, ingestion of a high fat diet for as little as 3-6 days has shown to decrease the activation of PDH at rest and during exercise, mediated through increased PDH kinase activity and PDK4 isoform expression (35; 115; 133).

Changes in glucose tolerance and insulin sensitivity/resistance

A high fat diet has also shown to induce insulin resistance, which results in an increase fat oxidation by suppressing CHO metabolism. Goedecke et al. (66) demonstrated that ingestion of a high fat diet for only 5 days reduced glucose tolerance as demonstrated by a significant increase in 30-min plasma glucose concentrations during an oral glucose tolerance test. It is well documented that increase FFA availability and increasing IMTG induce insulin resistance (115; 129). However, when the relationship between IMTG and insulin resistance was examined in endurance-trained athletes, the correlation generally disappears (183). This can be attributed to the fact that endurance trained athletes are markedly insulin sensitive (186). Therefore, the greater IMTG storage in the trained athlete as oppose to the elevated IMTG stores in obese and/or type 2 diabetics, represents an adaptive response to endurance training, allowing a greater contribution of the IMTG pool as a substrate source during exercise (186).

Despite adaptations that favor fat oxidation, and 'spare' muscle glycogen content following the ingestion of a high fat diet, the performance effect in response to a high fat diet is not clear.

1.3.2.2 The effect of a long-term (>4 days) high fat diet on exercise performance

There are a number studies that have manipulated dietary fat intake over longer periods (>5 days) in trained and untrained subjects, with majority of these investigating the effect of a high fat diet on moderate-intensity (60-85% VO_{2peak}) endurance capacity (exercise time to fatigue) (81; 84; 90; 118; 126; 137; 138; 140). The findings of these studies are not consistent, with nearly equal proportions of studies showing an improved (4 studies) (90; 118; 126; 137), no change (2 studies) (84; 138) or a decrement in endurance capacity (2 studies) (81; 140) (Table 1.3) in response to a high fat diet compared to a moderate to high CHO diet.

The four studies that have demonstrated an improved endurance capacity in response to a high fat diet recruited trained runners and cyclists to perform an exercise test to exhaustion at 50-80% VO_{2peak} . In a non-randomized design, Muoio et al. (126) demonstrated an 11% improvement in VO_{2peak} and a 20% improvement in endurance running capacity following the high fat compared to the high CHO diet (Table 1.3). However, these results should be interpreted with caution due to a number of reasons. The fat content in the high fat diet in this study was only 38%, a dietary fat content which is typically reported in habitual diets of sedentary South Africans (179) and would normally not be

Table 1.3: Effect of long-term (>5 days) high-fat diets on substrate utilization and endurance capacity (time to fatigue)

| Reference | Subjects | Dietary and training protocol | Performance protocol and dietary intake pre- and during exercise | Muscle glycogen (mmol/kg wet weight) and substrate utilization | Time to fatigue |
|---------------------------|----------|---|--|---|---|
| Pruett et al, 1970 (140) | 9 UT ♂ | A: 14 days high-fat (60% fat) diet B: 14 days control (9% fat) diet | 50% and 70% VO_{2peak} cycle to exhaustion. Overnight fasted + only water during exercise. | No change in fat oxidation or CHO oxidation on either diet. | <u>50%VO_{2peak}</u> A: 270 min (3%↑) B: 262 min <u>70%VO_{2peak} (n=4)</u> A: 164 min (14%↓) B: 193 min 1/4 increased performance on B |
| Phinney et al. 1983 (137) | 5 T ♂ | A: 1 week mixed (33% fat) diet B: 4 weeks ketogenic (85% fat) diet Non-randomized design. | 62-64% VO_{2peak} cycle to exhaustion. Overnight fasted + only water during exercise. | A: Muscle glyc pre 143, post 53 B: Muscle glyc pre 76, post 56 ↑ fat oxidation (↓RER), ketotic, ↓ [muscle glycogen], ↑ muscle glycogen sparing, ↓ glucose oxidation on B vs. A. | A: 147 min B: 151 min ↔ 3/5 increased performance on B |
| Muoio et al. 1994 (126) | 6 T ♂ | A: 7-day high-fat (38% fat) diet B: 7-day high CHO (15% fat) diet C: 7-day normal (25% fat) diet Non-randomized design. | VO_{2peak} , 85% VO_{2peak} run for 30 min, 75-80% VO_{2peak} run to exhaustion. Fasted + no CHO during exercise. | No change in fat oxidation (RER), ↑ [FFA], ↓ [glycerol] on A vs. B and C. | A: 91 min (↑ vs. B, C) B: 76 min C: 69 min VO_{2peak} ↑ on A vs. B,C |

CHO, Carbohydrate; VO_{2peak} , peak oxygen uptake; UT, untrained; T, trained; RER, respiratory exchange ratio; FFA, free fatty acid; IMTG, intramuscular triglycerides. Arrows represent significant ↑, increase; ↓ decrease; ↔, no change between treatments.

Table 1.3: Continue

| Reference | Subjects | Dietary and training protocol | Performance protocol and dietary intake pre- and during exercise | Muscle glycogen (mmol/kg wet weight) and substrate utilization | Time to fatigue |
|---------------------------------------|----------|---|--|--|--|
| Lambert et al. 1994 (118) | 5 T ♂ | A: 14 days high-fat (67% fat) diet B: 14 days high-CHO (12% fat) diet Randomized cross-over control | Wingate test, 90% VO_{2peak} cycle to exhaustion, 60% VO_{2peak} to exhaustion. Fasted? + no CHO during exercise | A: Muscle glyc pre-exercise = 68 B: Muscle glyc pre-exercise = 121 ↓RER, ↓ rates of CHO oxidation, ↑ muscle glycogen sparing on A vs. B. | ↔ in wingate ↔ in 90% VO_{2peak} A: 79.7 min (88%↑) B: 42.5 min |
| Pogliaghi and Veicsteinas. 1999 (138) | 14 UT ♂ | A: 4 weeks high-fat (55% fat) diet B: 4 weeks low-fat (15%fat) Non-randomized design. | VO_{2peak} , 75% VO_{2peak} cycle to exhaustion. Fed a high-fat or high-CHO meal 3 hours prior to exercise, only water during exercise. | No change in fat oxidation (RER) | ↔ in VO_{2peak} A: 46 min B: 48 min ↔ |
| Hoppeler et al. 1999 (90) | 7 T ♂ | A: 4 weeks high-fat (41% fat) diet B: 4 weeks high-CHO (18%fat) Non-randomized design. | VO_{2peak} , 80% VO_{2peak} run to exhaustion. Fed a high-fat or high-CHO meal 3 hours prior, only water during exercise. | ↑ [IMTG] on A vs. B. | ↔ in VO_{2peak} Endurance ↑21% after A vs. B |

CHO, Carbohydrate; VO_{2peak} , peak oxygen uptake; UT, untrained; T, trained; RER, respiratory exchange ratio; FFA, free fatty acid; IMTG, intramuscular triglycerides. Arrows represent significant ↑, increase; ↓ decrease; ↔, no change between treatments.

considered 'high-fat'. In fact, the CHO content in the 'high fat' was possibly high enough for the diet to be regarded a moderate CHO diet. Furthermore, the 'high fat' diet did not alter substrate utilization during exercise therefore the improvement in performance in this study was more likely related to an order effect as the diets were not randomized.

Lambert et al. (118) employed a cross-over design to investigate the effects of a high fat (67% fat) compared to high CHO diet on the capacity to perform a battery of exercise tests (Table 1.3). Despite a 44% lower muscle glycogen content after the high fat diet, supra-maximal performance and high-intensity performance was not compromised, but mean time to exhaustion during 60% VO_{2peak} was improved by 88% (118). However, it should be noted that the mean time to fatigue at 60% VO_{2peak} was only ~80 minutes, a duration that is less than would be expected from well trained athletes.

Finally, Hoppeler et al. (137) demonstrated a 21% improvement in endurance running following a high fat compared to a high CHO diet. However, similar to the study of Muoio et al. (126), the fat content of the high fat diet study was only 41%, only slightly higher than the fat content of a habitual mixed diet. Nonetheless, Hoppeler et al. (137) demonstrated a significant increase in IMTG that was associated with an increase in fat oxidation during exercise in response to the high fat diet compared to the high CHO diet. It is important to note that in all three studies, the subject sample size was small (5-7 subjects), possibly leading to type II error.

In contrast to these studies, Pruett et al. (140) demonstrated a decrease in endurance capacity at 70% VO_{2peak} in response to a 2 week high fat diet (59% fat) intervention in untrained subjects. It is well documented that exercise training induces adaptations that increase rates of fat oxidation at the same absolute intensities as discussed previously. Furthermore, only 4 subjects undertook the exercise trial at 70% VO_{2peak} and the authors demonstrated a variability in response with 3 of the 4 subjects improving performance following the high fat diet.

A study frequently cited to support the use of high fat diets to enhance endurance performance, despite subjects achieving similar endurance times in response to the two dietary interventions, is the study from Phinney et al. (137) who investigated the effects of 28 days of a high fat ketogenic diet on exercise time to fatigue in five well-trained cyclists (Table 1.3). Although the ketogenic diet (85% fat; <20g CHO/day) significantly reduced pre-exercise muscle glycogen concentrations compared the mixed moderate CHO diet, endurance capacity was not different between the two diets. However, the results should be interpreted with caution due to the large variability in response to the diets in the small group. Performance of 3 of the 5 subjects improved on the high fat diet, but the performance improvement ranged from 2-57%. In contrast, performance of 2 of the subjects was 28 and 36% slower. Moreover the results of the study were likely affected by an order effect as the moderate CHO diet was administered first in all the subjects. Hence the results of this study are difficult to interpret and could be misleading.

Similarly, Pogliaghi and Veicsteinas (138) also investigated the effect of 4 weeks of fat-adaptation compared to a high CHO diet on endurance capacity in 6 *untrained* men and failed to demonstrate differences in performance.

Together these results suggest that the ingestion of a high fat diet (>55% fat energy) for 7 to 14 days can potentially improve moderate intensity endurance capacity in trained athletes. However, exercise performance is not typically measured as time to fatigue, questioning the applicability of these results to athletes taking part in endurance races that are typically measured by the time to complete a set distance (time-trial). There are only a few studies (three) that have examined the effects of a high fat diet on time-trial performance that include aspects of a 'real life' race situation such as sprinting and pacing and allow subjects to 'compete' in a situation that mimics the demands of a 'real life' race situation.

None of the 3 studies documented in this review demonstrated a significant change in time-trial performance following 2 to 5 weeks of a high fat diet (53-69% fat energy) compared to moderate (65) or high CHO diet (154; 188). Using a cross-sectional design, Goedecke et al. (65) examined the effects of 5-15 days of high fat intake compared to moderate CHO intake in two groups of well-trained male cyclists. Although an increase in fat oxidation, with concomitant decrease in muscle glycogen utilization was reported after 5, 10 and 15 days of high fat intake, time to complete a 40-km time-trial following a 150 minute moderate-intensity constant-load cycle was not different between diets (Table 1.4). However, performance did improve in both groups in response to the

Table 1.4: Effect of long-term (>5 days) high-fat diets on substrate utilization and time-trial performance

| Reference | Subjects | Dietary and training protocol | Performance protocol and dietary intake pre- and during exercise | Muscle glycogen (mmol/kg wet weight) and substrate utilization | Performance |
|---------------------------------|----------|---|---|---|--|
| Goedecke et al. 1999 (65) | 16 T ♂ | A: 15 days high-fat (69% fat) diet B: 15 days control (30% fat) diet 2 Groups | 150 min cycle at 70% VO_{2peak} + 40 km cycle TT on days 0, 5, 10 and 15. ~14 g MCT 1.5 hours before + MCT (0.3 g/kg/hr) and CHO (0.8 g/kg/hr) during exercise. | ↑ total fat oxidation, ↓ total CHO oxidation, ↑ muscle glycogen sparing on A vs. B after 5, 10 and 15 days. | A: (5-d) 67.1 min B: (5-d) 68.2 min ↔ A: (10-d) 64.7 min B: (10-d) 64.9 min ↔ A: (15-d) 63.4 min B: (15-d) 65.6 min ↔ |
| Rowlands and Hopkins 2002 (154) | 7 T ♂ | A: 2 weeks high-fat (66% fat) diet B: 2 weeks high-CHO (16% fat) diet Non-randomized cross-over | Battery of performance protocols, including a 15-min performance test (short-endurance), and a 100-km cycle TT following 2.5-hours of cycling (ultra-endurance). Pre-exercise meal and sport bars and a 5% CHO solution during exercise. | ↑ [glycerol], ↑ fat oxidation | ↔ in VO_{2peak} ↔ in 100-km TT |
| Vogt et al. 2003 (188) | 11 T ♂ | A: 5 weeks high-fat (53% fat) B: 5 weeks control (17% fat) Randomized cross-over control | Testing during 5 th week of trial. VO_{2peak} on day 1, followed by 40 min sub-max + 20 min TT 48 hours later and a 21-km run another 48 hours later. Pre-exercise meal containing 40 g or 90 g CHO on A and B respectively. | ↑ fat oxidation (↓RER) at rest and during sub-maximal exercise and ↑ [IMTG] following A vs. B. | ↔ VO_{2peak} ↔ in 20 min TT ↔ in 21-km run |

CHO, Carbohydrate; VO_{2peak} , peak oxygen uptake; UT, untrained; T, trained; TT, time-trial; RER, respiratory exchange ratio; FFA, free fatty acid; IMTG, intramuscular triglycerides. Arrows represent significant ↑, increase; ↓ decrease; ↔, no change between treatments.

testing protocol, suggesting that the high fat diet did not compromise short-term training ability in the well-trained athletes (65). Similarly, Rowlands and Hopkins (154) investigated the effect of a high fat diet compared to a high CHO diet on ultra-endurance performance and also failed to demonstrate a performance benefit during a 100-km time-trial following 3-hours of riding in 7 trained athletes, despite a increase in fat oxidation in response to a high fat diet compared to a high CHO diet (Table 1.4). Finally, Vogt et al. (188) also demonstrated a increase in fat oxidation and higher IMTG stores in response to the high fat diet compared to the high CHO diet without demonstrating a performance effect during a 20 minute time-trial or a 21-km run in 11 trained runners (Table 1.4).

Since endurance training also induces metabolic adaptations that increase fat oxidation, a couple of studies have explored the effect of prolonged high fat intake in combination with a training program. Helge (81) examined the interaction between endurance training and dietary intervention during prolonged (7 weeks) high fat intake followed by 1 week of high CHO intake compared to 7 and 8 weeks of high CHO intake on endurance capacity in 10 untrained subjects (Table 1.5). Time to fatigue improved ($p < 0.05$) following 1 week of CHO intake in addition to 7 weeks of high fat intake compared to 7 weeks of high fat intake. However, exercise capacity following the high fat followed by high CHO diet was still 25% less compared to the ingestion of a high CHO diet for 7 or 8 weeks ($p < 0.05$). Helge (81) concluded that ingestion of a high fat diet during a prolonged endurance training program is detrimental to improvement in endurance.

A subsequent study from Helge et al. (84) also failed to report a difference in endurance capacity in response to a high fat compared to a high CHO diet in untrained subject that underwent 4 weeks of training in combination with a high fat or high CHO diet (Table 1.5). However, performance on both diets improved significantly following 4 weeks of training. This finding indicates that that the duration of the adaptation period might be important for the outcome of the dietary change, since performance was compromised after 7 weeks, but not 4 weeks of adaptation to a high fat diet.

In summary, in well-trained cyclists, 2 to 5 weeks of high fat intake increased fat oxidation and 'spared' muscle glycogen during exercise. This could be attributed to the metabolic and hormonal adaptations discussed in the previous section. Alternatively, an increase in fat oxidation could be due the significantly lower pre-exercise muscle glycogen stores seen after the ingestion of high fat compared to a high CHO diet. Despite an increase in fat oxidation, high fat intake for 2 to 5 weeks failed to demonstrate an improvement in time-trial performance. The failure to demonstrate a time-trial performance benefit might relate to muscle glycogen as time-trials are undertaken at a higher intensity and include sprints bouts during which muscle glycogen are the predominant fuel.

Table 1.5: Effect of long-term (>5 days) high-fat diets combined with exercise training on substrate utilization and exercise performance

| Reference | Subjects | Dietary and training protocol | Performance protocol and dietary intake pre- and during exercise | Muscle glycogen (mmol/kg wet weight) and substrate utilization | Performance |
|------------------------|----------|---|---|---|---|
| Helge et al. 1996 (81) | 20 UT ♂ | A: 7 weeks high-fat (62% fat) + 1 week high-CHO (65% CHO) B: 7 weeks high-CHO (65% CHO) + 1 additional week high-CHO. Supervised training program during first 7 weeks. 2 groups | VO _{2peak} , 81% VO _{2peak} cycle to exhaustion at 0, 7 and 8 weeks. Overnight fasted + 200 ml water every 20 min during exercise. | ↑ fat oxidation (↓RER) after 7 weeks on A vs. B, but ↔ on wk 8 after 1 week high-CHO, ↓ [muscle glycogen] after 7 weeks on A, but ↑ after 8 weeks, ↔ glycogen sparing | ↔ VO _{2peak} A: (7 wks) 65 min A: (8 wks) 77 min B: (7 wks) 102 min B: (8 wks) 102 min A (7 + 8 wks) ↓ vs. B |
| Helge et al. 1998 (84) | 15 UT ♂ | A: 4 weeks high-fat (62% fat) diet B: 4 weeks high-CHO (20% fat) diet Supervised training protocol during 4-week diet period 2 Groups | 80% VO _{2peak} cycle to exhaustion at 0, 2 and 4 weeks. (cycling) Overnight fasted + 200ml water every 20min during exercise. | A: Muscle glyc after 4 wks = 107 B: Muscle glyc after 4 wks = 151(↑) ↑ fat oxidation (↓RER) after 2 and 4 weeks, ↑ [IMTG] after 4 weeks on A vs. B. | A: (0 wks) 25.9 min B: (0 wks) 31.7 min A: (2 wks) 43 min B: (2 wks) 59.3 min ↑ A: (4 wks) 78.5 min B: (4 wks) 79.3 min *Performance on both diets ↑ wk 4 vs. wk 1. |

CHO, Carbohydrate; VO_{2peak}, peak oxygen uptake; UT, untrained; T, trained; TT, time-trial; RER, respiratory exchange ratio; FFA, free fatty acid; IMTG, intramuscular triglycerides. Arrows represent significant ↑, increase; ↓ decrease; ↔, no change between treatments.

1.3.2.3 Ingestion of a high-fat diet followed by CHO-loading

The restoration of muscle glycogen stores following a period of fat-adaptation could, theoretically, provide an athlete with the opportunity to enhance fuel provision during exercise from both glycolytic and lipolytic pathways (75). Furthermore, true differences in the rate of glycogen utilization during a standardized exercise bout following a high fat or high CHO diet can only be substantiated if subjects start exercise with similar muscle glycogen stores. Hence researchers examined the effect of a high fat diet followed by CHO loading on substrate utilization and exercise performance.

The 4 studies documented in this review that employed a fat-adaptation followed by CHO-loading strategy (HFD-CHO) are summarized in Table 1.6. Despite a increase in fat oxidation and a concomitant 'sparing' of muscle glycogen during exercise following the HFD-CHO dietary strategy in all the studies, only one study demonstrated a time-trial performance benefit for the HFD-CHO diet compared to a high CHO diet (117). The remaining studies demonstrated no change in performance (26; 28; 33).

The study that demonstrated an improved time-trial performance following a HFD-CHO dietary strategy investigated the effect of 10 days of high fat (>65% fat) intake followed by 3 days of CHO-loading on time-trial performance in 5 well-trained cyclists (Table 1.5). Lambert et al. (117) attributed the positive performance effect to a reduction in estimated rates of muscle glycogen utilization after the HFD-CHO compared to the habitual moderate CHO diet.

More recent studies examined the effect of a shorter duration fat-adaptation diet (5-6 days) followed by 1 days of CHO-loading (26; 28; 33). This was based on the findings from Goedecke et al. (66) who demonstrated that high fat intake for as little as 5-10 days induced adaptations that significantly increased fat oxidation during exercise. Burke et al. (26) investigated the effect of 5 days of high fat intake (>65% fat) followed by 1 day of CHO-loading compared to an iso-caloric high CHO diet on short duration (~25-30 min) time-trial performance following a 2-hour constant-load ride in 7 endurance trained male athletes (Table 1.6). One day of CHO-loading following the fat-adaptation period was sufficient to restore muscle glycogen concentrations to a similar high level compared to 6 days of high CHO intake. Despite similar starting muscle glycogen contents, muscle glycogen utilization was lower during the 2-hour moderate intensity constant-load ride following the HFD-CHO diet compared to the high CHO diet. Despite higher rates of fat oxidation and the 'sparing' of muscle glycogen during exercise in response to the HFD-CHO diet, overall time-trial performance was not significantly different between trials. However, mean time-trial time was 8% faster following the HFD-CHO diet, and 5 out of the 7 subjects improved short duration time-trial performance following the HFD-CHO diet compared to the high CHO diet (Table 1.6) (26).

In the above mentioned study, subjects completed the exercise trial in the fasted state. Since conditions in that trial were not in line with the typical nutritional practices of athletes, Burke et al. (28) conducted a follow-up study, employing the same dietary and performance protocol, but subjects were fed a

Table 1.6: Effect of fat-adaptation followed by carbohydrate-loading on substrate utilization and exercise performance

| Reference | Subjects | Dietary and training protocol | Performance protocol and dietary intake pre- and during exercise | Substrate utilization and muscle glycogen (mmol/kg wet weight) | Performance |
|---------------------------|----------|---|---|---|---|
| Burke et al. 2000 (28) | 8 T ♂ | <p>A: 5-day high-fat (>65% fat) combined with supervised training + 1-day CHO-loading (10g/kg) and rest.</p> <p>B: 5-day high-CHO (>70% CHO, 15% fat) combined with supervised training + 1-day CHO-loading (10g/kg) and rest.</p> <p>Randomized cross-over control</p> | <p>120 min cycle at 70% VO_{2peak} + 7kJ/kg cycling TT (~25-30 minutes).</p> <p>Overnight fasted + no CHO during exercise</p> | <p>A: Pre-exerc = 129, post-exerc = 68</p> <p>B: Pre-exerc = 141, post-exerc = 58</p> <p>↑ fat oxidation, ↓ CHO oxidation (↓RER), ↑ muscle glycogen sparing on A vs. B.</p> | <p>A: 30.7 min</p> <p>B: 34.2 min ↔</p> <p>5/8 increased performance on A</p> |
| Lambert et al, 2001 (117) | 5 T ♂ | <p>A: 10-day high-fat (65% fat) followed by 3 days high-CHO (>70% CHO).</p> <p>B: 10-day habitual (30% fat) followed by 3 days high-CHO (>70% CHO).</p> <p>Randomized cross-over control</p> | <p>150 min cycle at 70% VO_{2peak} + 20 km cycle TT. 400 ml 3,44% MCT solution 1 hr before and 600ml/hr 10% CHO, 3.44% MCT solution during exercise.</p> | <p>↑ [serum glycerol], ↑ fat oxidation, ↓ CHO oxidation (↓RER), ↑ muscle glycogen sparing on A vs. B.</p> | <p>A: 29.5 min</p> <p>B: 30.9 min</p> <p>A ↑ vs. B</p> |

CHO, Carbohydrate; VO_{2peak} , peak oxygen uptake; UT, untrained; T, trained; TT, time-trial; RER, respiratory exchange ratio; FFA, free fatty acid; IMTG, intramuscular triglycerides. Arrows represent significant ↑, increase; ↓ decrease; ↔, no change between treatments.

Table 1.6: Continue

| Reference | Subjects | Dietary and training protocol | Performance protocol and dietary intake pre- and during exercise | Substrate utilization and muscle glycogen (mmol/kg wet weight) | Performance |
|------------------------|----------|--|--|---|--|
| Carey et al. 2001 (33) | 7 T ♂ | <p>A: 6-day high-fat (69% fat) combined with supervised training + 1-day CHO-loading (11 g/kg) and rest.</p> <p>B: 6-day high-CHO (>70% CHO, 15% fat) combined with supervised training + 1-day CHO-loading (11 g/kg) and rest</p> <p>Randomized cross-over control</p> | 240 min cycle at 65% VO_{2peak} + 1-hour TT. CHO intake before (3g/kg) and during (1.3g/kg/hr) exercise | ↑ fat oxidation, ↓ CHO oxidation (↓RER), ↑ muscle glycogen sparing on A vs. B. ↑ fat oxidation. | <p>A: 312 Watts (11% higher vs. B)</p> <p>B: 279 Watts ↔</p> <p>5/7 increased performance on A</p> |
| Burke et al. 2002 (26) | 8 T ♂ | <p>A: 5-day high-fat (>65% fat) combined with supervised training + 1-day CHO-loading (10g/kg)</p> <p>B: 5-day high-CHO (>70% CHO, 15% fat) combined with supervised training + 1-day CHO-loading (10g/kg)</p> <p>Randomized cross-over control</p> | 120 min cycle at 70% VO_{2peak} + 7kJ/kg cycle TT (~25-30 minutes). CHO intake 2 hr before (2g/kg) and during (0.8g/kg/hr) exercise. | ↑ fat oxidation, ↓ CHO oxidation (↓RER) on A vs. B. ↑ fat oxidation. | <p>A: 25.53 min</p> <p>B: 25.45 min ↔</p> |

CHO, Carbohydrate; VO_{2peak} , peak oxygen uptake; UT, untrained; T, trained; RER, respiratory exchange ratio; IMTG, intramuscular triglycerides.

FFA, free fatty acid; Arrows represent significant ↑, increase; ↓ decrease; ↔, no change between treatments.

pre-exercise meal as well as CHO during the 2-hour constant-load ride (Table 1.6). The HFD-CHO diet again resulted in higher fat oxidation rates during the 2-hour moderate intensity constant-load cycle, despite the ingestion of CHO prior to and during exercise that is generally associated with increased insulin levels that suppresses lipolysis (92; 93). Similar to their previous study, overall time-trial performance was not different between diets.

The total duration of exercise in the two trials of Burke were only ~2.5 hours and possibly not long enough to deplete muscle glycogen stores (26; 28). Hence the increase in fat oxidation and concomitant “sparing” of muscle glycogen with this dietary strategy might not necessarily effect performance. Subsequently, Carey et al. (33) investigated the effect of fat-adaptation followed by CHO-loading on 1 hour time-trial performance following a 4-hour constant-load ride at 65% VO_{2peak} . The high-fat diet enhanced fat oxidation and reduced CHO oxidation, to such an extent that ~120g of endogenous CHO was “spared” during the 4 hour constant-load exercise bout. However, overall performance during the subsequent 1-hour time-trial was not different following the HFD-CHO diet compared to the CHO diet (33). Subjects in this study also ingested a breakfast prior to the trial and consumed plenty of CHO of CHO during the trial (~400 g/hour). Time-trial power output was 11% higher on the HFD-CHO diet compared to the high CHO trial once again individual differences were demonstrated with 5 of the 7 subjects improving performance on the HFD-CHO diet (33).

In summary, studies investigating a 5-6 day period of fat-adaptation, followed by a 1 day of high CHO intake in competitive athletes who completed a supervised program of 15-22hours/week of cycling, demonstrated decreased CHO stores compared to the a high CHO diet after 5 days. However, 1 day of rest in combination with a high-CHO diet was sufficient to super-compensate muscle glycogen concentrations to similar levels, independent of the preceding diet (26). In addition, this dietary strategy was associated with significantly higher rates of fat oxidation during sub-maximal exercise, despite the restoration of muscle glycogen levels prior to exercise, and was associated with muscle glycogen sparing (26; 28; 33). Despite the increase in fat oxidation and a concomitant 'sparing' of muscle glycogen during exercise, the available evidence for a potential ergogenic effect fat-adaptation followed by CHO-loading on prolonged endurance exercise is not clear-cut. However, the effectiveness of a high fat diet followed by CHO-loading on exercise performance has not been tested in self-paced endurance and ultra-endurance events.

The majority of studies that investigated the effect of a CHO-loading or a fat-adaptation followed by CHO-loading diet on exercise performance has demonstrated an individual variability in response. Some athletes improved performance, others demonstrated a decrease in performance and in some athletes performance remained unchanged in response to the specific dietary strategy. The performance suggests that some athletes respond to certain diets, and show true performance benefits, whereas others athletes do not respond.

1.4 RESPIRATORY EXCHANGE RATIO (RER) PHENOTYPES

Bosch et al. (18) studied non-CHO-loaded cyclists exercising at 70% $\text{VO}_{2\text{peak}}$ for 3 hours without ingestion any carbohydrates during exercise, and demonstrated that only 50% of the subjects were able to complete the 3-hour cycle. The subjects who fatigued early in this study tended to have higher rates of CHO oxidation throughout the exercise bout and seemed unable to increase the contribution of fat to oxidative metabolism. In contrast, the subjects who completed the exercise trials had lower relative rates of CHO oxidation and higher relative rates of fat oxidation throughout exercise (18). This data suggest that the ability of an athlete to oxidize fat may be an important determinant of performance during endurance exercise where muscle glycogen is limited.

Differences in substrate utilization have also been identified amongst athletes. Indeed, Goedecke et al. (66) found a large inter-individual variability in fasting, resting respiratory exchange ratio (RER) (~4-fold difference in fat oxidation) in trained athletes performing at a similar level that was normally distributed. Goedecke et al. (66) further demonstrated that at rest, type I muscle fibre content was the most significant determinant of RER, followed by muscle glycogen content which were both positively associated with RER. Training volume, dietary fat intake and resting FFA concentrations were also significantly (negatively) associated with RER (66). The variability in fuel utilization in these athletes was therefore dependent on factors intrinsic to the muscle (fibre type, enzymes) as well as modifiable factors such as diet and training (66).

Indeed, Cooling and Blundell (39) have identified two distinct dietary phenotypes, i.e. a high fat phenotype and a low fat phenotype, based on habitual dietary intake. The high-fat phenotype was able to increase fat oxidation in response to a high fat meal, whereas this was not the case for the low fat phenotype (40). In contrast, both groups increase CHO oxidation similarly in response to a high CHO meal. They also demonstrated significant differences in resting metabolic rate (basal metabolic rate), appetite and hormonal responses between the two dietary phenotypes that were independent of body fatness (40).

Furthermore, Goedecke et al. (66) examined the variability in RER during exercise of higher intensity and demonstrated that RER variability persisted during short bouts of low, moderate and high intensity exercise, despite a shift towards a greater reliance on carbohydrate (CHO) at higher exercise intensities (Figure 1.8) (66). During moderate intensity ($50\% W_{\text{peak}}$) exercise, muscle glycogen content, muscle citrate synthase (CS) activity, training volume, IMTG content and dietary fat intake contributed significantly to the model to predict RER, accounting for 42% of variance in exercising RER (66).

A further study from the same laboratory, divided athletes into two groups according to their fasting, resting RER, and demonstrated that CHO oxidation was higher in subjects with a high-RER compared to those with a low-RER during prolonged (3 hours), moderate intensity exercise ($55\% W_{\text{peak}}$) in the fasted state (64) (Figure 1.9).

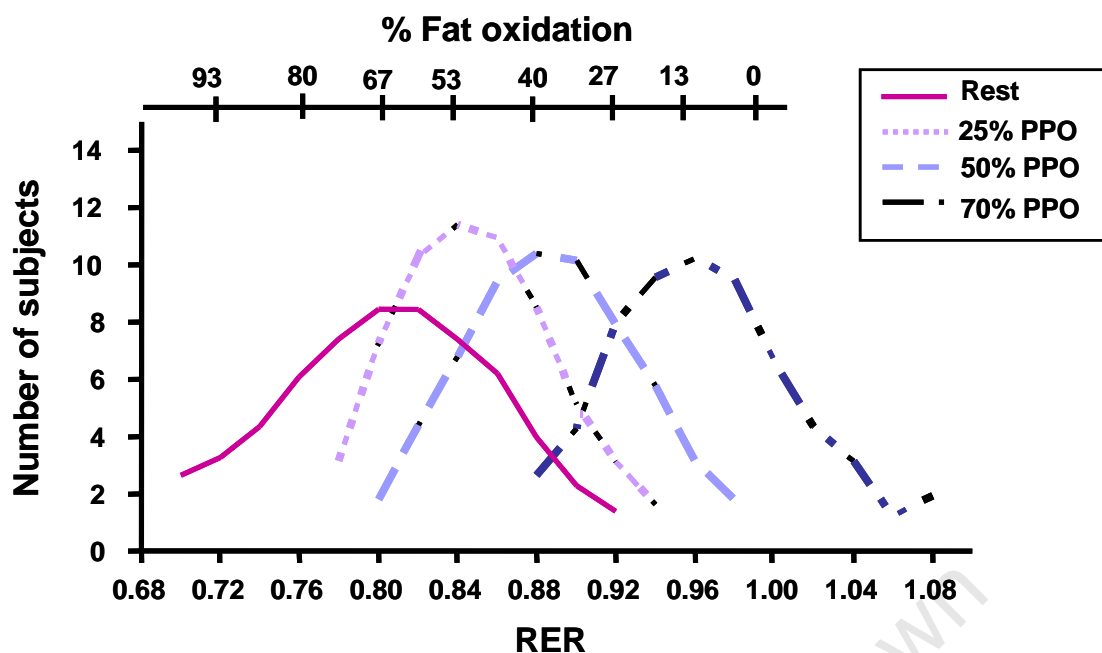


Figure 1.8: Variability in respiratory exchange ratio during rest and exercise of different intensities

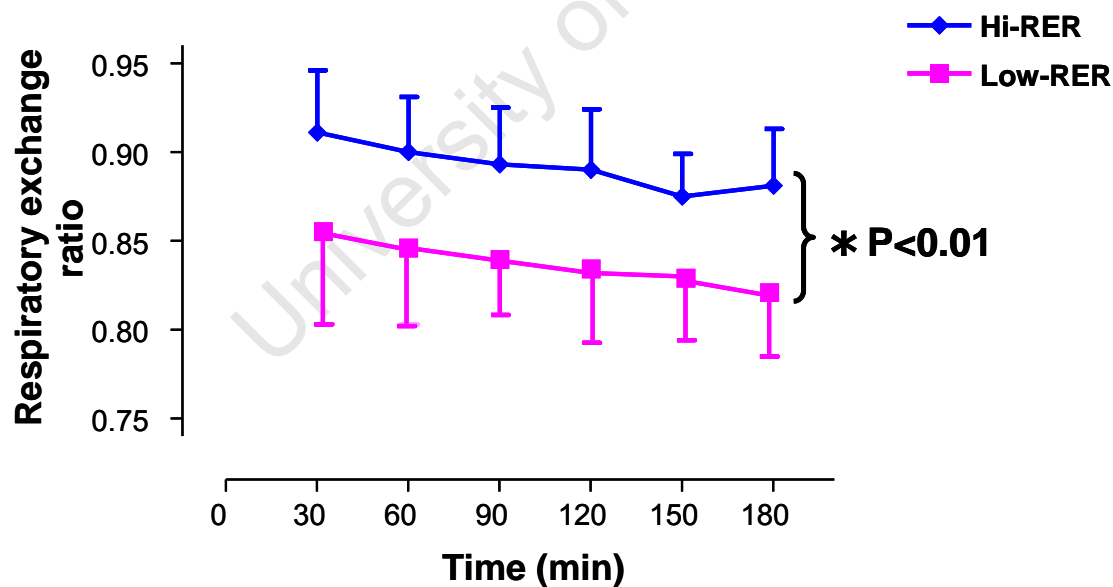


Figure 1.9: Variability in respiratory exchange ratio persists during prolonged moderate intensity ($55\%W_{peak}$) exercise in the high and low-RER groups.

Subsequently, the effect of CHO ingestion prior to and during the 3-hour moderate intensity cycle ride was investigated, and similar to the previous findings, CHO oxidation in the high-RER during exercise group was higher compared to the low-RER group (Figure 1.10) (45).

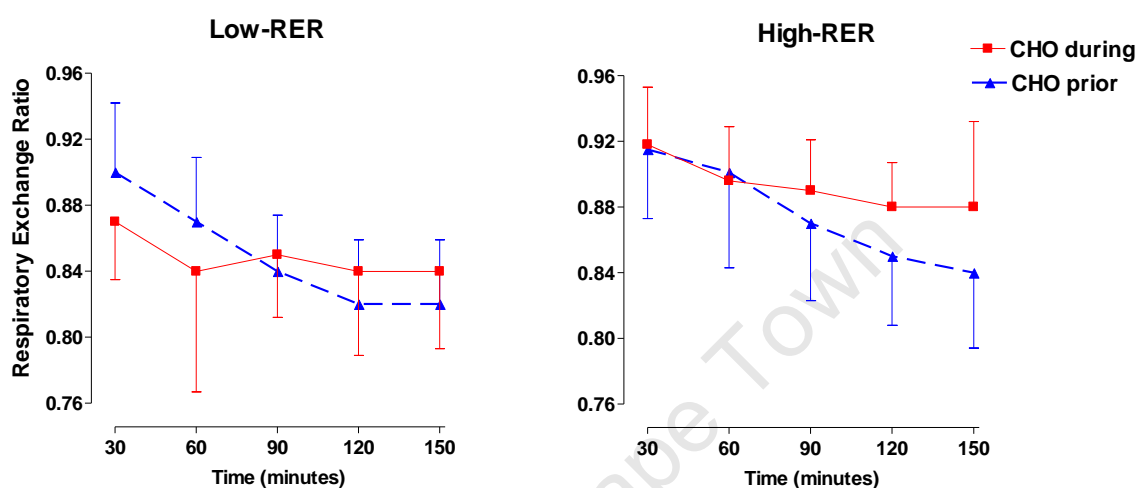


Figure 1.10: Variability in respiratory exchange ratio persists during prolonged moderate intensity ($55\%W_{peak}$) exercise in the high and low-RER groups despite the ingestion of CHO prior to and during exercise.

These studies provide evidence for the existence of distinct RER phenotypes in athletes. In addition to the external factors such as exercise intensity and duration, which alter fuel kinetics during exercise in response to dietary intake, RER phenotype may also effect fuel utilization in response to different diets.

The aims of this thesis are therefore: (1) to characterize the habitual dietary intakes of sub-elite male cyclists before and during an ultra-endurance event; (2) to investigate the effects of different dietary strategies aimed at increasing carbohydrate availability and ‘sparing’ muscle glycogen (e.g. CHO-loading and fat-adaptation), on substrate utilization and exercise performance during

simulated endurance and ultra-endurance exercise; and (3) to investigate the individual responsiveness of athletes to these dietary strategies.

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CHAPTER 2

Nutritional practices of male cyclists before and during an ultra-endurance event

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2.1 INTRODUCTION

The ingestion of a high carbohydrate (CHO) diet in the 3 days prior to an endurance event is a common practice used by athletes to enhance endurance performance. This practice, commonly referred to as “carbohydrate-loading” (CHO-loading), is based on findings from early studies that demonstrated a positive association between pre-exercise muscle glycogen concentrations and sub-maximal exercise duration to fatigue in trained (5; 12; 59) and untrained (4; 85) athletes. CHO-loading regimes have also been associated with a 2-3% performance improvement in endurance exercise in which a set distance is covered as quickly as possible (108; 146; 194; 196). Athletes competing in endurance events (>90 min) are therefore recommended to CHO-load prior to the event in order to maximize their endogenous CHO stores. To achieve this, general sports nutrition guidelines recommend CHO intakes of 7-10 g/kg body mass (BM) per day in the 3 days leading up to the event (27).

In a recent review, Burke (25) demonstrated that highly trained male athletes typically achieve CHO intakes within the recommended range during training and pre-competition. However, it is less certain whether recreational and sub-elite cyclists, who are not necessarily training and competing under the supervision of coaches and nutritionists, also meet recommended intakes prior to competition. Burke and Read (30) have demonstrated that in real life situations, marathon runners fail to reach the daily CHO intake targets of 7-10 g/kg BM without specific instructions or knowledge of nutrition and food composition. Similarly, Peters and Goetzsche (131) examined the pre-race dietary intake in a group of sub-elite ultra-distance runners prior to an 89-km

ultra-endurance marathon and reported CHO-intakes below the recommended range.

Although CHO-loading is associated with an increase in endurance capacity (5; 12; 23; 36; 59; 116), endogenous glycogen stores are limited and hepatic glucose production can only sustain euglycemia for ~1.5-2.5 hours of sub-maximal exercise in the fasted state (44). Several studies have shown that the ingestion of CHO during prolonged intense exercise will prevent the development of hypoglycemia by maintaining or raising the circulating glucose levels (37; 43; 44). In addition, exogenous CHO serves as the predominant fuel source in the latter stages (after 2-3 hours) of prolonged continuous exercise when muscle glycogen stores are low (38). Therefore, CHO ingestion during ultra-endurance events (>4-5 hours) is critical to prevent hypoglycemia and delay fatigue. General sports nutrition guidelines recommend CHO intakes of 30-60 g per hour of exercise (42).

Although there are a number of studies examining the effect of CHO-loading on endurance performance lasting ~1.5-4 hours, the available literature on pre-race nutritional strategies and CHO intakes, including information on the use of dietary supplements and sports foods, before and during ultra-endurance exercise (4-5 hours) in recreational and sub-elite athletes is limited. Therefore the aim of the study was to investigate the pre- and during race dietary and supplement intake of cyclists competing in a 210-km 1-day ultra-endurance cycle race.

2.2 METHODOLOGY

2.2.1 Subjects and study design

Forty five endurance-trained male cyclists participated in this dietary survey that was undertaken to characterize the pre- and during race dietary and supplement intakes of cyclists competing in a 210-km 1-day competitive mass participation ultra-endurance cycle race. Male cyclists that registered for the 2005 Double Century cycle race (an annual 210-km 1-day ultra-endurance cycle race held in the Western Cape, South Africa) were recruited from local cycle gyms and training centers. The course of the race was undulating (altitude between 300 and 800 m above sealevel) and included long climbs, steep down hills and flat sections. When the race profile was replicated in the laboratory, the relative intensity varied ($\sim 50\text{-}75\%W_{\text{peak}}$) along the course with an overall mean heart rate (HR) of $\sim 75\text{-}80\%$ HR max. The study was approved by the Research and Ethics Committee of the Faculty of Health Sciences of the University of Cape Town. The subjects were informed of the nature of the study and written informed consent was obtained prior to the start of the study.

2.2.2 Data collection

One week prior to participation in the 210-km cycle race, subjects were provided with the appropriate forms to complete during the study. The forms included a 3-day dietary record form with written instructions on how to accurately record dietary intake, as well as a questionnaire for the subjects to record their pre- and during race dietary intakes, as well as their systemic (i.e. headaches, dizziness, cramps) and/or gastro-intestinal (i.e. stomach cramps,

diarrhoea etc.) symptoms. In addition, the subjects were required to record their age, weight and height.

Three days prior to the 210-km cycle race, subjects completed the 3-day dietary record. Subjects were required to log any food, beverage or supplement, describing the nature of the product (i.e. *full cream* milk, *white* bread, *skinless* chicken etc.), the method of preparation (i.e. fried, grilled, roasted etc.) and the brand name where relevant (i.e. different supplement brands). Dietary intakes were quantified using household measures (i.e. 1 teaspoon, ½ cup, 1 slice of bread etc.), weight (grams), volume (i.e. 200 ml milk) and dimensions (i.e. 1 x 8 cm diameter pizza). Subjects were also required to indicate if the reported pre-race diet was representative of their usual daily intake and to stipulate reasons for any differences (i.e. they were CHO-loading or had social arrangements). On race day, cyclists were asked to record their pre-race meal, and then immediately after completing the race, to recall what they ate and drank during the race, describing the quantity and type of product (i.e. ½ a banana, 400 ml coke etc.), specifying the brand name where a supplement was used. Subjects were also required to report any systemic (i.e. headaches, dizziness, cramps) and/or gastro-intestinal (i.e. stomach cramps, diarrhoea etc.) symptoms that they might have encountered during the race.

The 3-day dietary records were analyzed with the Food Finder 3 programme (Medtech (Pty) Ltd, Medical Research Council, Tygerberg, South Africa) to determine the subjects' self-reported energy intake and macronutrient consumption. Data collection and analysis was performed by a registered

dietician. Supplements were analyzed according to the manufacturers' specifications as stipulated on the product label, and the macronutrient values were added to total dietary intakes. For the purpose of this paper, supplements included sport-specific supplements (i.e. carbohydrate drinks, carbohydrate gels, protein powders etc.) and sport-specific foods (i.e. energy bars, protein bars etc.). Vitamin and mineral supplements were excluded from the analysis.

The possibility of under-reporting of energy intake (E_i) in the present study was evaluated by the calculation of the mean reported E_i in relation to predicted basal metabolic rate (BMR) (E_i :BMR) (163) over the 3 days prior to the race according to the method of Goldberg (67). The E_i :BMR was adjusted for males with different physical activity levels (PAL)] as described by Black (16), with E_i :BMR values <1.21 (cut-off for males with a medium PAL) being considered as under-reporting. Although subjects in the present study were all endurance-trained with a presumably high PAL, it was assumed that subjects were tapering during the experimental period and reducing their training volumes, hence the cut-off value for medium PAL were used to identify under-reporters.

2.2.3 Statistical Analysis

Values are presented as the mean \pm standard deviation (SD). A One-way ANOVA to compare mean macronutrient intakes on the different days was performed using STATISTICA analysis software (Version 7, Statsoft, Tulsa, OK, USA). Independent T-tests were performed to test differences between CHO-loaders and non-loaders and Pearsons correlations were used to explore the associations between CHO intake (g/hour) and performance time (hour plus

fraction of hour). A multiple linear regression was used to explore the association between CHO intake and performance independent of ability. Statistical significance was accepted at $P < 0.05$.

2.3 RESULTS

2.3.1 Subject characteristics

The mean self reported age, weight and height of the cyclists who participated in this study were 39 ± 10 years, 75.6 ± 7.3 kg and 1.79 ± 0.07 m, respectively. Although the training status (VO_{2peak} and/or peak power output) of these athletes were not measured in the laboratory, the majority (91%) of the subjects completed an Argus Cycle Tour in the past two years, for which the finishing time and finishing position (expressed as a percentage of the total field) was used as a “marker” of training status/rider ability. The Argus Cycle Tour is a highly competitive annual mass participation 105-km cycle race held in the Western Cape that attracts ~30 000, riders ranging from recreational to elite. A finishing time of less than 3h15-3h30 min (mean speed ~30-32km/hour) for the Argus Cycle Tour can be regarded as well-trained. The mean finishing time for the subjects in the present study was $3h14 \pm 17$ min. Fifty percent of the subjects finished in the top 5% of the field (sub 3h10 min race time), 43% finished in the top 25% (3h10-3h40 min race time) and the remaining 7% finished in the top 45% of the field (3h40-4h05 min race time), suggesting that the majority of these cyclists were well trained. Mean 210-km race time was $7h18 \pm 1h03$ min, with the fastest cyclist finishing in 5h54 min and the slowest cyclist finishing in 10h25 min. Although the 210-km race was also a mass participation race which

attracted competitors and social riders, the majority of subjects in the present study raced the event with the mean finishing time within the top 35% of the field.

2.3.2. Habitual dietary intake 3 days prior to the race

Mean reported energy intake (kJ) in relation to calculated basal metabolic rate (kJ) (Ei:BMR) over the 3 days prior to the race was 1.8 ± 0.4 ranging from 1.23-2.87. Three subjects had an Ei:BMR value of >2.62 , which would normally be considered over-reporting. However, since the subjects were CHO-loading, the data was not excluded from the analysis. One subject was identified as an under-reporter (Ei:BMR <1.21) and his data was excluded from subsequent analysis.

The mean energy and macronutrient intakes over the 3-day period prior to the race are summarized in Table 2.1. CHO contributed the majority (54%) to total energy, whilst protein and fat contributed 15% and 31%, respectively. There was a weak, but significant correlation between mean CHO intake over the 3-day period prior to the 210-km race and race performance ($r=0.32$, $p<0.05$). Examining the three days individually (Table 2.2), the reported mean total energy intake for the day before the race (D-1) was significantly higher compared to D-2 and D-3 ($p<0.01$), largely due to a higher total CHO intake on D-1 ($p<0.01$), which was derived from both CHO supplements (75 ± 106 g vs. 42 ± 52 g and 47 ± 64 g, $p=0.057$) and dietary CHO sources (393 ± 149 g vs. 355 ± 116 g and 349 ± 118 g, $p=0.06$) on D-1 vs. D-2 and D-3, respectively. The

Table 2.1. Mean energy and macronutrient-intake over the 3-day period prior to race day (n=44).

| | Energy | | Carbohydrates | | | Protein | | | Fat | | |
|---------------------|------------|-----------|---------------|------------|-------------|----------|-----------|-------------|----------|------------|-------------|
| Intake | MJ | kJ/kg | g | g/kg | % TE | g | g/kg | % TE | g | g/kg | % TE |
| Total intake | | | | | | | | | | | |
| Mean±SD | 13.2±2.4 | 176±40 | 420±116 | 5.6±1.7 | 54±8.2 | 117±33 | 1.6±0.4 | 15±4 | 106±30 | 1.42±0.5 | 31±6.5 |
| Range | 9.5 – 19.8 | 113 – 330 | 261 – 817 | 3.2 – 11.3 | 41 – 77 | 65 – 231 | 0.9 – 2.9 | 9 – 25 | 51 – 211 | 0.47 – 3.5 | 14 – 44 |
| Diet | | | | | % CE | | | % PE | | | % FE |
| Mean±SD | 12.1±2.5 | 162±40 | 365±105 | 4.9±1.6 | 88±11 | 110±24 | 1.7±0.4 | 95±11 | 105±30 | 1.41±0.5 | 99.5±1.5 |
| Range | 7.6 – 19.7 | 109 – 328 | 242 – 790 | 3.2 – 11 | 61 – 100 | 65 – 179 | 0.9 – 2.6 | 46 – 100 | 51 – 211 | 0.7 – 3.5 | 93 – 100 |
| Supplements | | | | | % CE | | | % PE | | | % FE |
| Mean±SD | 1.1±1.07 | 14±14 | 55±55 | 0.7±0.7 | 12±11 | 7.5±21 | 0.1±0.3 | 5.0±10.5 | 0.6±1.6 | 0.01±0.0 | 0.5±1.5 |
| Range | 0 – 4.07 | 0 – 52 | 0 – 200 | 0 – 2.7 | 0 – 39 | 0 – 124 | 0 – 1.6 | 0 – 54 | 0 – 7.3 | 0 – 0.1 | 0 – 7 |

TE = Total energy, CE = Carbohydrate energy, PE = Protein energy, FE = Fat energy, SD = Standard deviation.

Table 2.2. Mean energy and macronutrient-intake 3 days (D-3), 2 days (D-2) and 1 day (D-1) prior to the race (n=44).

| | Energy | | Carbohydrates | | | Protein | | | Fat | | |
|--------------|------------|-----------|---------------|------------|---------|----------|-----------|--------|----------|-----------|---------|
| Total Intake | MJ | kJ/kg | g | g/kg | % TE | g | g/kg | % TE | g | g/kg | % TE |
| Day-3 | | | | | | | | | | | |
| Mean±SD | 12.5±3.1 | 167±46 | 396±132 | 5.3±1.9 | 53±9.6 | 114±41 | 1.5±0.5 | 16±5.0 | 100±35 | 1.3±0.5 | 31±8.5 |
| Range | 6.6 – 19.4 | 80 – 323 | 135 – 840 | 1.9 – 11.5 | 34 – 79 | 34 – 222 | 0.4 – 2.8 | 7 – 30 | 35 – 190 | 0.4 – 3.2 | 14 – 49 |
| Day-2 | | | | | | | | | | | |
| Mean±SD | 12.5±2.9 | 168±46 | 397±129 | 5.3±1.9 | 54±9.7 | 114±38 | 1.5±0.5 | 16±4.5 | 101±39 | 1.4±0.6 | 30±8.1 |
| Range | 8.5 – 22.8 | 100 – 317 | 219 – 999 | 3.1 – 14.1 | 36 – 83 | 60 – 220 | 0.8 – 2.8 | 8 – 24 | 35 – 206 | 0.5 – 3.4 | 10 – 47 |
| Day-1 | | | | | | | | | | | |
| Mean±SD | 14.5±3.6* | 193±55* | 468±171* | 6.3±2.4* | 54±11 | 123±45 | 1.6±0.6 | 15±5.4 | 116±48 | 1.6±0.6 | 31±9.0 |
| Range | 9.3 – 25.2 | 127 – 365 | 183 – 893 | 2.1 – 12.4 | 28 – 81 | 64 – 250 | 0.9 – 3.2 | 8 – 33 | 48 – 239 | 0.6 – 4.0 | 11 – 49 |

TE = Total energy, SD = Standard deviation, * p<0.01 for D-1 vs. D-2 and D-3.

relative contribution (% of total energy) of CHO, protein and fat to total energy intake were very similar on the three days (Table 2.2).

Fifty seven percent (n=25) of the subjects indicated that they CHO-loaded for 1-3 days before the race. Although it is general practice to CHO-load for 3 days, ingesting 7-10 g CHO/kg BM, almost half (n=12) of these subjects indicated that they CHO-loaded for only 1 day (D-1), achieving a mean CHO intake of 6.0 ± 1.9 g/kg BM on D-1 (Table 2.3). The subjects that indicated that they did not CHO-load, reported a lower mean CHO intake over the 3-day period prior to the race compared to the CHO-loaders ($p=0.052$). Mean reported CHO intake on D-1 was also lower, although not significantly for the non-loaders compared to the CHO-loaders (Table 2.3).

Table 2.3. Mean reported carbohydrate intakes of the carbohydrate-loaders vs. the non-loaders prior to the race (n=44).

| Loading period | Mean Reported CHO intake (g/kg/day) | | P-level | |
|--|-------------------------------------|------------------------|--------------------------|--------------------|
| | CHO-loaders (n=25) | | | Non-loaders (n=19) |
| | Loaded 3 days (n=13) | Loaded 1 day (n=12) | | |
| Full 3-day period before the race | | | | |
| Mean \pm SD (range) | 6.4 \pm 2.3 (3.6-11.3) | - | 5.1 \pm 1.3 (3.2-7.9) | 0.052 |
| % achieving intakes between 7-10g/kg | 8% | | 10.5% | |
| % achieving intakes \geq 10g/kg | 15% | | 0% | |
| One day before the race (D-1) | | | | |
| Mean \pm SD (range) | - | 6.0 \pm 1.9 (4.1-10) | 5.6 \pm 1.9 (3.4-10.7) | 0.572 |
| % achieving intakes between 7-10g/kg | | 17% | 10.5% | |
| % achieving intakes \geq 10g/kg | | 8% | 5% | |

SD = Standard deviation.

Overall, 14% of all subjects reported CHO intakes of ≥ 7 g/kg BM over the 3 days prior to the race, 15% reported a moderate-to-high CHO intake (6-7 g/kg BM), 62% reported a moderate CHO intake (4-6 g/kg BM) and 9% reported a low CHO intake (< 4 g/kg BM) over the 3 days prior to the race. The most popular dietary CHO-rich foods were pasta and pizza, with 89% of the subjects reporting that they had either pasta or pizza or a combination of both during the 3 days leading up to the race.

The majority (84%) of the subjects indicated that they used some form of supplement during the 3 days leading up to the event. During this period, the use of 36 different supplements was reported and included mostly CHO (64%) and protein (28%) supplements. The different types of supplements used are summarized in Table 2.4.

2.3.3 Pre-event breakfast/meal

The majority of subjects (95.5%) consumed a pre-event breakfast/meal. Mean total energy intake for the pre-event meal was 1.9 ± 0.6 MJ (24 ± 8 kJ/kg). Mean CHO intake was 76 ± 32 g (1.0 ± 0.4 g/kg) and contributed 70% to total energy whilst protein and fat contributed 16% and 14%, respectively. Breakfast cereal, including oats, corn flakes, a local cereal brand ("ProNutro") and muesli, which were served with milk or yoghurt, were the most popular breakfast choice (75%). Other options included bananas, bread or toast, muffins and raisin buns. Coffee was the most popular beverage to accompany breakfast (24%), followed by energy drinks (21%) and fruit juice (7%). Forty one percent of the subjects that consumed breakfast ingested a supplement with breakfast on the morning

Table 2.4. Supplements used 1-3 days prior to and during the race.

| Supplements | Pre-race | | During race | |
|---------------------------------|----------|----|-------------|----|
| | % | n | % | n |
| Carbohydrate Supplements | | | | |
| CHO-loading drinks (>15% CHO) | 21 | 9 | 2 | 1 |
| CHO energy drinks (7-10% CHO) | 34 | 15 | 27 | 12 |
| CHO energy powders (6-10% CHO) | 36 | 16 | 77 | 34 |
| CHO energy gels | 5 | 2 | 50 | 22 |
| CHO energy bars | 9 | 4 | 64 | 28 |
| Protein Supplements | | | | |
| Protein Bars | 5 | 2 | 7 | 3 |
| Protein Powders/Recovery Shakes | 23 | 10 | 5 | 2 |
| High protein energy gels | 5 | 2 | 9 | 4 |
| Other Supplements | | | | |
| Caffeine drinks | 2 | 1 | 2 | 1 |
| Dehydroepiandrosterone (DHEA)* | 2 | 1 | 0 | 0 |

Values are presented as a % of total number in group (frequency of usage) and number (n) of subjects recording usage of supplements. *Supplement that acts as a hormone precursor, claimed to increase muscle mass.

of the race. Supplements included CHO-loading drinks, CHO energy drinks or powders, protein shakes/meal replacement formulas and energy bars. Despite the popular use of supplements, especially on D-1, the majority of energy was still derived from dietary food sources (92% food energy vs. 8% supplement energy).

2.3.4 Dietary and supplement intake during the race

Energy and CHO intakes during the 210-km cycle race are presented in Table 2.5. The majority of energy (86%) was derived from CHO with a mean

estimated CHO intake of 445 ± 139 g. Mean reported CHO intake per hour of riding was 63 ± 23 g, with CHO intakes ranging from 28-145 g/hour. The majority of subjects (98%) achieved the recommended CHO intake of 30-60 g/hour of riding that is required to maintain euglycemia during prolonged (>90 minutes) exercise (42). CHO supplements accounted for most of the CHO energy consumed ($76 \pm 20\%$). The majority of subjects (98%) indicated that they used some form of supplement during the race, with 9% of the subjects using only supplements and 89% using supplements and food. One subject relied on food and water alone. There was a significant positive correlation between CHO intake during the race (g/hour) and 210-km race performance ($r=0.44$, $p<0.01$).

Table 2.5. Energy and Carbohydrate intake during the race (n=44).

| Intake | Energy | | Carbohydrates | | |
|---------------------|----------------|----------------|---------------|-------------|-------------|
| | MJ | MJ/hr | g | g/hr | %TE |
| Total intake | | | | | |
| Mean \pm SD | 8.77 ± 2.7 | 1.2 ± 0.4 | 445 ± 139 | 63 ± 23 | 86 ± 12 |
| Range | 4.0 – 15.3 | 0.6 – 2.6 | 208 – 870 | 28 – 145 | 66 – 91 |
| Diet | | | | | % CE |
| Mean \pm SD | 2.2 ± 1.5 | 0.3 ± 0.2 | 100 ± 71 | 14 ± 10 | 76 ± 20 |
| Range | 0 – 6.5 | 0 – 0.9 | 0 – 267 | 0 – 45 | 0 – 100 |
| Supplements | | | | | % CE |
| Mean \pm SD | 6.5 ± 0.5 | 0.9 ± 0.05 | 345 ± 146 | 49 ± 24 | 24 ± 20 |
| Range | 0 – 13.3 | 0 – 2.2 | 0 – 750 | 0 – 125 | 0 – 100 |

TE = Total energy, CE = Carbohydrate energy, SD = Standard deviation.

Thirty nine supplements were used during the race and included mostly CHO-rich energy drinks (19 different brands of CHO drinks), gels (10 different brands

of energy gels) and bars (9 different brands of bars). The different categories of supplements that were used during the race are summarized in Table 2.4. Most of the dietary CHO were derived from bananas, baby potatoes, fruit cake, sandwiches and sugary sweets (Table 2.6).

Table 2.6 Dietary food and beverage intake during the race.

| Food or Beverages | % | Food or Beverages | % |
|---------------------------------|----------|---|----------|
| Salty snacks | | Sweets and bars | |
| Baby Potatoes | 23 | Sugary sweets (i.e. jelly babies) | 16 |
| Dried meat/dried beef sausage | 5 | Nougat | 7 |
| Peanuts | 2 | Cereal bars | 7 |
| Ham/cheese/tomato/peanut butter | | Peanut butter bar | 2 |
| Sandwiches | 20 | | |
| Fruit and dried fruit | | Cakes/Breads with sweet fillings | |
| Bananas | 36 | Muffins | 16 |
| Other fruit | 2 | Fruit cake | 23 |
| Raisins | 14 | Fruit pies | 2 |
| Other dried fruit | 5 | Banana loaf | 2 |
| Beverages | | Biscuits | 2 |
| Coke | 27 | Jam + Peanut butter and jam | |
| Fruit juice | 2 | Sandwiches | 18 |

Values are presented as a % of total number in group.

Reported total mean fluid intake (water, beverages and supplements) during the 210-km cycle race was 4310 ± 1311 ml, corresponding to a mean fluid intake of 600 ± 178 ml/hour and ranged from 282-1167 ml/hour. When expressed relative to body weight, mean reported fluid intake was 8.0 ± 2.4 ml/kg/hour and ranged

from 3.1-16.7 ml/kg/hour. Fluid intake during the 210-km cycle race was not associated with race performance ($r=-0.13$, $p=0.404$).

2.3.5 Reported symptoms during the race

Forty one percent of the subjects ($n=18$) reported some form of adverse symptom/s during the race. The main symptoms included muscle cramps (33%) and abdominal discomfort/nausea (33%). Other symptoms included loss of appetite ($n=2$), headache ($n=1$) and low blood sugar levels (“hitting the wall”) ($n=2$).

2.4 DISCUSSION

Fatigue during prolonged (>90 minutes) exercise is associated with, amongst other factors, the depletion of glycogen stores in trained (5; 12; 59) and untrained (4; 85) athletes. Athletes are therefore recommended to CHO-load (7-10 g CHO/kg BM/day) during the 3 days leading up to an event (27). Despite these recommendations, we found that the mean self-reported CHO intakes achieved during the 3 days leading up to the 210-km race were only 5.6 ± 1.7 g/kg BM/day, which is well below that recommended.

Bussau et al. (31) demonstrated that a CHO intake of ~ 10 g/kg BM/day combined with active rest for as little as 1 day can maximize muscle glycogen stores in well trained athletes. It is important to note that the subjects in this study undertook their last training session the day before the loading protocol officially started, so in essence the true loading phase was ~ 36 hours (31).

Although in the present study the subjects reported a significantly higher CHO intake on the day before the race (D-1) compared to D-2 and D-3, the mean self-reported CHO intake on D-1 was only 6.3 ± 2.4 g/kg BM. In fact, only 32% (n=14) of all subjects in the present study achieved CHO intakes ≥ 7 g/kg BM on D-1, of which only four had intakes ≥ 10 g/kg BM.

In contrast to our findings, a review characterizing the dietary intake of highly-trained endurance cyclists reported pre-competition CHO intakes of 6.8-7.8 g/kg BM/day (51). Reported pre-competition energy intakes were also higher compared to the present study (205-252 kJ/kg vs. 176 kJ/kg) (51). More recently, Burke et al. (25; 27) demonstrated that highly trained male athletes typically achieve CHO intakes within the recommended range. The lower mean reported CHO and energy intake in the 1-3 days prior to the ultra-endurance event in the present study could possibly be explained by two factors, namely, the level of athletes and the dietary period of interest in this study. Firstly, the majority of the available information on the nutritional intake of road cyclists includes males ranging from national level to world-class professionals. The level of cyclists taking part in the present study, based on Argus Cycle Tour time that was used as a "marker" of training status, ranged from moderate (4h02 min, top 45% of Argus Cycle Tour) to elite (2h50 min, top 0.7% of Argus Cycle Tour) with the majority of athletes (93%) being classified as well-trained (<3h30 min, finished within the top 5-25% of Argus Cycle Tour finishers). In fact, when we stratify the data into quartiles according to Argus Cycle Tour times, mean self-reported energy (13.2 ± 2.4 MJ vs. 12.7 ± 1.5 MJ, $p=0.058$) and CHO (5.6 ± 1.7 vs. 4.6 ± 1.0 g/kg BM, $p=0.149$) intakes tended to be higher in the top

25% compared to the lowest 25%. In a qualitative study, Robins and Hetherington (149) demonstrated a variation in nutrition knowledge in a group of triathletes that was related to the level of competition. Food choices, especially those of the more competitive athletes, were based on past experience and 'trial and error', with choices been made to improve performance. The less competitive athletes were extremely interested in the idea of manipulating pre-race nutrition to improve performance, but still tended to be in the 'trial and error' phase (149).

Secondly, the period of interest that was investigated in the majority of studies examined a specific occasion (e.g. racing in a 5-day tour), a typical training diet and/or the athlete's 'every-day' or 'typical' diet. The period of interest in the current study, i.e. the 1-3 days prior to the 210-km ultra-endurance event does not necessarily represent a 'typical', 'training' or 'competition' diet. The only other study that measured dietary intake in the same period (i.e. 1-3 days prior to a 1-day ultra-endurance event), and in the same level of athletes prior to an ultra-endurance marathon reported CHO intakes of 441 ± 255 g (6.1 g/kg) (131). Mean reported total pre-race energy, macronutrient and supplement intake in the present study were also similar to those reported by Peters and Goetzsche (131).

There was a weak, but significant correlation between mean self-reported CHO intake over the 3-day period prior to the 210-km cycle race and race performance ($r=0.32$, $p=0.034$). However this finding was possibly confounded by the suggestion that higher level athletes might have a better nutritional

knowledge than recreational athletes (149), and that the top 25% of athletes in the present study reported a higher CHO intake over the 3-day dietary period compared to the lowest 25%. Indeed, when covarying for Argus Cycle Tour time (“marker” of training status), we failed to demonstrate a correlation between mean self-reported CHO intake over the 3-day period prior to the 210-km cycle race and race performance. Although it is well documented that CHO-loading elevates muscle glycogen stores (5; 12; 59; 169), prolongs endurance time to fatigue (5; 12; 23; 59; 116), and enhances time-trial performance in trained (108; 146; 181; 194; 196) and untrained (181) individuals, in events lasting ~90-180 minutes, the benefits of CHO-loading requires confirmation in a randomized placebo-controlled trial in athletes of different ability. In addition, to our knowledge, there are no studies that have specifically examined the effects of CHO-loading on ultra-endurance (>4-5hrs) exercise performance. Nonetheless, CHO-loading is generally accepted as the dietary strategy of choice for an event of this nature.

Despite general recommendations for athletes to CHO-load (7-10 g CHO/kg BM/day) 1-3 days prior to endurance and ultra-endurance events, only 57% (n=25) of the subjects indicated that they did this. Moreover, the mean self-reported CHO intake for the CHO-loaders prior to the race was below the recommendations for CHO-loading, with only 23% of CHO-loaders achieving the minimum recommended CHO intake (≥ 7 g/kg BM) in the 3-days prior to the race. This demonstrates a marked discrepancy between perceived and actual intakes of CHO. Burke and Read (30) demonstrated similar findings in a group of 76 marathon runners who failed to reach daily CHO intake targets of 7-10

g/kg BM after practicing a variety of methods that they believed would achieve these intakes. Burke (24) suggested that in a free-living situation, without specific instructions or knowledge of nutrition and food composition, athletes are limited in their ability to achieve the dietary requirements of CHO-loading. A typical example of the lack of knowledge regarding nutrition and food composition is the choice of pizza as a CHO-rich food by a quarter of the subjects in this study. A typical medium thin-base pizza is high in fat (~20 g per pizza) and contains ~60 g CHO. In contrast, a plate of low-fat tomato-based pasta contains ~120 g CHO and less than 8 g of fat. In our study, the subjects who achieved higher CHO intakes, ingested more CHO in the form of supplements compared to those not meeting the requirements (21% of CHO energy vs. 11% of CHO energy, respectively). In addition, these subjects chose the more concentrated CHO-loading (>15% CHO) and energy drinks, which are energy dense, practical to use and easy to consume.

Although the majority of subjects failed to meet recommended CHO intakes during the 1-3 days prior to the race, this was not the case on race day, as on average, the cyclists met the recommended CHO and fluid intakes. Mean self-reported CHO intake on the morning of the race was 1.0 ± 0.4 g/kg BM, within the recommended range of 1-4 g of CHO/kg BM (73). Mean self-reported CHO intake during the race was 63 ± 23 g/hour, which was slightly higher than the recommended CHO intake (i.e. 30-60 g/hour) to maintain normal blood glucose levels (42). The upper range of 60 g/hour is based on the maximal rate of exogenous glucose oxidation (~1 g/min) (104). However, when different sugars (i.e. glucose and fructose) are combined, higher rates of exogenous CHO

oxidation have been reported (~1.3 g/min) (98; 99), suggesting that CHO intakes of greater than 60 g/hour of exercise are not necessarily redundant. There was a significant positive correlation between self-reported CHO intake during the 210-km race and race performance ($r=0.44$, $p<0.01$), however, similar to the findings for the association between pre-exercise CHO intake and performance, this was not longer significant when adjusting for athletes ability. Exogenous CHO serves as the predominant fuel source in the latter stages (after 2-3 hours) of prolonged continuous exercise when muscle glycogen stores are low (38), and is critical to prevent hypoglycemia and delay fatigue (37; 43; 44). The majority of energy and CHO during exercise was derived from supplements such as CHO energy drinks, gels and bars that provided a compact and practical source of available energy.

Mean self-reported fluid intake, expressed relative to cycling time was 600 ± 178 ml/hour (8.0 ± 2.4 ml/kg/hour). According to the 2007 American College of Sports Medicine (ACSM) position statement on exercise and fluid replacement (159), it is suggested that athletes should drink enough to prevent excessive dehydration (>2% body loss from water deficit) and excessive changes in electrolyte balance (generally 400-800 ml/hour, depending on environmental conditions). The mid-day temperature on race day was ~28-30 degrees Celcius. The majority of subjects (81%) achieved fluid intakes between 400-800 ml/hour. It was interesting to note that the majority of subjects who experienced muscle cramps reported a low fluid intake of less than 500 ml/hour ($5.9-7.6$ ml/kg/hour). In early anecdotal reports, exercise associated muscle cramping (EAMC) was associated with, amongst other things, profuse sweating and

dehydration, as well as warm environmental conditions (83). However, recent studies have failed to support these findings (168). Premature muscle fatigue and abnormal neuromuscular control at the spinal level in response to fatiguing exercise has been proposed as a possible alternative explanation for EAMC (167).

As with any dietary survey study, there are limitations to the accurate assessment of dietary intake (161; 162). We have attempted to reduce the likelihood of misreporting by excluding under-reporting. However, this was difficult due to the fact that the dietary period of interest in the present study represented a typical pre-race diet and not a typical habitual diet. A cut-off value for athletes with a medium physical activity level ($E_i:BMR < 1.21$) was used to identify under-reporters since it was assumed that subjects were tapering during the experimental period and reducing their training volumes prior to the 210-km race, but not necessarily altering their total energy intake. A further limitation to the present study was that household measures were used to quantify food intake instead of food scales due to limited facilities (requirement of 50 individual scales). However, a recent review from Burke et al. (27) showed that most surveys in athletic populations used a 3- to 4-day food diary with the quantification of intake described by household measures. Finally, we did not record the subjects' training history or their official training status in the laboratory, but Argus Cycle Tour race performance times and finishing position provide some form of criteria to characterize the ability of the riders.

In conclusion, the majority of subjects in the present study failed to meet the typical recommended pre-race CHO intake of ≥ 7 g/kg BM per day. In addition, there was a discrepancy between perceived and actual self-reported intakes of CHO. Laboratory-based studies are required to determine if CHO is in fact beneficial for ultra-endurance performance in athletes of different ability, with a view to devise scientifically-based recommendations for sub-elite athletes competing in ultra-endurance events. Once scientific recommendations are known, sports nutritionists and coaches can advocate specific pre-race CHO intakes and ensure that their athletes are educated in terms of nutrition and food composition, and given specific instructions on how to achieve optimal CHO intakes (i.e. type and quantities of CHO-rich foods) prior to ultra-endurance events.

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CHAPTER 3

Comparison of a high carbohydrate diet vs. a moderate carbohydrate diet on substrate utilization and performance in athletes with different RER phenotypes

3.1 INTRODUCTION

The ingestion of a high carbohydrate (CHO) diet is generally recommended prior to an endurance event, however in Chapter 2 we demonstrated that the majority of cyclists (86%) failed to meet the recommended CHO intake (≥ 7 g CHO/kg body mass) in the 3 days prior to the 210-km ultra-endurance cycle race. Moreover, not all the cyclists (only 57%) chose to consume a high CHO diet prior to the endurance event. Although CHO-loading has been shown to improve prolonged (>90 minutes) endurance capacity (time to fatigue) compared to low ($<10\%$ CHO energy) (5; 12; 36; 59) and moderate CHO (~ 4 -6 g CHO/kg) diets (23; 116), there is limited data on the effect of CHO-loading on self-paced time-trial performance, which is more representative of real-life race situations. Of the few studies that have examined the effect of a high CHO compared to a mixed or moderate CHO diet on prolonged (>90 minutes) time-trial performance, some have shown a performance benefit (108; 145; 146; 194; 196), while others have shown no effect (6; 29). However, most of these studies demonstrated a variability in response to the different diets, suggesting that not everyone may respond in a similar manner to a certain diet.

The variability in response may be attributed, in part, to an athletes' oxidative or respiratory exchange ratio (RER) phenotype. Indeed, Bosch et al. (18) demonstrated that non-CHO-loaded subjects with a high exercising RER tended to fatigue early during 3 hours of moderate intensity ($70\% \text{VO}_{2\text{peak}}$) cycling in the fasted state. In contrast, subjects with a low exercising RER were able to complete the 3-hour moderate intensity cycle (20). Goedecke et al. (66) found a

large inter-individual variability in fasting, resting RER that ranged from 0.72 to 0.93 (mean \pm SD was 0.82 \pm 0.05) in well-trained cyclists. This variability in substrate utilization was linked to factors intrinsic to the muscle (fibre type, enzymes), as well as modifiable factors such as diet and training (66). Goedecke et al. (66) further demonstrated that RER variability persisted during short bouts of low, moderate and high intensity exercise, despite a shift towards a greater reliance on CHO at higher exercise intensities (66). Despite this shift, a normal distribution was demonstrated at rest and at all three exercise intensities (66). A subsequent study from the same laboratory, categorized athletes according to their fasting, resting RER, and demonstrated that CHO oxidation in the high-RER group remained higher during prolonged (3 hours) moderate intensity (55% W_{peak}) exercise in the fasted state compared to the low-RER group (64). Taken together, these studies suggested that there are distinct RER phenotypes, and that these phenotypes may be associated with resistance to fatigue in the fasted state.

However, endurance exercise is not usually undertaken in the fasted state and athletes typically ingest CHO prior to and during exercise. Hence a subsequent study examined the effect of CHO ingestion prior to (pre-exercise meal containing ~80 g CHO) and during (60 g/hour) a 2.5-hour moderate intensity (55% W_{peak}) cycle followed by a 250 kJ time-trial on subjects categorized according to a high or low fasting resting RER phenotype. Similar to the findings of Goedecke et al. (64), this study demonstrated that CHO oxidation in the high-RER group was increased relative to the low-RER group during constant-load exercise, despite CHO ingestion prior to and during exercise (45). However,

there were no differences in subsequent time-trial performances between the two RER phenotype groups (45).

No previous studies have examined the changes in substrate oxidation during exercise in response CHO-loading in athletes with low and high RER phenotypes. This is an important question in that CHO-loading is a dietary strategy typically recommended for athletes taking part in endurance events (27). Individuality in response to CHO-loading may possibly explain why some athletes fail to observe a performance benefit in response to CHO-loading compared to a mixed or moderate CHO diet and why some athletes may choose not to CHO-load prior to an endurance event (Chapter 2). The aim of this study was therefore to investigate the effects of a high CHO vs. a moderate CHO diet on substrate utilization and performance in athletes with low or high fasting resting RER phenotypes.

3.2 METHODOLOGY

3.2.1 Subjects and study design

Twenty endurance-trained male cyclists, nine with a high-RER phenotype ($RER \geq 0.84$) and eleven with a low-RER phenotype ($RER \leq 0.80$), based on preliminary screening as described below, participated in this study, which was approved by the Research and Ethics Committee of the Faculty of Health Sciences of the University of Cape Town. All subjects were free from known metabolic conditions and were currently not taking any medications for chronic conditions such as high blood pressure or stimulants for conditions such as

asthma. The subjects were informed of the nature of the study and written informed consent was obtained prior to the start of the study.

Each subject completed two trials in a randomized, single-blind crossover manner, with a 2 week washout period separating the trials. Each trial consisted of a 3-day dietary period during which the subjects ingested either a moderate or high CHO diet, followed by 1 day of testing during which the subjects were required to complete a 2.5-hour constant-load cycle at 55% W_{peak} followed by a 250 kJ time-trial. The diet and testing protocol are summarized in Figure 3.1.

3.2.2 Preliminary testing

Screening

Prior to inclusion in the study, subjects were screened and selected on the basis of their resting fasting respiratory exchange ratio (RER) and assigned to either a high-RER ($RER \geq 0.84$) or a low-RER group ($RER \leq 0.80$). The cut-points for the RER groups were based on the distribution of substrate oxidation from Goedecke et al. (66) in which the resting fasting RER of more than 40 well-trained male athletes was measured. In this study, the RER was normally distributed and the upper and lower tertiles were used as the cut points for the high-RER and low-RER groups.

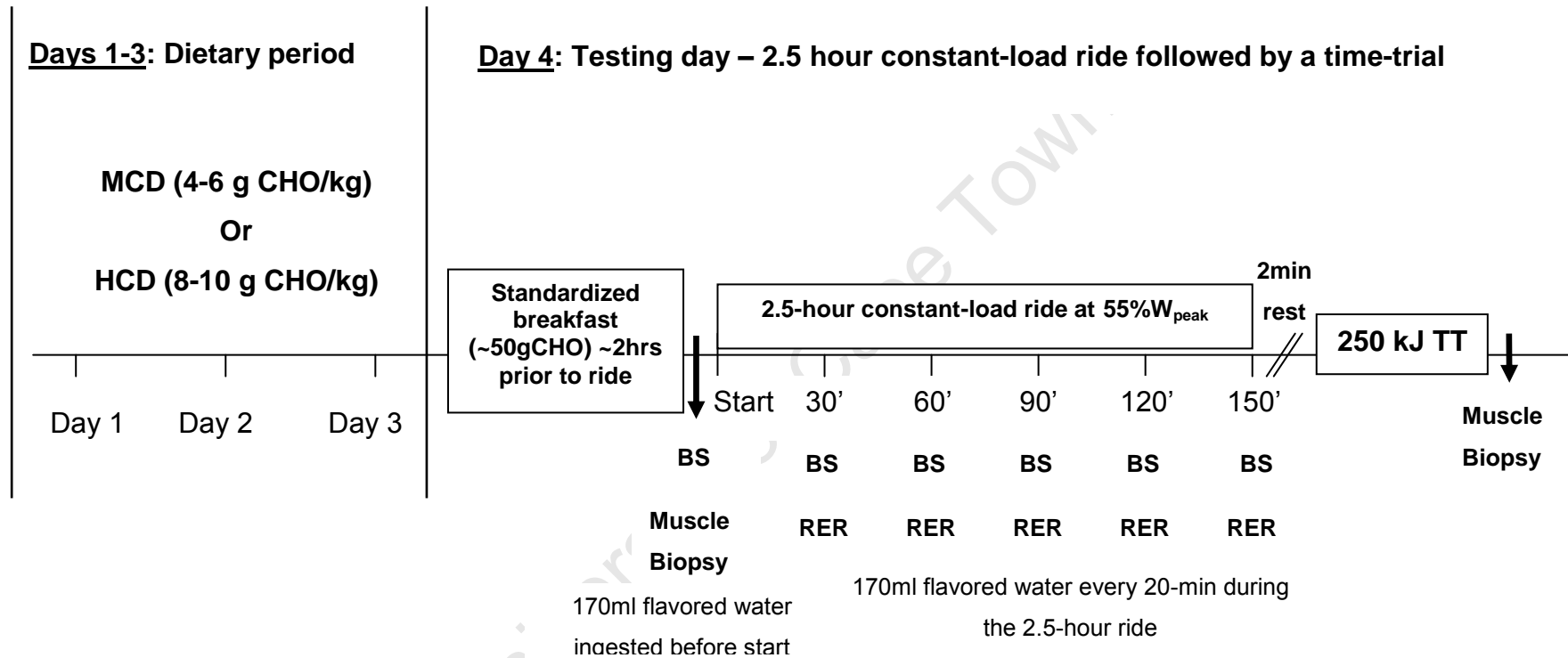


Figure 3.1: Dietary and experimental protocol.

MCD, moderate CHO diet; *HCD*, high CHO diet; *BS*, blood sample; *RER*, respiratory exchange rate; *TT*, time-trial

For the screening, subjects reported to the laboratory after a 10-12 hour overnight fast. Oxygen uptake (VO_2) and CO_2 production (VCO_2) were measured for 15-20 minutes to determine resting RER using a breath-by-breath Oxycon Alpha analyzer (Jaeger-Mijnhardt, Bunnik, The Netherlands) while the subjects was seated in a resting position and breathing through a mouthpiece attached to a mask. Before each test, the gas meter was calibrated with a Hans Rudolph 3-L syringe (Vacumed, Ventura, CA) and the analysers were calibrated with room air and a 4% CO_2 and 96% N_2 gas mixture. Only the subjects who had a resting fasting RER of ≤ 0.80 or ≥ 0.84 were selected to participate in the study. The subjects with a resting fasting RER between 0.80-0.84 were excluded from participation in the study.

Anthropometry

Body weight (kg) and height (m) were measured to the nearest decimal place. The percent body fat was determined from measurements of skinfold thickness, using the equations of Durnin and Womersley (50).

Peak power output (W_{peak}) and peak oxygen consumption ($\text{VO}_{2\text{peak}}$)

Peak oxygen consumption ($\text{VO}_{2\text{peak}}$) and peak power output (W_{peak}) were measured on an electronically braked cycle ergometer (Lode, Groningen, Holland) modified with toe clips and racing handlebars, as described by Hawley and Noakes (76). Work rates were started at 3.33 W/kg body mass and increased first by 50 W and then by 25 W every 150 seconds until the subject was exhausted. W_{peak} was defined as the highest exercise intensity the subject completed for 150 seconds (in watts), plus the fraction of time spent in the final

workload. During the progressive exercise test, ventilation volume (V_E), VO_2 and VCO_2 were measured over 15-second intervals using a breath-by-breath Oxycon Alpha analyzer (Jaeger-Mijnhardt, Bunnik, The Netherlands) as described previously. Heart rate was recorded continuously by means of a PolarTM Heart Rate Monitor (Polar Electro, Kempele, Finland). W_{peak} values were used to set the work rate for the 2.5-hour constant-load ride during the experimental trial corresponding to 55% of W_{peak} .

Familiarization ride

Two weeks prior to the trial, subjects were required to complete a familiarization trial consisting of a 2.5-hour constant-load ride at 55% W_{peak} followed by a 250 kJ time-trial on an electronically braked cycle ergometer (Lode, Groningen, Holland). This trial provided subjects with an opportunity to familiarize themselves with the duration, distance and pacing in the laboratory. The familiarization ride also served as a screening trial to establish if the subjects were adequately trained and able to complete the trial.

Dietary Analysis and training history

Subjects were also required to complete a 3-day dietary record representing their habitual dietary intake (2 week days and one weekend day). The dietary records were analyzed with the Food Finder 3 programme (Medtech (Pty) Ltd, Medical Research Council, Tygerberg, South Africa) to determine the subjects' self-reported energy intake and macronutrient consumption. The habitual dietary information was used as a guideline to devise the two experimental diets. To aid adherence to the diets, subjects were also required to indicate their

food preferences. In addition, subjects were required to complete a training diary. The type, duration and intensity of training were recorded and converted to metabolic energy equivalents (METS).

3.2.3 Dietary manipulations

Subjects were required to ingest either a moderate CHO diet (MCD, 4-6 g CHO/kg) or an iso-caloric high CHO diet (HCD, 8-10 g CHO/kg) for 3 days prior to the experimental trial. A registered dietician formulated individualized menus. In order to control dietary intake, all the meals were pre-packed and provided for the subjects together with a diary to record any deviations from the diet. Efforts were made to blind the diets by covertly manipulating the macronutrient compositions of the diets.

3.2.4 Exercise trial

Following 3 days of dietary intervention, subjects reported to the laboratory ~2 hours after ingesting a standardized breakfast (1 cup of corn flakes with ½ cup of low-fat milk and a slice of toast with a scraping of margarine and marmite) containing ~50 g carbohydrates. A muscle biopsy was taken from the vastus lateralis muscle, using the percutaneous needle biopsy technique of Bergstrom (11), as modified by Evans et al. (53). Muscle samples were immediately frozen in liquid N₂ and stored at -80° for subsequent analysis of muscle glycogen content.

Twenty minutes after the muscle biopsy, subjects consumed 170 ml of flavored water and commenced the 2.5-hour constant-load ride at 55% of W_{peak} on the

electronically braked cycle ergometer. During the 2.5-hour constant-load ride, VO_2 and VCO_2 values were measured for 4-5 minutes every 30 minutes, using the on-line Oxycon computerized system for the calculation of total CHO and fat oxidation and the determination of exercising RER. In addition, a blood sample was drawn at rest and again at 30-minute intervals throughout the constant-load ride for the subsequent analysis of plasma glucose, lactate and insulin levels and serum free fatty acid (FFA) concentrations. Subjects were also required to consume 170 ml of flavored water (energy free) every 20 minutes throughout the 2.5-hour constant-load ride. The present study aimed to test the robustness of the RER phenotype in response to CHO-loading therefore subjects ingested a placebo drink during exercise, and not an energy drink containing carbohydrates. On completion of the 2.5-hour constant-load ride subjects were allowed to rest for 2 minutes before completing a 250 kJ time-trial as fast as possible. A second muscle biopsy was performed immediately on completion of the 250 kJ time-trial.

3.2.5 Total CHO and fat oxidation

The overall rates of CHO and fat oxidation (grams per minute) were calculated from the formulae of Frayn (57), assuming a non-protein respiratory exchange ratio.

$$\begin{aligned}\text{Total CHO oxidation} &= 4.55\text{VCO}_2 - 3.21\text{VO}_2 \\ \text{Total fat oxidation} &= 1.67(\text{VO}_2 - \text{VCO}_2)\end{aligned}$$

In these formulae, VCO_2 is the volume of CO_2 in the expired air (liters per minute) and VO_2 is the corresponding oxygen uptake (liters per minute).

3.2.6 Blood sampling and analysis

Venous blood samples (~8 ml) were drawn at rest and at 30-minute intervals during the 2.5-hour constant-load ride by inserting a flexible 20-gauge cannula into a forearm antecubital vein and attaching it to a three-way stopcock. The cannula was kept patent by flushing with 1 ml sterile saline after each blood sample. One aliquot (2 ml) was placed into a vacutainer containing potassium oxalate and sodium fluoride for subsequent analysis of plasma glucose and lactate concentrations. Another aliquot (3 ml) was placed into a vacutainer containing lithium heparin for analysis of plasma insulin concentrations. The remaining aliquot (3 ml) was placed into a vacutainer containing gel and clot activator for determination of serum FFA concentrations. All samples were kept on ice and then centrifuged at 3000 rpm at 4°C for 10 minutes at the end of the trial. The supernatants were stored at -80°C (insulin) and -20°C (glucose, lactate, and FFA) for later analysis.

Plasma glucose concentrations were determined using the glucose oxidase method (Glucose Analyser 2 (intra CV = 0.64%); Beckman Instruments, Fullerton, CA, USA). Plasma lactate and serum FFA concentrations were determined by spectrophotometric measurements (Model 35; Beckman, Fullerton, CA, USA) using commercial kits (Lactate Pap; Bio-Merieux, Marcy-L'Etiole, France; and FFA Half-micro test; Boehringer, Mannheim, Germany) (intra CV = 0.43% and 2.08% for lactate and FFA, respectively). Plasma insulin concentrations were determined using immuno-chemiluminescence and the ADVIA Centaur insulin assay as described by El Kenz and Bergmann (52).

3.2.7 Analysis of muscle biopsies

For determination of muscle glycogen levels, the muscle samples were freeze-dried and dissected free of any visible fat and connective tissue. Muscle glycogen content was determined as glucose residues with the glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA, USA) after hydrolyses of the muscle samples in 2 M HCL at 95°C for 3 hours (130).

3.2.8 Statistical Analysis

Values are presented as the mean \pm standard deviation (SD). A 3-way analysis of variance (ANOVA) with repeated measures and Tukey post hoc analysis were performed to test group \times diet \times time interactions using STATISTICA analysis software (Version 7, Statsoft, Tulsa, OK, USA). A 2-way ANOVA with repeated measures and Tukey post hoc analysis were performed to test group \times diet interactions. Independent T-tests were performed to test differences in single variables between the high-RER and low-RER groups (i.e. habitual energy and macronutrient intakes). A Pearson correlation was performed to examine the association between pre-exercise muscle glycogen content and performance. Statistical significance was accepted at $P < 0.05$. $P < 0.1$ was regarded a trend. Magnitude based inferences, as described by Batterham and Hopkins (8), were also used to calculate the mean effect of the diets (\pm 90% confidence limits) on time-trial performance, and to estimate the chances that an effect was meaningful.

3.3 RESULTS

3.3.1 Subject characteristics

The characteristics of the subjects are summarized in Table 3.1. By design, there was a significant difference ($p < 0.001$) in fasting resting RER between the high-RER and low-RER group. Despite differences in resting RER, mean age, weight, height, body fat percentage and training status (W_{peak} and $VO_{2\text{peak}}$) were remarkably similar (Table 3.1).

Table 3.1. Subject Characteristics

| Characteristic | High-RER (n=9) | Low-RER (n=11) |
|---------------------------------|----------------|----------------|
| Resting RER | 0.87±0.01* | 0.77±0.02* |
| Age (years) | 34±6.0 | 31±7.0 |
| Weight (kg) | 76.2±8.8 | 77.6±4.8 |
| Height (m) | 1.77±0.08 | 1.77±0.05 |
| Body fat% | 15.9±2.9 | 15.4±4.0 |
| $VO_{2\text{peak}}$ (ml/kg/min) | 59.5±6.6 | 58.9±4.3 |
| W_{peak} (W) | 355±34 | 355±30 |
| Power:Weight (W/kg) | 4.7±0.5 | 4.6±0.3 |
| Training METS/week | 3610±1839 | 4952±1382 |

Values are means ± standard deviations (SD). RER, respiratory exchange ratio; $VO_{2\text{peak}}$, peak oxygen uptake; W_{peak} , peak power output; W, watts; METS, metabolic energy equivalents. * $p < 0.05$ group effect.

3.3.2 Dietary control

All the subjects followed the experimental diets, ingested the food that was provided during both trials and achieved the recommended CHO target. The

Table 3.2. Habitual dietary intake and dietary intake during the dietary treatments (n=20)

| | Energy (MJ) | CHO | | | FAT | | | PROTEIN | | |
|---------------------|----------------|---------|----------|--------|---------|----------|--------|---------|---------|--------|
| | | g | g/kg | %E | g | g/kg | %E | g | g/kg | %E |
| Habitual | | | | | | | | | | |
| High-RER | 11.9±2.5 | 333±63 | 4.3±0.8 | 50±5 | 109±27 | 1.4±0.3 | 35.5±3 | 94±22 | 1.2±0.3 | 14.5±1 |
| Low-RER | 11.2±3.0 | 313±81 | 4.1±1.1 | 50.5±9 | 96±39 | 1.2±0.5 | 33±7 | 107±38 | 1.4±0.5 | 16.5±4 |
| Experimental | | | | | | | | | | |
| HCD | 14.8±1.6 | 732±94* | 9.5±0.8* | 81±2* | 29±6* | 0.4±0.1* | 7±2* | 107±26 | 1.4±0.3 | 12±2 |
| MCD | 14.4±2.9 | 436±83* | 5.6±0.9* | 52±6* | 131±35* | 1.7±0.4* | 34±5* | 122±29 | 1.5±0.3 | 14±1 |

Values are means ± standard deviations. *Significant trial effect (p<0.001). CHO, carbohydrate; E, energy; HCD, high carbohydrate diet; MCD, moderate carbohydrate diet.

subjects' habitual diet and mean dietary intakes during both trials are presented in Table 3.2. There were no differences in habitual intake or macronutrient composition between the groups. By design, there was a significant difference ($p < 0.001$) in the CHO and fat contents of the HCD and MCD trials, but protein content was kept as constant as possible.

3.3.3 Respiratory exchange ratio (RER) during exercise

Mean exercising RER recorded at 30-minute intervals during the 2.5-hour constant-load cycle ride is presented in Figure 3.2. Mean exercising RER was higher in response to the HCD compared to the MCD ($p < 0.05$). RER decreased significantly ($p < 0.05$) during the 2.5-hour constant-load ride in both groups and in response to both dietary treatments (Figure 3.2). However, the high-RER group had a higher exercising RER compared to the low-RER group in both diets, but the HCD resulted in a higher exercising RER in the high-RER group compared to the low-RER group ($p = 0.077$ for group \times diet \times time, Figure 3.2).

The mean change in exercising RER (delta RER) during the 2.5-hour constant-load trial tended to be greater in the low-RER group vs. the high-RER group in response to the HCD ($p = 0.095$, diet \times group), suggesting a lower ability to sustain higher rates of CHO-oxidation towards the end of the 2.5-hour ride following the HCD compared to the MCD in the low-RER group.

3.3.4 Total CHO and fat oxidation during exercise

Accordingly to the exercising RER results, the high-RER group had higher rates of total CHO oxidation ($p < 0.05$, Figure 3.3a) and lower rates of fat oxidation

3-way ANOVA

2-way ANOVA

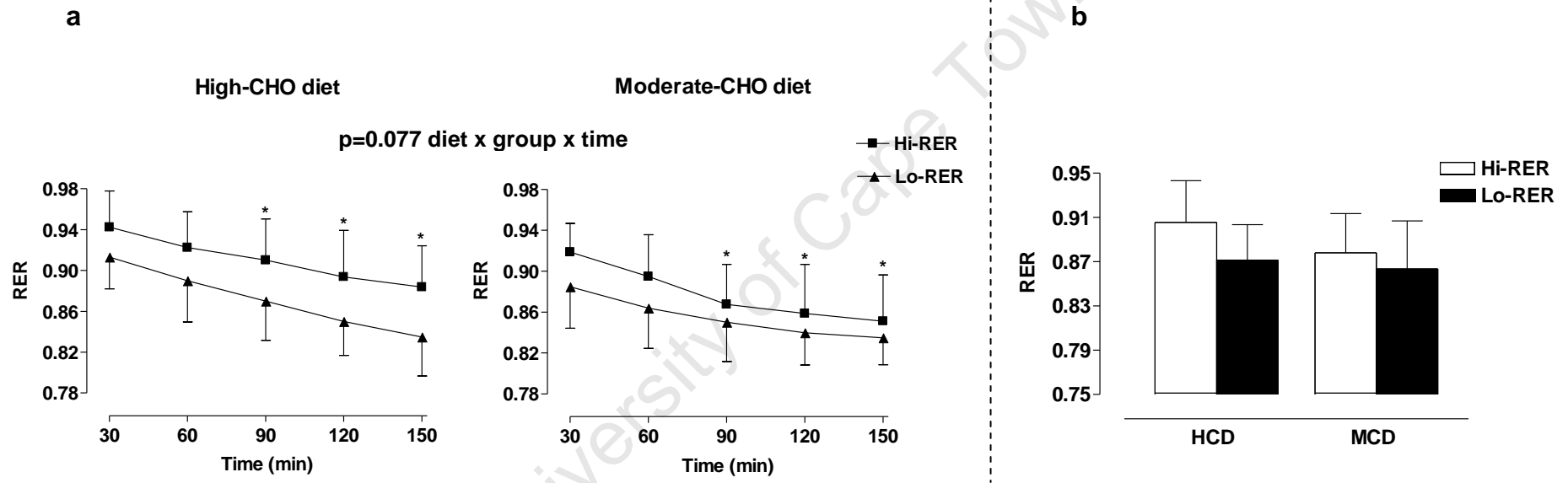


Figure 3.2a. RER during 2.5-hour moderate intensity constant-load exercise in response to the high and moderate CHO diet in the high-RER and low-RER groups. Fig 3.2b represents overall mean RER during the 2.5-hour constant-load exercise ($n=20$).
* $p < 0.05$ time.

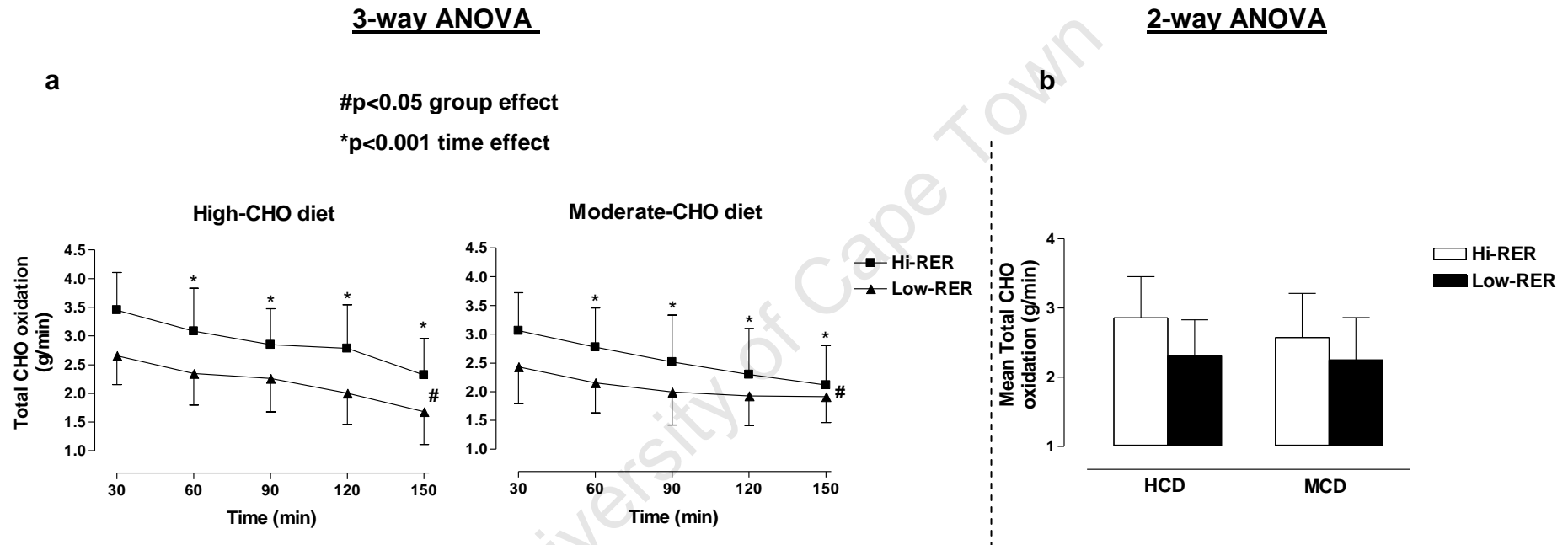


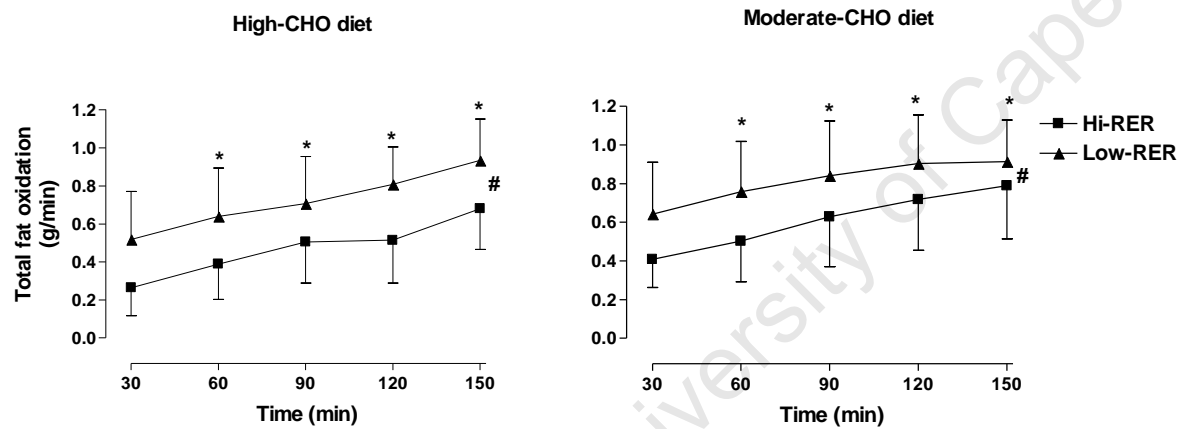
Figure 3.3a. Total CHO oxidation during the 2.5-hour moderate intensity constant-load exercise in response to the high and moderate CHO diet in the high-RER and low-RER groups. Fig 3.3b represents overall mean total CHO oxidation during the 2.5-hour constant-load exercise (n=20).

3-way ANOVA

p<0.05 group effect

*p<0.001 time effect

a



b

2-way ANOVA

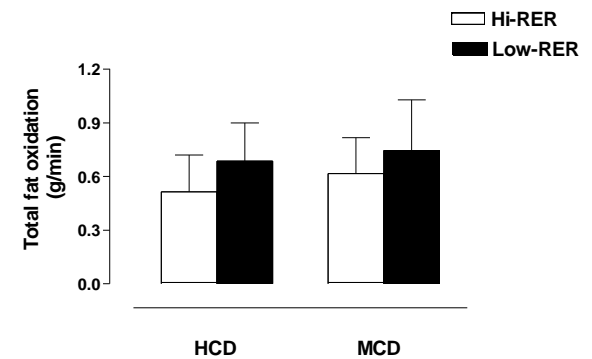


Figure 3.4a. Total fat oxidation during the 2.5-hour moderate intensity constant-load exercise in response to the high and moderate CHO diet in the high-RER and low-RER groups. Fig 3.4b represents overall mean total fat oxidation during the 2.5-hour constant-load exercise (n=20).

($p < 0.05$, Figure 3.4a) during the 2.5-hour constant load cycle compared to the low-RER group. Overall mean CHO and fat oxidation were not different between the two dietary treatments (Figures 3.3b and 3.4b).

Total CHO oxidation decreased significantly whilst total fat oxidation increased significantly during the 2.5-hour constant-load ride in both groups and in response to both dietary treatments ($p < 0.001$, Figures 3.3a and 3.4a).

3.3.5 Circulating hormone and substrate concentrations

There was a significant 3-way interaction effect ($p < 0.05$) for plasma lactate concentrations measured at rest and during exercise in the high-RER and low-RER groups in response to the two diets (Figure 3.5a). Despite similar resting levels, exercising lactate concentrations were higher in the high-RER compared to the low-RER phenotype in response to both diets ($p < 0.001$). Ingestion of a HCD increased overall mean exercising lactate concentrations to a greater degree in the high-RER compared to the low-RER group ($p < 0.05$ diet \times group \times time, Figure 3.5)

Circulating glucose, FFA and insulin concentrations, measured at rest and during the 2.5-hour moderate intensity constant-load ride are summarised in Table 3.3. Mean plasma glucose concentrations decreased similarly over time in both groups in response to the HCD and MCD diets ($p < 0.001$). All subjects, except for 1 in the low-RER group (glucose decreased to 2.9 mmol/l), remained euglycemic (glucose concentration ≥ 3.5 mmol/l) throughout the 2.5-hour constant-load trials following both dietary treatments. Serum FFA

3-way ANOVA

2-way ANOVA

a

b

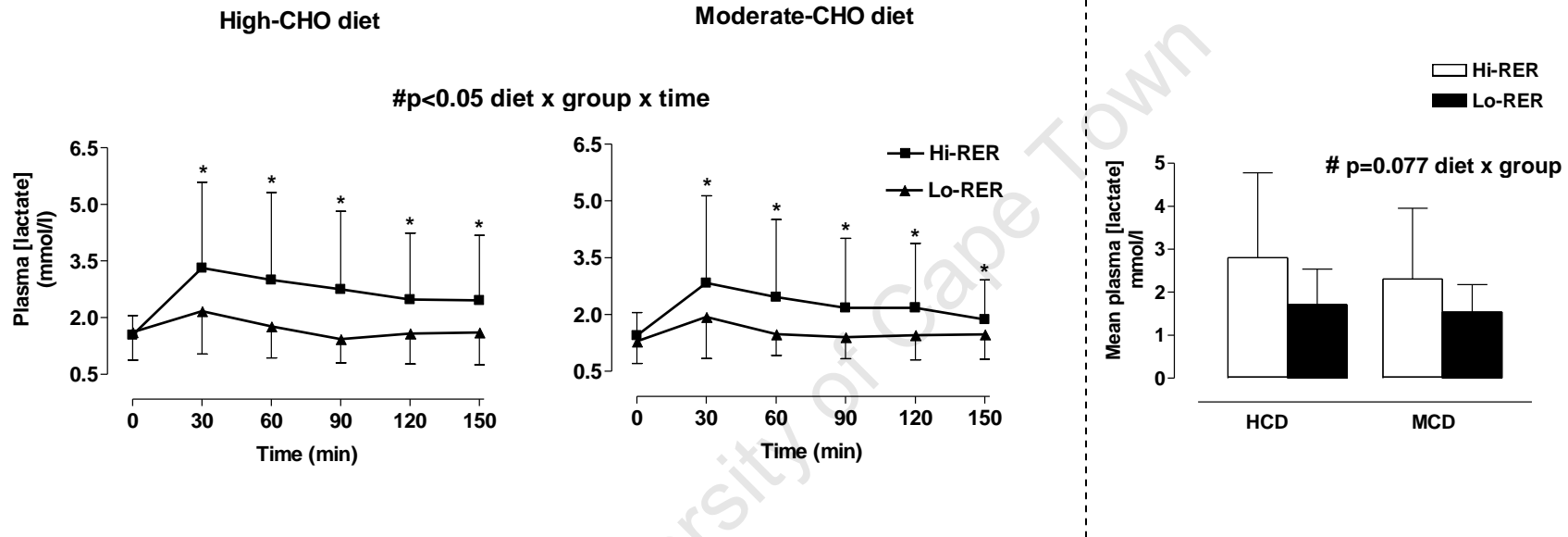


Figure 3.5a. Plasma lactate concentrations at rest and during 2.5-hour moderate intensity constant-load exercise in response to the high and moderate CHO diet in the high-RER and low-RER groups. Fig 3.3b represents the overall mean exercising plasma lactate concentrations (n=20).

Table 3.3. Circulating blood concentrations during 2.5-hour moderate intensity constant-load exercise in response to the high and moderate CHO diet in the high-RER and low-RER groups (n=20).

| | Rest | | 30 min | | 60 min | | 90min | | 120 min | | 150 min | |
|--------------------------------|-----------|-----------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| | Hi-RER | Lo-RER | Hi-RER | Lo-RER | Hi-RER | Lo-RER | Hi-RER | Lo-RER | Hi-RER | Lo-RER | Hi-RER | Lo-RER |
| Plasma glucose (mmol/l) | | | | | | | | | | | | |
| HCD | 4.9±0.31 | 5.0±0.45 | 5.2±0.42 | 5.3±1.09 | 5.1±0.44 | 4.9±0.47 | 4.9±0.49* | 4.6±0.60* | 4.7±0.39* | 5.2±0.54* | 4.6±0.50* | 4.3±0.62* |
| MCD | 5.3±0.85 | 4.9±0.44 | 5.1±0.76 | 5.3±1.09 | 5.1±0.61 | 4.7±0.40 | 4.9±0.50* | 4.6±0.57* | 4.6±0.59* | 4.5±0.54* | 4.4±0.79* | 4.3±0.61* |
| Serum FFA (mmol/l) | | | | | | | | | | | | |
| HCD | 0.34±0.25 | 0.30±0.24 | 0.27±0.20 | 0.22±0.12 | 0.35±0.23* | 0.29±0.15* | 0.51±0.28* | 0.45±0.19* | 0.67±0.35* | 0.66±0.22* | 0.87±0.38* | 0.81±0.23* |
| MCD | 0.24±0.13 | 0.23±0.24 | 0.22±0.16 | 0.25±0.15 | 0.34±0.16* | 0.37±0.20* | 0.50±0.23* | 0.50±0.22* | 0.77±0.25* | 0.76±0.22* | 0.95±0.25 | 0.93±0.23* |
| Plasma Insulin (mU/l) | | | | | | | | | | | | |
| HCD | 6.63±2.65 | 5.17±2.18 | 4.25±0.65* | 3.34±1.08* | 4.48±2.46* | 3.13±1.40* | 3.43±2.13* | 2.31±1.27* | 2.70±1.19* | 1.74±0.96* | 1.66±1.25* | 1.41±0.87* |
| MCD | 5.70±2.16 | 5.27±2.34 | 4.05±1.09* | 4.24±2.89* | 2.98±0.68* | 3.63±1.96* | 2.73±0.72* | 2.50±1.54* | 1.78±0.75* | 1.97±1.13* | 1.35±0.62* | 1.43±1.13* |

Values are means ± standard deviations. HCD, high carbohydrate diet; MCD, moderate carbohydrate diet; FFA, free fatty acids. *p<0.001 time.

concentrations increased significantly throughout exercise ($p < 0.001$) but were not different between diets or groups (Table 3.3). Conversely, mean plasma insulin concentrations decreased significantly over the 2.5-hour constant-load ride ($p < 0.001$) but were also not different between groups in response to the two diet interventions (Table 3.3).

3.3.6 Muscle Glycogen Content

The pre- and post exercise muscle glycogen levels, as well as glycogen utilization during the 2.5-hour moderate intensity constant-load ride for the high-RER and low-RER groups are summarized in Table 3.4. The HCD significantly increased total pre-exercise muscle glycogen content compared to the MCD ($p < 0.001$), but no group differences were demonstrated (Table 3.4).

Table 3.4. Pre- and post exercise muscle glycogen content in response to the high and moderate CHO diet in the high-RER and low-RER groups

| | High-RER (n=8) | Low-RER (n=10) |
|--|-------------------|-------------------|
| Pre-exercise glycogen (mmol/kg dry weight) | | |
| High CHO diet | 671±177* | 657±106* |
| Moderate CHO diet | 513±180* | 565±97* |
| Post-exercise glycogen (mmol/kg dry weight) | | |
| High CHO diet | 221±138* | 262±177* |
| Moderate CHO diet | 142±63* | 171±55* |
| Glycogen utilization (mmol/kg dry weight) | | |
| High CHO diet | 450±149 | 395±146 |
| Moderate CHO diet | 371±207 | 394±110 |

Values are means ± standard deviations. * $p < 0.05$ HCD vs. MCD.

Muscle glycogen utilization was not significantly different between groups or diets. Consequently, post-exercise muscle glycogen levels were higher following the HCD compared to the MCD ($p < 0.05$), with no significant differences between the high-RER and low-RER groups (Table 3.4).

3.3.7 Time Trial Performance

Three subjects failed to complete one or both of the time-trials. Hypoglycemia was possibly the reason why one of the subjects with a low-RER phenotype was unable to complete the time-trial on the HCD trial (blood glucose was 2.9mmol/l towards the end). One subject (high-RER phenotype) failed to complete the time-trial following the HCD and the MCD while the third subject (low-RER phenotype) failed to complete the time-trial following the MCD. These two subjects failed to complete one or both of the experimental time-trials despite the fact that they both successfully completed the familiarization ride. The muscle biopsies that were only performed prior to the experimental trials could possibly have caused discomfort during the experimental trials, resulting in early perceived fatigue.

Overall time-trial performance was 36 seconds faster following the HCD compared to the MCD ($p = 0.096$). When using magnitude based inferences, there was an 89% likelihood that the mean difference in overall performance between the 2 diets had meaningful significance (Table 3.5). The low-RER group tended to performed better than the high-RER group on both diets ($p = 0.083$), however, time trial performance was not significantly different

between the two groups in response to the high CHO or moderate CHO diet (Figure 3.6).

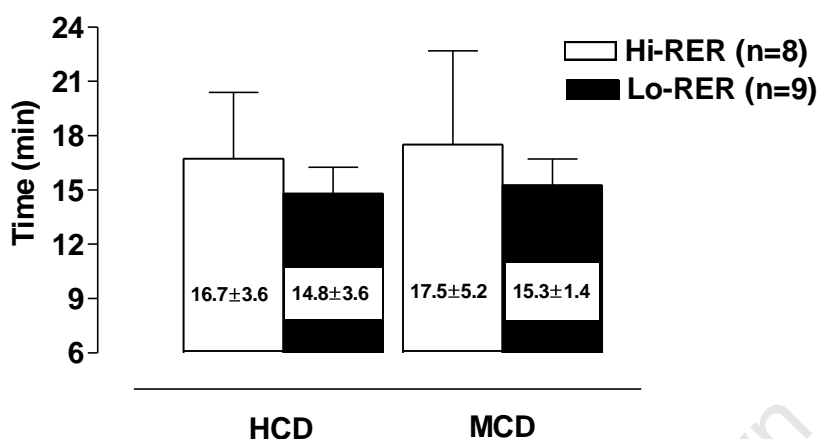


Figure 3.6: Time-trial performance in response to the high and moderate CHO diet in the high-RER and low-RER phenotype groups.

However, when using magnitude based inferences to investigate the performance differences between the high CHO and moderate CHO diet withing each RER group, there was an 80% likelihood that the mean improvement on the HCD in the high-RER group had meaningful significance (Table 3.5). Furthermore, there was an 81% likelihood that the mean improvement on the MCD in the low-RER group had meaningful significance (Table 3.5).

Table 3.5. Performance difference in response to a high vs. a moderate CHO diet using magnitude based inferences (n=17).

| Group | Mean performance difference [min (±90% CI)] | P-value | % difference | Probability |
|----------|---|---------|--------------|-------------|
| Overall | +0.62 (±3.7) | 0.096 | +3.79% | 89% likely |
| High-RER | +0.78 (±7.1) | 0.276 | +4.43% | 80% likely |
| Low-RER | -0.48 (±4.2) | 0.206 | -3.12% | 81% likely |

3.4 DISCUSSION

There is evidence to suggest the existence of distinct resting respiratory exchange ratio (RER) phenotypes in athletes (45; 64; 66). In addition to the external factors such as exercise intensity and duration, which alter fuel kinetics and exercise performance in response to dietary interventions such as CHO-loading, an athletes' RER phenotype may also determine their response to a specific dietary intervention and affect exercise performance. However, the response of different RER phenotypes to a high CHO diet compared to a moderate CHO diet has not been investigated previously. The aim of this study was therefore to examine the effects of a 3- day high CHO diet (7-10 g CHO/kg) vs. a 3-day moderate CHO intake (4-6 g CHO/kg) on substrate utilization and performance in athletes with a high or low fasting resting RER phenotype.

The first main finding of the study was that RER phenotypes persisted as demonstrated by a higher exercising RER and greater CHO oxidation rates in the high-RER vs. low-RER group. These findings therefore suggest that the RER phenotypes are relatively robust.

The higher rates of CHO oxidation during exercise in the high-RER vs. the low-RER group in response to both dietary interventions is consistent with studies previously conducted in our laboratory that investigated the effect of prolonged moderate exercise on fuel utilization in trained athletes with high and low fasting resting RER's. CHO oxidation was higher in subjects with a high-RER compared to those with a low-RER during 3 hours of cycling at 55% W_{peak} in the fasted state (64) and when CHO was ingested prior to and during exercise (45).

The increase in CHO oxidation in the high-RER group in response to the high CHO diet was supported by greater rates of glycogen utilization (~80 mmol/kg dry weight more on the high CHO compared to moderate CHO diet, Table 3.4) and significantly higher mean exercising plasma lactate concentrations (Figure 3.5), suggesting increased glycogenolysis in the high-RER compared to the low-RER group. Furthermore, the high-RER group was able to sustain a higher rate of CHO-oxidation throughout the exercise bout in response to the high CHO diet compared to the low-RER group.

Differences in fuel utilization between high-RER and low-RER phenotypes have partly been explained by certain modifiable factors such as diet and training status, as well as factors intrinsic to the muscle (66). Anthropometric measurements (age, weight, height and body fat %), training status (VO_{2peak} and W_{peak}) and habitual dietary intake and macronutrient composition were remarkably similar between the two RER groups. The low-RER reported a higher, although not significant training volume compared to the high-RER group (an additional 1300 METS/week, equivalent to ~1.5-2 hours of intensive cycling/week). An extra 1.5-2 hours of intensive riding per week could have been enough to potentially lower muscle glycogen in the low-RER group, resulting in lower day-to-day muscle glycogen levels in the low-RER compared to the high-RER group. This could potentially explain the lower RER in the low-RER group compared to the high-RER group despite similar habitual dietary and macronutrient intakes. The extra training could also potentially have changed the oxidative potential of the muscle (i.e. enzyme activity, increased capillarization, hormonal changes etc) increasing the low-RER groups' capacity to oxidize fat. The extra training could also possibly explain why overall time-trial performances tended to

be faster for the low-RER group compared to the high-RER (Figure 3.6). Goedecke et al. (66) demonstrated that at 50% W_{peak} , an exercise intensity similar to the exercise intensity in the present study, training volume were significantly negatively correlated with exercising RER.

Goedecke et al. (66) also demonstrated that factors within the muscle such as muscle fibre type and muscle enzyme activity were correlated with exercising RER. They demonstrated that CS activity, a marker of training status (155) was negatively correlated with exercising RER (66). In contrast, PFK-to-CS and HK-to-CS ratios, both representing glycolytic flux, were positively correlated with RER at 50% W_{peak} (66). Although we did not measure muscle fibre type or muscle enzyme activity in the present study, the higher rates of CHO oxidation during exercise, greater muscle glycogen utilization and a significantly higher mean exercising lactate concentration following the high CHO diet in the high-RER compared to the low-RER group suggests higher glycolytic flux (possibly due to a higher PFK-to-CS and HK-to-CS ratio) in the high-RER vs. the low-RER group (66).

Although several studies have demonstrated a positive relationship between the initial muscle glycogen concentration and the subsequent rate of glycogenolysis (18; 74; 147; 193), the present study demonstrated a dissociation between pre-exercise muscle glycogen levels and the rate of glycogenolysis in athletes with a high or low RER phenotype. It appears that the high-RER phenotype group is better equipped to oxidize the available CHO stores compared to the low-RER group, which may be beneficial during exercise where muscle glycogen is not a

limiting factor. The low-RER group on the other hand, seems to be better equipped to oxidize fat, as they are able to sustain a higher rate of fat oxidation compared to the low-RER group, regardless of the pre-exercise muscle glycogen content. This may be beneficial during ultra-endurance events where muscle glycogen is indeed limited and where an increased reliance on fat can provide an alternative fuel and spare the available glycogen stores.

As we were able to demonstrate the robustness of the RER phenotypes, we were able to, for the first time, examine the differential performance responses of the RER phenotypes to pre-competition high and moderate CHO diets. CHO-loading regimes have typically been used to supercompensate pre-exercise muscle glycogen levels with the aim of prolonging sub-maximal exercise duration to fatigue (5; 12; 23; 59; 108) and improving time-trial performance (108; 146; 194; 196). In the present study the high CHO diet resulted in significant higher pre-exercise muscle glycogen concentrations ($p < 0.001$) and tended to improve overall time-trial performance compared to the moderate CHO diet ($p = 0.096$). When comparing the performances of the RER phenotypes in response to the 2 diets, we found that both groups performed faster following the high CHO compared to the moderate CHO diet (48 and 30 seconds faster, for the high-RER and low-RER group, respectively). Despite the differences in substrate utilization during exercise in the two RER groups in response to the high and moderate CHO diet, this study failed to demonstrate any performance differences between the two groups. Perhaps the failure to show a difference in performance was due to the short duration time-trial (~14.5-17.5 minutes) that was used as a marker of performance and was

maybe not representative of a 'real-life' race situation. In addition, the duration of exercise was not long enough to deplete muscle glycogen stores which have been associated with an increase in effort perception and the onset of fatigue. Indeed, post-exercise muscle glycogen levels were greater than 220 and 140 mmol/kg dry wt following the high and moderate CHO diet, respectively for both groups. Thirdly, the sample size was possibly too small to detect significant differences using a 3-way ANOVA.

In conclusion, despite the increase in glycogen availability as a result of CHO-loading, the RER phenotypes persisted during prolonged moderate exercise, as demonstrated by greater rates of CHO oxidation in the high-RER compared to the low-RER groups. However, there was no difference in performance between the two RER groups in response to the two diets. Further studies are required to compare the response of the RER phenotypes to different dietary interventions during more prolonged exercise where glycogen is limiting and higher rates of fat oxidation may be beneficial. This is with a view to formulating individualized dietary guidelines for athletes with different RER phenotypes.

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CHAPTER 4

The effect of fat-adaptation followed by carbohydrate-loading on 100-km time-trial performance

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4.1 INTRODUCTION

It is generally recommended that athletes ingest a high carbohydrate (CHO) diet (7-10 g CHO/kg body mass/day) in the 1-3 days prior to prolonged endurance (>90 minutes) exercise. However, in Chapter 2 we have demonstrated that only 57% of sub-elite cyclists chose to CHO-load in the 1-3 days prior the 210-km ultra-endurance cycle race, and that only 23% of these cyclists actually achieved the recommended CHO intake. Moreover, in Chapter 3 we demonstrated that although the ingestion of high CHO diet significantly increased pre-exercise muscle glycogen levels, it only tended ($p=0.096$) to improve short time-trial performance following 2.5-hours of moderate intensity constant-load exercise compared to a moderate CHO (4-6 g/kg body mass) diet.

Therefore, in this chapter we will explore an alternative strategy aimed at increasing fat oxidation and reducing CHO oxidation. This dietary strategy encompasses 5-6 days of fat-loading (68% fat energy), followed by 1 day of CHO-loading (~10 g/kg BM) prior to the event, and has previously been shown to increase fat oxidation at rest and during exercise (26; 28; 33), even when carbohydrate was ingested prior to and during exercise (28; 33). This strategy has also been shown to increase muscle glycogen stores, but reduce muscle glycogen utilization during exercise (26). Despite this muscle glycogen “sparing” effect, overall improvements in performance have not been demonstrated (26; 28; 33), possibly due to an increase in sympathetic nervous system activation

(158) and an increase in effort perception that have been associated with high-fat intake (81; 141; 178).

However, the effects of this particular dietary strategy on performance have only been tested under time-trial conditions (~25-60 minutes) following prolonged sub-maximal constant-load exercise (2-4 hours at 65%-70% of VO_{2peak}) (26; 28; 33). The effects of 5-6 days of fat-loading, followed by 1 day of CHO-loading (90% CHO) has not been investigated during exercise that simulates race conditions, which includes high intensity (>85% VO_{2peak}) sprints. Since muscle glycogen is the predominant fuel source during high-intensity exercise (151), we hypothesize that a dietary strategy that optimizes muscle glycogen stores, but also promotes glycogen sparing would most likely benefit endurance exercise that simulates a 'real-life' race situation that include high intensity exercise bouts. A 'real-life' race situation will also allow cyclists to select their own race-pace and adopt a pacing strategy, which is also an important factor for performance (56).

The aim of the present study was therefore to investigate the effect a high-fat diet followed by 1 day of CHO-loading compared to a high CHO diet on substrate utilization, heart rate variability as a proxy measure of sympathetic activation, effort perception and performance during a 100-km cycling time-trial including high-intensity sprints, simulating race situations.

4.2 METHODOLOGY

4.2.1 Subjects and study design

Eight endurance-trained male cyclists participated in this study, which was approved by the Research and Ethics Committee of the Faculty of Medicine of the University of Cape Town. All subjects were free from known metabolic conditions and were currently not taking any medications for chronic conditions such as high blood pressure or stimulants for conditions such as asthma. The subjects were informed of the nature of the study and written informed consent was obtained prior to the start of the study.

Each subject completed two trials in a randomized, single-blind crossover design with a 2 week washout period separating each trial. Each trial consisted of an 8-day diet, training and testing period, during which subjects reported to the laboratory on days 1, 3, 5, 7 and 8 to undertake supervised training and testing (Figure 4.1).

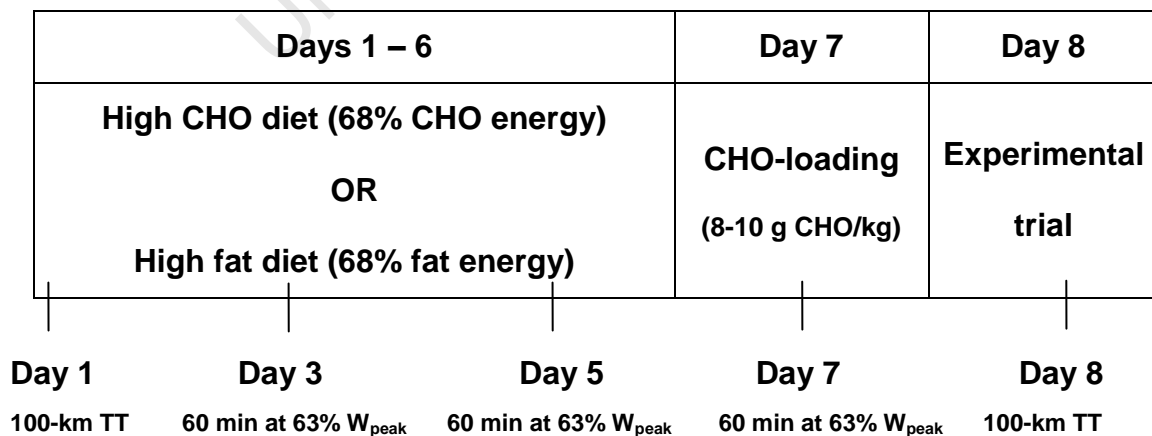


Figure 4.1. Diet, training and testing protocol.

4.2.2 Preliminary testing

Anthropometry

Prior to the start of the trial, body weight and height were measured to the nearest decimal place. The percent body fat was determined from measurements of skinfold thickness, using the equations of Durnin and Womersley (50).

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

In addition, peak oxygen consumption (VO_{2peak}) and peak power output (W_{peak}) were measured during a progressive exercise test as described previously in Chapter 3. W_{peak} values were used to set the work rates in the experimental trials to correspond to 63% of each subject's W_{peak} (approximately 70% VO_{2peak}).

Dietary Analysis

The subjects were further instructed to complete a 3-day dietary record consisting of 2 week days and 1 weekend day. These dietary records were analyzed with the Food Finder 3 programme (Medtech (Pty) Ltd, Medical Research Council, Tygerberg, South Africa) to determine the subjects' self-reported energy intake and macronutrient consumption. This dietary information was used as a guideline to devise the two experimental diets. To aid adherence to the diets, subjects were also required to indicate their food preferences.

4.2.3 Dietary manipulations

During the trial, subjects were required to ingest either a high fat diet (~68% energy (E) from fat) for 6 days followed by 1 day CHO-loading (~90% E from CHO), or an equal energy high CHO diet (68% E from CHO) for 6 days followed by 1 day of CHO-loading (~90% E from CHO). A registered dietician formulated individualized menus. In order to control dietary intake, all the meals were pre-packed and provided for the subjects together with a diary to record any deviations from the diet. Efforts were made to blind the diets by covertly manipulating the macronutrient compositions of the diets.

4.2.4 Exercise training sessions

On days 1, 3 and 5, subjects reported to the laboratory after a 10-12 hour fast and completed an exercise training session. On the first day of training (day 1), subjects completed a 100-km familiarization time-trial on their own bicycles mounted on a Kingcycle Trainer (EDS Portaprompt, Ltd., UK). The calibration and reliability of the Kingcycle has been described in detail previously (128). The 100-km time-trial is a highly reliable laboratory test [between-test CV = 0.93 (95%CI 0.79 to 0.89); within-subject CV = 1.7% (95%CI 1.1 to 2.5%) (160)] that included five 1-km sprint distances after 10, 32, 52, 72 and 99 km, as well as four 4-km sprint distances after 20, 40, 60 and 80 km, during which subjects were requested to cycle “as fast as possible”. Mean sprint performance also showed similar good reliability (within-subject variation and correlations for the 1-km and 4-km sprint times were 1.9%, 2.0%, 0.93 and 0.81, respectively (160). The familiarization time-trial also served as a screening trial to ascertain whether the subjects were adequately trained to complete the trial.

On days 3 and 5, subjects completed a 60 minute constant-load cycle at 63% W_{peak} (70% $VO_{2\text{peak}}$) on a lode bike, maintaining their cadence at 90 rpm. The constant-load training sessions on days 3 and 5 were undertaken to ensure consistency in the subjects' training during the trial, as well as monitor their physiological and metabolic responses to the dietary interventions. During the constant-load cycle, heart rate was recorded continuously by means of a PolarTM Heart Rate Monitor (Polar Electro, Kempele, Finland) and VO_2 and VCO_2 values were measured for 4-5 minutes every 15 minutes (15, 30, 45 and 60 minutes), using the on-line computerized system (Oxycon Alpha Analyzer, Jaeger-Mijnhardt, Bunnik, The Netherlands). Rate of perceived exertion (RPE) scores were also recorded at 15 minute intervals, using the validated Borg 6-20 RPE Scale (17). Printed scale instructions together with a verbal explanation of how the scale works, were given to the subjects prior to the trial in order to familiarize them with the operation of the scales.

Prior to exercise on all three days, VO_2 and VCO_2 values were measured while the subject was seated in a resting position for 15-20 minutes to determine the resting respiratory exchange ratio (RER), using the on-line computerized system, as previously described. Heart rate variability (HRV) was also recorded prior to exercise using a heart rate monitor (Body IQ, Cape Town, South Africa). HRV has been implicated as an indirect measure of autonomic nervous system activation (68). During the HRV test, heart rate measurements were recorded while subjects breathed rhythmically (12 breaths/min) for 5 minutes of supine lying, followed by 5 minutes of standing. Power spectrum analysis for low frequency (LF, indicative of sympathetic activation) and high frequency (HF,

indicative of parasympathetic activation) was performed based on the HRV interval, using MATLABTM software (The MathWorks Inc.). The natural logarithm of LF and HF power, as well as the ratio of LF power to HF power was calculated from the power spectrum values.

4.2.5 Experimental trials

On day 7, subjects reported to the laboratory after a 10-12 hour overnight fast. HRV, VO_2 and VCO_2 values were measured at rest as described earlier. Subjects then completed a 60 minute constant-load cycle at 63% W_{peak} , during which heart rate was recorded continuously and VO_2 and VCO_2 values were measured for 4-5 minutes every 15 minutes (15, 30, 45 and 60 minutes). Blood samples were drawn at rest and at 15 minute intervals (15, 30, 45 and 60 minutes) during the constant-load exercise for the subsequent analysis of plasma glucose, lactate, free fatty acids (FFA) and catecholamine concentrations. In addition, RPE were recorded at 15 minute intervals.

On day 8, CHO-loaded subjects reported to the laboratory after a 10-12 hour overnight fast. HRV, VO_2 and VCO_2 values were measured at rest as described earlier. Following a 5 minute warm-up, subjects completed a 100-km performance time-trial. During the 100-km time-trial, a blood sample was drawn at rest and again immediately prior to the 1-km sprints at 32, 52, 72 and 99 km for the subsequent analysis of plasma glucose, lactate, FFA and catecholamine concentrations. Power output and heart rate were measured continuously throughout the trial. RPE was recorded immediately prior to and after every sprint. The only feedback the subjects received during the 100-km time-trial was

their elapsed distance. During both trials, subjects ingested a 10% glucose polymer solution at regular intervals (200 ml every 20 minutes) to maintain euglycemia.

4.2.6 Blood sampling and analysis

Venous blood samples (~8 ml) were drawn during the constant-load cycle on day 7 and during the 100-km time-trial on day 8, as described in Chapter 3. One aliquot (1 ml) was placed into a vacutainer containing potassium oxalate and sodium fluoride for subsequent analysis of glucose and lactate concentrations. Two aliquots (2 x 2 ml) were placed into vacutainers containing lithium heparin for analysis of plasma epinephrine and norepinephrine concentrations. The remaining aliquot (3 ml) was placed into a vacutainer containing gel and clot activator for determination of serum FFA concentrations. All samples were kept on ice and then centrifuged at 3000 rpm at 4°C for 10 minutes at the end of the trial. The supernatants were stored at -80°C (epinephrine and norepinephrine) and -20°C (glucose, lactate, insulin and FFA) for later analysis.

Plasma glucose, FFA and lactate concentrations were determined as described in Chapter 3. Plasma catecholamine concentrations were analyzed by High Performance Liquid Chromatography (HPLC), according to the method described by Forster and MacDonald (55) (intra CV = 8.74% and 9% for norepinephrine and epinephrine, respectively).

4.2.7 Statistical Analysis

Values are presented as the mean \pm standard deviations (SD). A two-way analysis of variance (ANOVA) with repeated measures and the Tukey post hoc analysis were performed using STATISTICA analysis software (Version 6, Statsoft, Tulsa, OK, USA). Statistical significance was accepted at $P < 0.05$.

4.3 RESULTS

4.3.1 Subject characteristics

The characteristics of the subjects are summarized in Table 4.1. The majority of subjects were well trained with a mean VO_{2peak} of greater than 55 ml/kg/min and a power to weight ratio of 4.5 watts/kg body weight (Table 4.1).

Table 4.1. Subject Characteristics (n=8).

| Characteristic | Mean + SD | Range |
|---------------------------|-----------------|--------------|
| Age (yr) | 26 \pm 3 | 22 – 32 |
| Weight (kg) | 81.3 \pm 9.6 | 74.1 – 100.2 |
| Height (m) | 1.80 \pm 0.10 | 1.65 – 1.91 |
| Body fat (%) | 14.0 \pm 2.8 | 8.90 – 18.1 |
| VO_{2peak} (ml/kg/min) | 57.8 \pm 5.5 | 51.1 – 67.2 |
| W_{peak} (W) | 361 \pm 36 | 290 – 419 |
| W_{peak} :Weight (W/kg) | 4.5 \pm 0.6 | 3.6 – 5.2 |

Values are means \pm standard deviations (SD). VO_{2peak} , peak oxygen uptake;

W_{peak} , peak power output; W, watts.

4.3.2 Training and dietary control

All subjects followed the experimental diets, ingested the food that was provided during both trials and achieved the recommended target for fat and CHO intake. The mean dietary intakes during both trials are presented in Table 4.2. According to design, there was a significant difference ($p < 0.001$) between the CHO and fat contents of the HCD and HFD consumed. The protein content in the two experimental diets was kept the same. Although diets were blinded and covertly manipulated, subjects were able to distinguish that the diets were different, but were unaware of their composition.

All the subjects attended all the training sessions, but 2 subjects were only able to complete 45 minutes of the 60 minute constant-load training session on day 5, after 4 days of high-fat intake. Although the remaining subjects successfully completed all the training sessions, four subjects experienced difficulties during the constant-load cycle on day 3 and/or day 5 on the HFD treatment, complaining of “tired” and “burning” legs or having difficulties in maintaining the training cadence at the defined workload.

4.3.3 Resting variables

The mean resting fasting RER was significantly lower during the HFD compared to the HCD trial ($p < 0.001$). RER decreased over 6 days of the high-fat intake (0.84 ± 0.04 day 1 to 0.78 ± 0.04 day 7) and remained low (0.77 ± 0.02) on day 8, despite 1 day of CHO-loading on day 7 (Figure 4.2). In contrast, RER in the HCD trial did not change significantly over the 8-day trial period.

Table 4.2. Dietary intake during the experimental treatments (n=8).

| | Energy (MJ) | CHO | | | FAT | | | PROTEIN | | |
|------------|----------------|---------|----------|---------|---------|-----------|-----------|---------|---------|----------|
| | | g | g/kg | %E | g | g/kg | %E | g | g/kg | %E |
| HFD | 14.9±1.0 | 151±10* | 1.9±0.1* | 17±0.4* | 268±18* | 3.3±0.2* | 68±0.6* | 133±9 | 1.6±0.1 | 15±0.9 |
| HCD | 14.8±1.2 | 603±41* | 7.5±0.4* | 68±1.2* | 67±4.6* | 0.8±0.04* | 17.5±0.7* | 128±11 | 1.6±0.2 | 14.5±0.7 |

Values are means ± standard deviations. CHO, carbohydrate; E, energy; HFD, high-fat diet; HCD, high carbohydrate diet. *Significant trial effect (p<0.001).

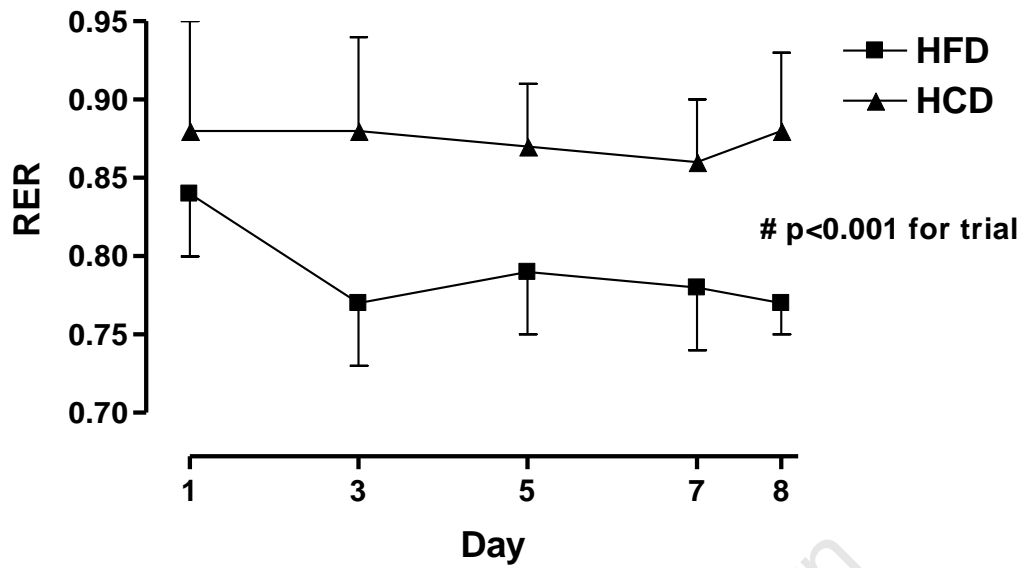


Figure 4.2. Resting RER in response to the HFD-CHO and HCD-CHO dietary interventions (n=8).

The mean normalized heart rate variability values for low frequency (LF), reflecting sympathetic modulation for supine and standing, are presented in Table 4.3. No significant differences between trials were demonstrated for high frequency (HF) or LF:HF ratio (data not shown), but there was a tendency towards a significant trial effect ($p=0.056$) for the LF supine values (Table 4.3). No significant differences in mean resting RPE or heart rate were found between the two diet treatments (data not shown).

4.3.4 RER, HR and RPE during constant-load training rides

Mean exercising variables, including heart rate, RER, and RPE, measured at 15 minute intervals during the 60 minute constant-load cycle on days 3, 5 and 7 are presented in Table 4.4.

Table 4.3. Mean resting heart rate variability (expressed as the natural log [ln]) in response to the HFD and HCD (n=7)

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

Values are means ± standard deviations. LF, low frequency; HFD, high-fat diet; HCD, high carbohydrate diet; NS, not significant

Mean exercising RER on days 3, 5 and 7 was significantly lower on the HFD compared to the HCD ($p < 0.05$) (Table 4.4). Conversely, mean exercising heart rate was significantly higher in response to the HFD treatment ($p < 0.05$) during the three constant-load rides (Table 4.4). There was a tendency ($p = 0.063$) for mean effort perception during the three training rides to be higher when ingesting the HFD compared to the HCD (Table 4.4).

Table 4.4. Mean exercising values during the 60 minute constant-load cycle on days 3, 5 and 7 in response to the HFD and HCD treatments (n=8).

| | Day 3 | Day 5 | Day 7 | P-level |
|-------------------------|-----------|-----------|-----------|-----------------|
| RER | | | | |
| HFD | 0.85±0.04 | 0.86±0.03 | 0.87±0.03 | |
| HCD | 0.93±0.04 | 0.92±0.01 | 0.93±0.02 | <0.05 for trial |
| Heart Rate (bpm) | | | | |
| HFD | 153±6 | 152±6 | 151±9 | |
| HCD | 149±7 | 146±7 | 147±8 | <0.05 for trial |
| RPE | | | | |
| HFD | 14±1 | 14±1 | 13±0.6 | |
| HCD | 13±1 | 13±1 | 13±0.7 | 0.063 for trial |

Values are means ± standard deviations. RER, respiratory exchange ratio; RPE, rating of perceived exertion; HFD, high-fat diet; HCD, high carbohydrate diet (n=8).

4.3.5 Experimental constant-load cycle on day 7

Table 4.5a and 4.5b summarizes the metabolic responses during the constant-load cycle on day 7, after 6 days of high fat intake.

RER, heart rate and RPE during the constant-load ride on day 7

Mean RER was significantly lower ($p < 0.01$) on the HFD compared to HCD (Table 4.5a). Heart rate increased during the exercise bout ($p < 0.05$) and was significantly higher for HFD ($p < 0.05$) compared to HCD (Table 4.5a). Despite the increase in heart rate following HFD, mean RPE was not different between trials (Table 4.5a).

Circulation hormone and substrate concentrations (day 7)

Euglycemia was maintained during the constant-load cycle during both trials and plasma glucose concentrations were not different between the HFD and HCD (Table 4.5b). An interaction effect was demonstrated for plasma lactate concentrations ($p < 0.05$) in response to the dietary interventions with the mean plasma lactate response being significantly lower ($p < 0.01$) following HFD compared to HCD (Table 4.5b). In contrast, plasma FFA concentrations were significantly higher ($p < 0.001$) at rest and during the constant-load cycle following the HFD compared to HCD. Plasma catecholamine concentrations were not different between trials (Table 4.5b).

4.3.6 Metabolic and performance data during the 100-km time-trial*Circulation hormone and substrate concentrations (day 8)*

Circulating hormone and substrate concentrations, obtained immediately prior to the 1-km sprints at 10, 32, 52, 72 and 99 km during the 100-km time-trial, are summarised in Table 4.6.

Table 4.5a. Mean exercising values during the 60 minute constant-load cycle on day 7 in response to the HFD and HCD treatments (n=8).

| | 15 min | 30 min | 45 min | 60 min | p-level |
|-------------------------|-----------|-----------|-----------|-----------|------------------|
| RER | | | | | |
| HFD | 0.89±0.03 | 0.86±0.03 | 0.84±0.02 | 0.85±0.03 | <0.005 for trial |
| HCD | 0.96±0.03 | 0.91±0.02 | 0.91±0.02 | 0.91±0.02 | <0.001 for time |
| Heart Rate (bpm) | | | | | |
| HFD | 149±9 | 152±7 | 156±7 | 158±7 | <0.05 for trial |
| HCD | 143±11 | 148±8 | 151±6 | 154±8 | <0.001 for time |
| RPE | | | | | |
| HFD | 12±2 | 13±1 | 14±1 | 14±2 | |
| HCD | 11±2 | 12±1 | 13±1 | 13±1 | <0.001 for time |

Values are means ± standard deviations. RER, respiratory exchange ratio; RPE, rating of perceived exertion; HFD, high-fat diet; HCD, high carbohydrate diet.

Table 4.5b. Mean circulating blood concentrations during the 60 minute constant-load cycle on day 7 in response to the two dietary treatments.

| | 15 min | 30 min | 45 min | 60 min | P-level |
|--|-----------|-----------|-----------|------------|--------------------|
| Plasma glucose (mmol/l) | | | | | |
| HFD | 3.9±0.61 | 4.3±0.86 | 4.3±0.70 | 4.0±0.52 | NS |
| HCD | 4.5±0.78 | 4.5±0.78 | 4.4±0.78 | 4.5±0.91 | |
| Plasma lactate (mmol/l) | | | | | |
| HFD | 2.5±0.94 | 2.55±1.05 | 2.56±0.98 | 2.57±0.90 | <0.05 time x trial |
| HCD | 4.01±1.28 | 4.26±1.31 | 3.88±1.28 | 3.85±1.31 | |
| Serum free fatty acids (mmol/l) | | | | | |
| HFD | 0.27±0.09 | 0.37±0.11 | 0.41±0.12 | 0.47±0.12 | <0.005 for trial |
| HCD | 0.20±0.07 | 0.24±0.10 | 0.29±0.12 | 0.35±0.15 | <0.001 for time |
| Plasma epinephrine (nmol/l) | | | | | |
| HFD | - | 0.99±0.22 | 1.15±0.28 | 1.27±0.27 | |
| HCD | | 1.02±0.08 | 1.14±0.22 | 1.15±0.08 | <0.001 for time |
| Plasma norepinephrine (nmol/l) | | | | | |
| HFD | - | 8.42±2.43 | 8.81±2.34 | 10.36±2.48 | |
| HCD | | 8.10±1.20 | 8.55±2.25 | 9.48±1.96 | <0.001 for time |

Values are means ± standard deviations. HFD, high-fat diet; HCD, high carbohydrate diet. NS, not significant.

Table 4.6. Circulating blood concentrations during the 100-km time-trial in response to the HFD-CHO and HCD-CHO treatments

| | 10 km | 32 km | 52 km | 72 km | 99 km | p-level |
|--|-----------|------------|------------|------------|------------|-------------|
| Plasma glucose (mmol/l) | | | | | | |
| HFD-CHO | 4.3±0.47 | 4.4±0.70 | 4.5±0.66 | 4.2±0.35 | 4.3±0.43 | NS |
| HCD-CHO | 4.4±1.23 | 4.7±0.75 | 4.7±0.83 | 4.4±0.76 | 4.4±0.70 | |
| Serum free fatty acids (mmol/l) | | | | | | |
| HFD-CHO | 0.31±0.09 | 0.28±0.14 | 0.35±0.23 | 0.46±0.29 | 0.78±0.38 | |
| HCD-CHO | 0.28±0.12 | 0.25±0.12 | 0.30±0.18 | 0.38±0.16 | 0.77±0.35 | <0.001 time |
| Plasma lactate (mmol/l) | | | | | | |
| HFD-CHO | 1.18±0.26 | 4.69±2.26 | 4.51±2.04 | 4.04±1.83 | 2.95±0.98 | 0.069 trial |
| HCD-CHO | 1.58±0.37 | 5.35±3.45 | 5.15±3.33 | 5.00±2.87 | 4.23±2.19 | <0.001 time |
| Plasma epinephrine (nmol/l) | | | | | | |
| HFD-CHO | 0.25±0.04 | 0.91±0.30 | 1.10±0.31 | 1.46±0.65 | 2.86±1.58 | |
| HCD-CHO | 0.21±0.05 | 0.95±0.38 | 1.20±0.58 | 1.58±0.28 | 3.61±1.49 | <0.001 time |
| Plasma norepinephrine (nmol/l) | | | | | | |
| HFD-CHO | 1.68±0.49 | 11.40±6.13 | 12.25±6.93 | 14.01±7.33 | 15.42±8.48 | |
| HCD-CHO | 1.90±0.53 | 12.72±7.99 | 12.88±6.17 | 14.56±4.98 | 19.69±7.47 | <0.001 time |

Values are means ± standard deviations. HFD-CHO, 6d high-fat diet + 1d carbohydrate loading; HCD-CHO, 6d high carbohydrate diet + 1d carbohydrate loading. NS, not significant.

Plasma glucose concentrations were not significantly different between the HFD-CHO and HCD-CHO and subjects remained euglycemic throughout the 100-km time-trial following both diet treatments. Plasma FFA concentrations increased significantly during both trials ($p < 0.001$), but were not different in response to the two diet interventions. Similarly plasma lactate concentrations increased during both trials ($p < 0.001$) and there was a tendency ($p = 0.069$) for the levels to be higher after the HCD-CHO compared to the HFD-CHO. Plasma catecholamine concentrations also increased significantly during both trials ($p < 0.001$) but were not different between the two dietary treatments.

100-km Time-trial performance

Overall 100-km time-trial performance was not significantly different between trials ($p = 0.23$), however mean performance time was 3 minutes 44 seconds slower on the HFD-CHO compared to the HCD-CHO (Figure 4.3).

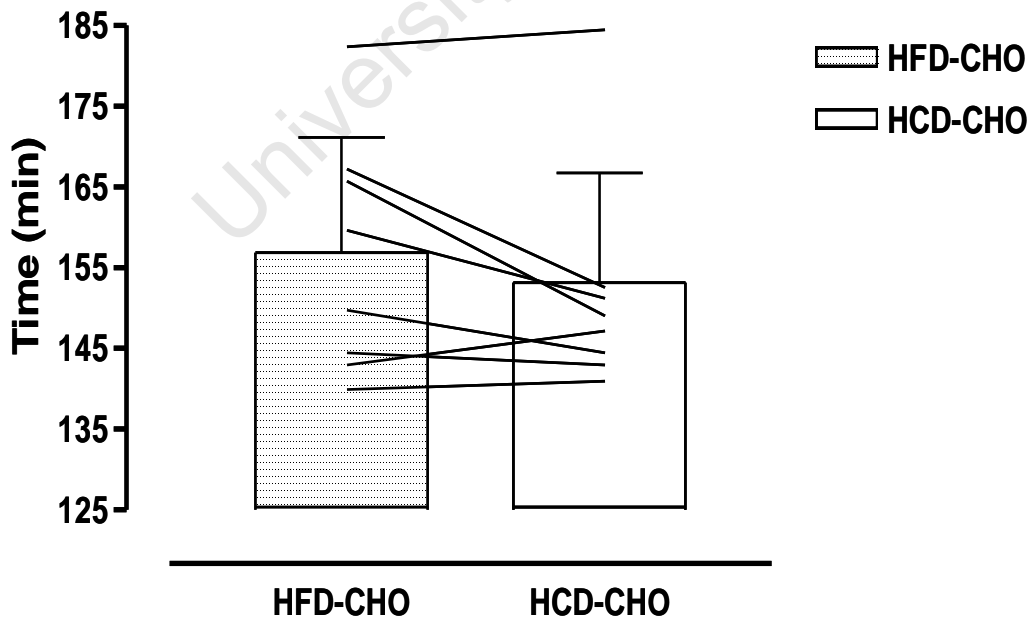


Figure 4.3: 100-km time-trial performance in response to the HFD-CHO and HCD-CHO dietary interventions. The lines on the graph represent individual performances ($n=8$).

When using magnitude based inferences, as described in Chapter 3, we found that there was an 76% likelihood that the mean difference in 100-km performance [3.74 min (\pm 3.7)] between the 2 diets had meaningful significance. Performance of 5 of the 8 subjects declined on the HFD-CHO, with no order effect observed ($p=0.28$). Variables recorded during the 4-km sprints are summarized in Table 4.7. No differences were demonstrated during the 4-km sprints between the HFD-CHO and HCD-CHO. Mean power output and sprint time recorded during 4-km sprints decreased significantly over time during both trials ($p<0.01$, Table 4.7). Conversely RPE recorded immediately after 4-km sprints increased similarly over time in both trials ($p<0.001$, Table 4.7).

Table 4.7. Variables measured during the 4-km sprints in response to the HFD-CHO and HCD-CHO treatments ($n=8$).

| 4-km sprints | 20-km | 40-km | 60-km | 80-km | p-level |
|--------------------------|--------------|--------------|--------------|--------------|-------------|
| Sprint time (sec) | | | | | |
| HFD-CHO | 336 \pm 26 | 338 \pm 26 | 340 \pm 24 | 347 \pm 29 | |
| HCD-CHO | 327 \pm 27 | 330 \pm 31 | 328 \pm 28 | 335 \pm 31 | <0.05 time |
| Heart Rate (bpm) | | | | | |
| HFD-CHO | 166 \pm 6 | 166 \pm 8 | 167 \pm 6 | 168 \pm 7 | |
| HCD-CHO | 166 \pm 7 | 166 \pm 9 | 164 \pm 10 | 166 \pm 9 | NS |
| RPE | | | | | |
| HFD-CHO | 17 \pm 2 | 18 \pm 1 | 18 \pm 2 | 19 \pm 1 | |
| HCD-CHO | 16 \pm 3 | 17 \pm 2 | 18 \pm 2 | 18 \pm 2 | <0.001 time |

Values are means \pm standard deviations. RPE, rating of perceived exertion; HFD-CHO, 6d high-fat diet + 1d carbohydrate loading; HCD-CHO, 6d high carbohydrate diet + 1d carbohydrate loading; NS, not significant.

In contrast to the overall and 4-km performance, mean power output recorded during the high intensity 1-km sprints was significant lower after the HFD-CHO

vs. the HCD-CHO diet ($p < 0.05$ time \times trial, Figure 4.4). Consequently, 1-km sprint times tended to be slower ($p = 0.07$, Table 4.8) following the HFD-CHO compared to the HCD-CHO.

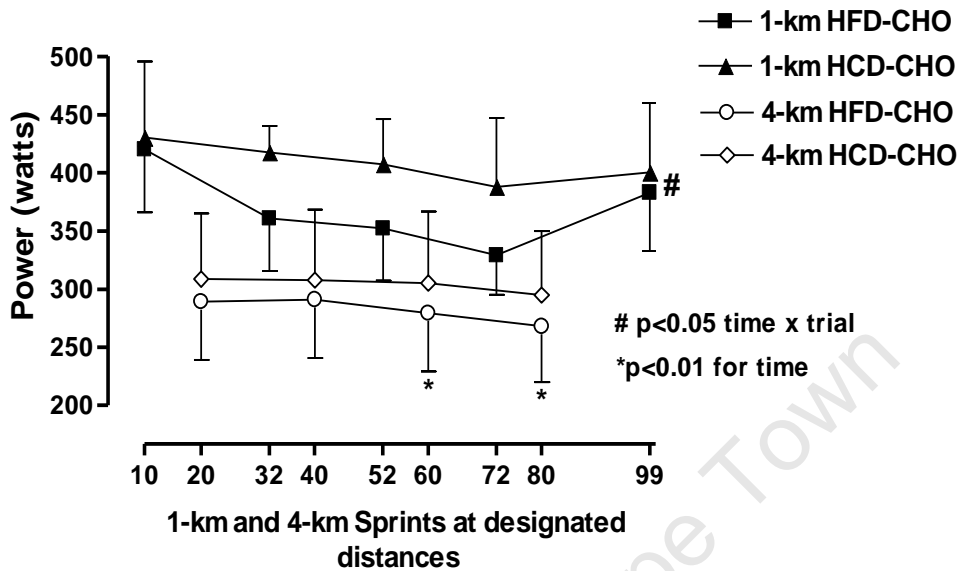


Figure 4.4. Power output during the 1-km and 4-km sprint distances during the 100-km TT in response to the HFD-CHO and HCD-CHO dietary interventions ($n=8$).

Mean heart rate was similar for both treatments (Table 4.8). RPE recorded immediately after the 1-km sprints rose progressively over time ($p < 0.01$) but was not different between treatments (Table 4.8).

4.4 DISCUSSION

In this chapter, we examined the effects of 6 days of a high fat intake, followed by 1 day of CHO-loading compared to a high CHO diet, on substrate utilization, heart rate variability, effort perception and performance during a 100-km endurance cycle time-trial. The study is unique in that it is the first study to investigate the effect of high fat feeding, followed by CHO-loading on endurance

Table 4.8. Variables measured during the 1-km sprints in response to the HFD-CHO and HCD-CHO treatments (n=8).

| 1-km sprints | 10-km | 32-km | 52-km | 72-km | 99-km | p-level |
|--------------------------|-------|-------|-------|-------|-------|--------------|
| Sprint time (sec) | | | | | | |
| HFD-CHO | 73±3 | 77±5 | 78±4 | 80±5 | 77±5 | |
| HCD-CHO | 72±5 | 72±3 | 74±3 | 76±5 | 74±5 | p=0.07 trial |
| Heart Rate (bpm) | | | | | | |
| HFD-CHO | 168±5 | 168±4 | 170±4 | 167±4 | 173±5 | |
| HCD-CHO | 166±8 | 170±2 | 170±5 | 168±7 | 172±7 | NS |
| RPE | | | | | | |
| HFD-CHO | 16±2 | 17±1 | 18±1 | 19±1 | 20±1 | |
| HCD-CHO | 15±2 | 18±1 | 18±1 | 18±1 | 20±1 | <0.01 time |

Values are means ± standard deviations. RPE, rating of perceived exertion; HFD-CHO, 6d high-fat diet + 1d carbohydrate loading; HCD-CHO, 6d high carbohydrate diet + 1d carbohydrate loading; NS, not significant

exercise including *high intensity sprints* that simulate actual race situations. It was hypothesized that the potential glycogen sparing effect of this dietary strategy (26) would be most beneficial for exercise that included high intensity sprint bouts, where muscle glycogen is the predominant fuel (151). However, in contrast to our hypothesis, the HFD-CHO strategy actually compromised high intensity 1-km sprint performance (Figure 4.4). This is a novel finding and, to our knowledge, has not previously been reported.

The ingestion of a HFD for 6 days resulted in a shift in substrate metabolism towards a greater reliance on fat and a reduction in CHO oxidation. The increase in fat oxidation in the present study persisted *despite* 1 day of CHO-loading on day 7 as demonstrated by the lower resting RER (0.77 ± 0.02 vs. 0.88 ± 0.05 , Figure 4.3) and higher circulating FFA (Table 4.6) during exercise

after HFD-CHO compared to HCD-CHO on day 8. These findings are consistent with the findings of Burke et al. (26; 28) and Carey et al. (33) who also demonstrated an increase in fat oxidation with a short-term high fat diet that persisted even after restoration of CHO stores. Burke et al. (26) demonstrated that 1 day of rest and CHO-loading was sufficient to restore muscle glycogen levels to above baseline levels in both dietary treatments (470 ± 24 to 554 ± 45 mmol/kg dry wt after HFD-CHO; 470 ± 24 to 608 ± 51 mmol/kg dry wt after HCD-CHO). Although muscle glycogen was not measured in our study, it is assumed that muscle glycogen levels were restored on day 8 as a similar dietary strategy was used to that of Burke et al. (26) in which muscle glycogen levels were measured directly. The increase in fat oxidation with this dietary regime can therefore not be explained by low glycogen stores (192), and may be related to changes in insulin sensitivity (65), increased fatty acid uptake into the muscle (32) and changes in skeletal muscle enzyme activities that favor fat oxidation (134; 175). In addition to an increase in fat oxidation, Burke et al. (26) have shown that the ingestion of a HFD-CHO resulted in a significant reduction in muscle glycogen utilization (~ 100 mmol/kg dry wt) during a 120 minute cycle at $\sim 70\%$ VO_{2max} with the HFD-CHO compared to the HCD-CHO dietary strategy.

Ingestion of a HFD for 6 days was associated with a significant increase in heart rate, as well as a tendency towards a higher effort perception during training on days 3 and 5. Six of the eight subjects complained of fatigue and difficulty in maintaining the defined workload during the steady state cycle, with 2 subjects failing to complete the 60 minute training session. Burke et al. (26) reported similar subject complaints whilst training on a HFD. The increased effort perception and heart rate may be attributed to low glycogen stores and an

increase in sympathetic activation (Table 4.3, 4.4) (81; 158). This has practical implications for athletes ingesting a low CHO/high fat diet for example the Atkins diet, in terms of their ability to train at high intensities. Moreover, athletes that rely on heart rate to set their training loads may fail to achieve a desired power output and hence training stimulus.

Although the HFD-CHO dietary strategy was associated with an increase in fat oxidation and an apparent “sparing” of muscle glycogen stores on day 8, overall 100-km time-trial (156min 54sec for HFD-CHO vs. 153min 10sec for HCD-CHO) and 4-km sprint performance times following the two dietary treatments were not significantly different. Similarly, Burke et al. (26) and Carey et al. (33) demonstrated no overall improvements in performance during a 7 kJ/kg time-trial (lasting ~ 25 minutes) following a 2-hour sub-maximal constant-load cycle (26) or a 1-hour time-trial following 4 hours of constant-load exercise (33). However in both studies, there were individual differences in performance. In the first study, time-trial time was 8% faster in 5 of the 7 subjects during the HFD-CHO compared to the HCD-CHO trials (26). Similarly, Carey et al. (33) demonstrated improved performance in 5 of the 7 subjects following the high-fat diet. However, these studies did not simulate race conditions where high intensity sprint bouts (>90% of PPO) are integral to performance. Mean power output during the 25 min time-trial in the two studies of Burke et al (26; 28) after fat adaptation was 281 W (76% of PPO) and 302 W (76% of PPO) respectively, and mean power output during the 1-hour time-trial of Carey et al. (33) was 312 ± 15 (77.4% of PPO). The present study is the first study that included high intensity sprints (mean power output during 1-km sprints >90% of PPO) with endurance exercise, simulating race conditions. In contrast to the original

hypothesis, we found that HFD-CHO dietary strategy actually compromised high intensity 1-km sprint power output. Power output during the 1-km high-intensity sprints was even compromised in the 3 subjects whose overall 100-km time-trial performance was improved on the HFD-CHO dietary strategy.

We postulated that this reduced performance might be related to changes in sympathetic activation associated with high fat feeding, as we demonstrated an increase in low frequency power spectrum for heart rate variability, suggestive of increased sympathetic activation following high-fat intake that persisted after 1 day of CHO-loading. Heart rate variability has previously been shown to be a non-invasive, practical and reliable measure of sympathetic modulation (68). In addition, heart rate was similar during the 1-km sprints (Table 4.8) despite reduced 1-km power output following the HFD-CHO diet, suggesting increased sympathetic activation during the HFD-CHO trial. Previous research (158) has demonstrated an increase in sympathetic activation during exercise, as measured by plasma noradrenaline levels, with 7 days of high-fat feeding which was associated with low muscle glycogen stores. However, Helge et al. (81) demonstrated that the increase in sympathetic activation during exercise with a high fat diet *persisted* despite the restoration of muscle glycogen stores. An increase in sympathetic activation in response to a high-fat intake, as suggested by findings in the present study, has previously been associated with increased effort perception (81; 141; 178). The present study showed similar ratings of perceived exertion immediately following the 1-km sprints for both trials (Table 4.8), despite a reduced power output during the second, third and fourth 1-km sprint in the HFD-CHO trial (Figure 4.4). The subjects therefore experienced a similar effort perception for less work produced.

Ingestion of the HFD-CHO may have compromised the ability to oxidize the available glycogen at a sufficient rate to fuel the high-intensity sprint bouts. The 1-km sprints were performed at an intensity of more than 90% of PPO, during which muscle glycogen is the predominant fuel source (151). In contrast, power output during the 4-km sprints was performed at a lower intensity (~78-84% of PPO) and was not affected by the high-fat intake. Therefore the 'glycogen sparing' effect of the HFD-CHO strategy, which was thought to be beneficial for endurance performance, may in fact compromise high intensity sprint performance. This may possibly be mediated by changes in pyruvate dehydrogenase (PDH) activity (47; 134; 175). Indeed, studies investigating the effects of a high fat intake for between 3 days and 3 weeks demonstrated a decrease in the active form of PDH, suggesting reduced glycogenolysis and reduced CHO oxidation (47; 134). Furthermore, data from Stellingwerff et al. (175) using a similar HFD-CHO strategy to the present study, also demonstrated a decrease in mean active PDH activity during constant-load exercise. Further studies however, are required to examine this hypothesis.

In conclusion, ingestion of a HFD for 6 days, followed by 1 day of CHO-loading compared to 7 days of high CHO intake, increased fat oxidation, but reduced high-intensity sprint power performance, which was associated with increased effort perception and heart rate. The mechanisms associated with the decrement in performance are not clear, but could possibly be related to increased sympathetic activation and/or the inability to oxidize the available CHO during the high intensity sprints. Further research is required to determine the effectiveness of the dietary strategy in ultra-endurance (>4-5 hours) exercise where very high rates of CHO oxidation are not necessarily required.

In addition, further research is required to investigate the mechanisms underlying compromised high-intensity exercise performance associated with high fat feeding.

University of Cape Town

CHAPTER 5

***The effect of fat-adaptation followed by carbohydrate-loading
on simulated ultra-endurance race performance***

5.1 INTRODUCTION

In the previous chapter we hypothesized that the glycogen “sparing” effect of the fat-adaptation followed by CHO-loading (HFD-CHO) strategy might benefit endurance exercise including very high-intensity sprints ($>85\%$ VO_{2peak}) during which CHO is the predominant fuel source (151). In contrast to the hypothesis, the HFD-CHO dietary strategy did not alter overall time-trial performance, but compromised 1-km sprint ($>90\%$ W_{peak}) performance, possibly due to an increase in sympathetic nervous system activation, a higher effort perception that were associated with high fat intake, as well as an inability to oxidize the available CHO (175). With a failure to demonstrate a clear performance benefit for endurance exercise (26; 28), and the growing body of evidence to suggest that fat-adaptation strategies may actually impair the ability of athletes to work at high exercise intensities (175), the potential of this dietary strategy to enhance moderate to high intensity exercise performance is questioned.

We therefore propose an alternative hypothesis that 6 days of high fat intake followed by 1 day of CHO-loading (HFD-CHO) may benefit ultra-endurance (>4 - 5 hours) events that are typically undertaken at sub-maximal exercise intensities where very high rates of CHO oxidation are not necessarily required. Carey et al. (33) investigated the effect of fat-adaptation followed by CHO-loading on 1-hour time-trial performance following a 4-hour constant-load ride at 65% VO_{2peak} . Although total duration (5 hours) of exercise in that study was sufficient to deplete muscle glycogen stores, overall performance was not different following the fat-adaptation trial compared to the CHO trial (33).

However, the 4-hour ride was undertaken at a fixed workload (65% VO_{2peak}) and is not representative of a 'real-life' race situation. The effect of a high-fat diet, followed by CHO-loading on simulated 'real-life' ultra-endurance (>5 hours) race performance has not been previously investigated. None of the subjects investigated in Chapter 2 reported using this alternative dietary strategy diet prior to the 210-km cycle race. Therefore a randomized control trial to explore the effects of a HFD-CHO dietary strategy on simulated 'real-life' race performance will provide novel and valuable insight regarding the use of this alternative pre-exercise dietary strategy. In addition, there are no studies that have examined the responsiveness of athletes with different RER phenotypes (as described in Chapter 3) to this dietary strategy. Indeed, Carey et al. (33) demonstrated a variability in performance in response to the HFD-CHO strategy with 5 of the 7 subjects improving performance on the HFD-CHO.

Therefore the aims of this study were to i) investigate the effect of a high fat diet followed by 1 day of CHO-loading compared to a high CHO diet on substrate utilization, effort perception (RPE) and performance during a simulated self-paced 200-km cycling time-trial; and ii) to undertake a preliminary investigation to explore the responsiveness of subjects with a high or low RER phenotype to a HFD-CHO compared to a high CHO diet. The 200-km time-trial in this study simulated the ultra-endurance Double Century cycle race that the subjects completed in Chapter 2.

5.2 METHODOLOGY

5.2.1 Subjects and study design

Nine endurance-trained male cyclists participated in this study, which was approved by the Research and Ethics Committee of the Faculty of Health Sciences of the University of Cape Town. All subjects were free from known metabolic conditions and were currently not taking any medications for chronic conditions such as high blood pressure or stimulants for conditions such as asthma. The subjects were informed of the nature of the study and written informed consent was obtained prior to the start of the study.

Each subject completed two trials in a randomized, single-blind crossover design with a 2 week washout period separating each trial. Each trial consisted of an 8-day diet, training and testing period, during which subjects reported to the laboratory on days 1, 3, 5, 7 and 8 to undertake supervised training and testing (Figure 5.1).

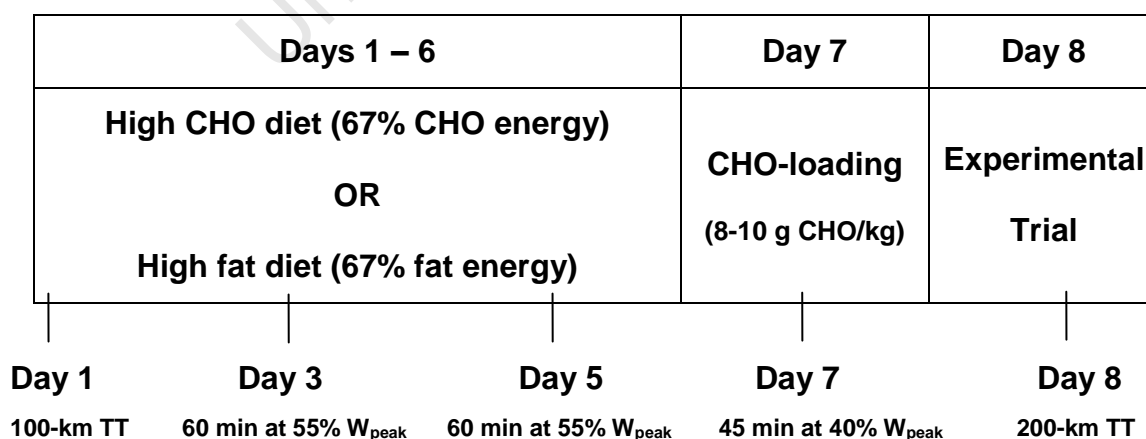


Figure 5.1. Diet, training and testing protocol.

To undertake a preliminary investigation exploring the effects of the RER phenotypes on substrate utilization and performance in response to a HFD-CHO vs. a high CHO diet, subjects were divided into a high-RER or low-RER group. Due to the difficulties in recruiting subjects for a trial of this nature, subjects were not screened prior to the trial and selected according to a resting fasting RER, as in Chapter 3. Subjects were stratified into a RER group based on the mean fasting resting RER recorded on day 1 of each trial. Subjects with a resting RER of >0.825 were assigned to the high-RER group and subjects with a resting RER <0.825 were assigned to the low-RER group. This cut point was based on the combined data of two studies [Goedecke et al. (66) and (64)] that measured the resting RER of more than 100 trained athletes and demonstrated a variability in resting fasted RER that was normally distributed with a mean of 0.825. The results of the RER groups are to be regarded as preliminary due to the small sample size of each group and are presented separately from the combined results in the second part of the results section.

5.2.2 Preliminary testing

Anthropometry

Prior to the start of the trial, body weight and height were measured to the nearest decimal place.

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

Peak oxygen consumption (VO_{2peak}) and peak power output (W_{peak}) were measured on an electronically braked cycle ergometer as described in Chapter

3. Heart rate was recorded continuously throughout the incremental test to voluntary fatigue by means of a Suunto T6 Heart Rate Monitor (Suunto Oy, Finland). W_{peak} values were used to set the work rates for the constant-load training rides on days 3 and 5 corresponding to 55% W_{peak} and for the constant-load training ride on day 7 corresponding to 40% of W_{peak} .

Familiarization ride

Two weeks prior to the trial, subjects were required to complete a simulated self-paced 200-km time-trial on their own bicycles, mounted on an electronically braked cycle ergometer (CompuTrainer Pro 3D, Racermate, Seattle, US). This trial provided subjects with an opportunity to familiarize themselves with the distance and pacing in the laboratory. The familiarization 200-km time-trial also served as a screening trial to establish if the subjects were adequately trained and able to complete the trial. To motivate the subjects and to ensure that they would complete the 200-km time-trials as fast as possible, at least 2 subjects (mostly training partners) performed the familiarization ride together. Pairs were matched for subsequent experimental trials based on their familiarization time-trial performance.

Dietary Analysis

The subjects were also instructed to complete a 3-day dietary record consisting of 2 week days and 1 weekend day, reflecting their habitual dietary intake. In order to assess habitual *pre-race* dietary intake, subjects were required to record their dietary intake in the 3 days prior to the familiarization 200-km time-trial. The subjects were instructed to prepare for the familiarization time-trial as

they would normally prepare for an ultra-endurance race. These dietary records were analyzed with the Food Finder 3 programme (Medtech (Pty) Ltd, Medical Research Council, Tygerberg, South Africa) to determine the subjects' self-reported habitual and pre-race energy intake and macronutrient consumption. The habitual dietary information was used as a guideline to devise the two experimental diets. To aid adherence to the diets, subjects were also required to indicate their food preferences.

5.2.3 Dietary manipulations

Subjects were required to ingest either a high-fat diet (~67% energy (E) from fat) for 6 days followed by 1 day of CHO-loading (8-10 g/kg CHO), or an isocaloric CHO diet (67%E from CHO) for 6 days followed by 1 day of CHO-loading (8-10 g/kg CHO). A registered dietician formulated individualized menus. In order to control dietary intake, all the meals were pre-packed and provided for the subjects together with a diary to record any deviations from the diet. Efforts were made to blind the diets by covertly manipulating the macronutrient compositions of the diets.

5.2.4 Exercise training sessions

On days 1, 3, 5, and 7, subjects reported to the laboratory after a 10-12 hour fast and completed an exercise training session. On the first day of training (day 1), subjects completed a 100-km time-trial on their own bicycles mounted on a cycle ergometer (Computrainer Pro 3D, RacerMate, Seattle, USA). The 100-km time-trial served to deplete muscle glycogen stores and included five 1-km

sprint distances and four 4-km sprint distances, during which subjects were requested to cycle “as fast as possible”, as described in Chapter 4.

On days 3 and 5, subjects completed a 60 minute constant-load cycle at 55% W_{peak} on a Lode bike, maintaining their cadence at 90 rpm. The constant-load training sessions on days 3 and 5 were undertaken to ensure consistency in the subjects’ training during the trial, as well as monitor their physiological and metabolic responses to the dietary interventions. Subjects were tested in the fasted state in order to measure the changes in their metabolic response at rest and during exercise over the course of the trial in response to the two dietary interventions.

During the constant-load cycle, heart rate was recorded continuously by means of a Suunto T6 Heart Rate Monitor (Suunto Oy, Finland) and VO_2 and VCO_2 values were measured for 4-5 minutes every 15 minutes (15, 30, 45 and 60 minutes), using the on-line computerized system (Oxycon Alpha Analyzer, Jaeger-Mijnhardt, Bunnik, The Netherlands). Finally, rate of perceived exertion (RPE) scores were recorded at 15 minute intervals, using the validated Borg 6-20 RPE Scale (17). Printed scale instructions together with a verbal explanation on how to use the scale, were given to the subjects prior to the trial in order to familiarize them with the operation of the scales.

On day 7 subjects completed a 45 minute constant-load cycle at 40% W_{peak} on a Lode bike, maintaining their cadence at 90 rpm. Heart rate was recorded continuously and RPE was scores were recorded at 15 minute intervals. The

intensity and duration of the constant-load exercise on day 7 was lower compared to days 3 and 5, and compared to that reported on day 7 in Chapter 4 (40% W_{peak} for 45 min vs. 63% W_{peak} for 60 min, respectively), in order to mimic a 'real-life' pre-race training week, during which cyclists would typically only perform a low-intensity 30-45 minute 'leg loosener' on the day before a ultra-endurance (>5 hours) event.

Resting measurements

Prior to exercise on all four days, VO_2 and VCO_2 values were measured while the subjects were seated in a resting position for 15-20 minutes to determine RER, using the on-line computerized system, as previously described. Heart rate variability (HRV) was also recorded prior to exercise using the Suunto T6 heart rate monitor (Suunto Oy, Finland) as described in Chapter 4. Power spectrum analysis for low frequency (LF, indicative of sympathetic activation) and high frequency (HF, indicative of parasympathetic activation) was performed based on the HRV interval, using MATLAB™ software (The MathWorks Inc.). The natural logarithm of LF and HF power, as well as the ratio of LF power to HF power was calculated from the power spectrum values.

5.2.5 Experimental trial

On day 8, following 7 days of dietary intervention, subjects reported to the laboratory after a 10-12 hour overnight fast. HRV, and VO_2 and VCO_2 were measured at rest as described previously to determine RER. Subjects then completed a simulated self-paced 200-km performance time-trial on their own

bicycles, mounted on an electronically braked indoor cycle ergometer (CompuTrainer Pro 3D, Racermate, Seattle, US).

200-km Performance time-trial profile

The profile of the 200-km time-trial simulated an actual race (the Double Century race described in Chapter 2) and consisted of 3 major sections; 100-km undulating hills followed by a 34-km climb section and the final 66-km downhill/home stretch (Figure 5.2). The actual race profile was recorded with a Garmin GPSMAP 76CS Global Positioning System (Garmin Ltd. USA) and the data was downloaded onto the Computrainer.

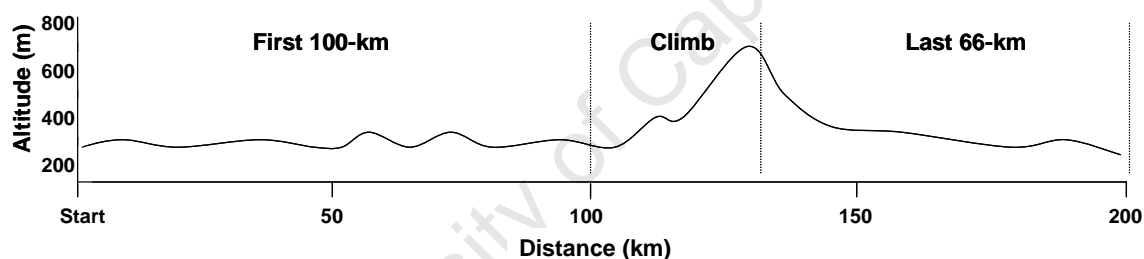


Figure 5.2. Profile for the 200-km performance time-trial (Double Century).

Calibration of the cycle ergometer (Computrainer)

The subjects' bicycle was attached to the ergometer by the rear axle quick release mechanism and supported under the front wheel by a plastic support. To standardize the load generator contact pressure of the Computrainer, rear tyres were inflated to 800 kPa and subjects were required to spin for 6 minutes to allow the tyres to warm up. After the 6 minute warm-up period, calibration of the Computrainer was performed automatically during the calibration mode by accelerating the bicycle to 40 km/hour and then allowing the bicycle to coast

until the rear wheel stopped. Calibration and load generator contact pressure was adjusted manually to achieve a pressure between 2.00-2.15 lbs. The average calibration value recorded during the study was 2.09 ± 0.03 lbs. This value provides the highest levels of accuracy when compared to power measured by a SRM (Schoberer Rad Messtechnik, Weldorf, Germany) ergometer (48).

Physiological measurements

During the 200-km time-trial, a blood sample was drawn at rest and at 25-km intervals throughout the trial for the subsequent analysis of plasma glucose, lactate and catecholamines levels and serum free fatty acid (FFA) concentrations. RPE was also recorded at 25-km intervals during the 200-km time-trial. In addition, VO_2 and VCO_2 values were measured for 4-5 minutes at 55, 90, 140 and 170 km, using the on-line Oxycon computerized system for the determination of exercising RER and the calculation of total CHO and fat oxidation, using the formulae from Frayn (57), as described in Chapter 3. Power output and heart rate were measured continuously throughout the trial (Figure 5.3). Subjects were allowed a 4-5 minute rest period after 98 and 150 km to stretch their legs, eat and use the toilet if needed.

Dietary intake during the trial

To maintain euglycemia in both trials, subjects were required to ingest at least 60 g of CHO per hour by consuming 150-200 ml of an 8-10% CHO drink every 20 minutes plus a snack containing ~25 g of CHO every hour. The feeding schedule is summarized in Figure 5.4. To ensure consistency of CHO intake

during the 200-km time-trials, each subject's CHO intake (type, quantity and timing of intake) was recorded during his first experimental trial and duplicated during the second trial. The mean CHO intake per hour of cycling during the 200-km time-trial was 68 ± 5 and 67 ± 7 g for the HFD-CHO and HCD-CHO, respectively. CHO intake did not vary by more than 5 g/hour of cycling.

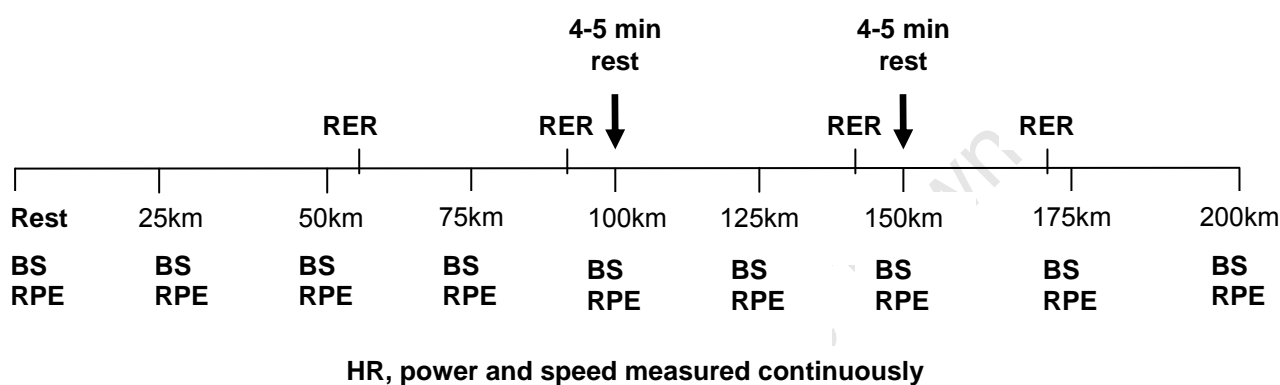
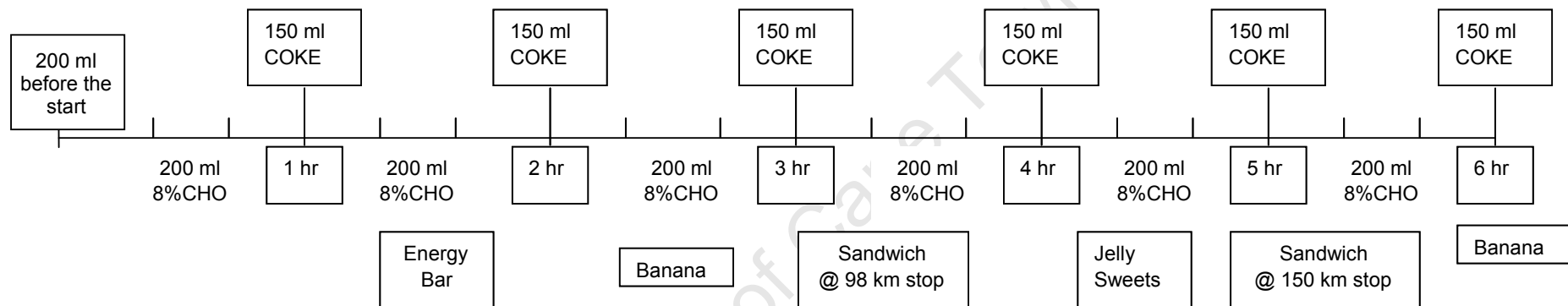


Figure 5.3. Measurements during the 200-km time-trial. BS, blood sample; RPE, rate of perceived exertion; RER, respiratory exchange ratio; HR, heart rate.

5.2.6 Blood sampling and analysis

Venous blood samples (~12 ml) were drawn at 25-km intervals during the 200-km time-trial, as described in Chapter 3, for the subsequent analysis of plasma glucose, lactate, epinephrine and norepinephrine and serum FFA concentrations. The samples were analyzed and as described in Chapter 3 (glucose, lactate, FFA) and Chapter 4 (catecholamines).



At least 1 x 200 ml 8% CHO drink per hour at 20 or 40 minutes, 150 ml COKE at every hour, and a ~25 g CHO snack per hour after the first hour. Water when thirsty (ad libitum)

Figure 5.4. Feeding schedule during the 200-km experimental time-trials.

5.2.7 Statistical Analysis

Values are presented as means \pm standard deviation (SD). A two-way analysis of variance (ANOVA) with repeated measures and the Tukey post hoc analysis were performed using STATISTICA analysis software (Version 7, Statsoft, Tulsa, OK, USA). Paired T-tests were performed to test differences in single variables between trials.

Where differences between RER groups were examined, a 3-way ANOVA with repeated measures and Tukey post hoc analysis were performed to test group x diet x time interactions and a 2-way ANOVA analysis were performed to test mean (area under the curve) group x diet interactions. Paired T-tests were performed to test differences in single variables between trials. Statistical significance was accepted at $P < 0.05$. Due to the limited sample size, $P < 0.1$ was regarded as a trend.

5.3 RESULTS

5.3.1 Combined results (n=9)

5.3.1.1 Subject characteristics

The characters of the subjects are summarized in Table 5.1. The subjects were well trained with a mean VO_{2peak} of greater than 55 ml/kg/min and a power to weight ratio of greater than 4.5 watts/kg body weight (Table 5.1).

Table 5.1. Subject Characteristics (n=9)

| Characteristic | Mean + SD | Range |
|----------------------------------|------------------|--------------|
| Age (years) | 32 ± 2 | 29 – 36 |
| Weight (kg) | 80.8 ± 10.4 | 70.0 – 96.0 |
| Height (m) | 1.84 ± 0.06 | 1.75 – 1.93 |
| VO _{2peak} (ml/kg/min) | 55.5 ± 5.77 | 49.0 – 63.2 |
| W _{peak} (W) | 364 ± 35.4 | 310 – 425 |
| W _{peak} :Weight (W/kg) | 4.54 ± 0.45 | 3.9 – 5.3 |

Values are means ± standard deviations (SD). VO_{2peak}, peak oxygen uptake;

W_{peak}, peak power output; W, watts.

5.3.1.2 Training and dietary intake

All the subjects attended and successfully completed all the training and exercise testing sessions. In addition, all subjects followed the experimental diets, ingested the food that was provided during both trials and achieved the recommended target for fat and CHO intake. The subjects' habitual diet, pre-familiarization time-trial diet and dietary intake during the experimental trials are presented in Table 5.2. By design, there was a significant difference ($p < 0.001$) in the CHO and fat content of the HCD and HFD diets. Although diets were blinded and covertly manipulated, subjects were able to distinguish that the diets were different, but were unaware of their composition.

The mean energy content of the experimental diets was similar to the reported energy content of the habitual diet (Table 5.2). Although not significant, mean total energy and CHO content were higher for the pre-familiarization time-trial diet compared to the habitual diet (Table 5.2). In contrast, mean reported

protein intake tended to be lower for the pre-familiarization time-trial diet compared to the habitual diet ($p=0.072$), but the reported fat intake was very similar between the two diets (Table 5.2).

5.3.1.3 Resting variables

The mean fasting resting RER was significantly lower during the HFD compared to the HCD trial ($p<0.001$). RER decreased over 6 days of the high-fat diet (0.84 ± 0.04 day 1 to 0.76 ± 0.04 day 7) and remained lower than the HCD on day 8 despite 1 day of CHO-loading on day 7 (0.83 ± 0.06 vs. 0.89 ± 0.02 , $p<0.001$) (Figure 5.5).

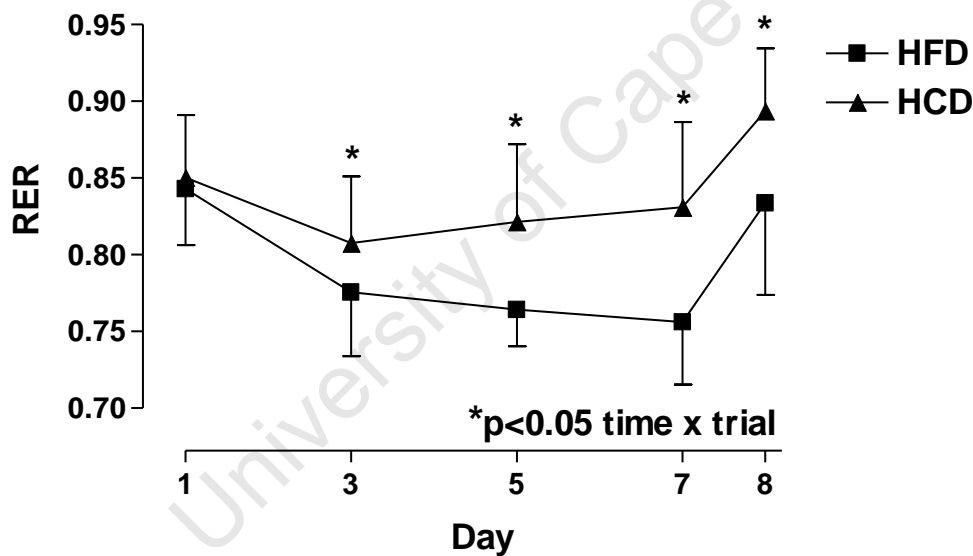


Figure 5.5. Resting RER over the trial in response to the HCD and HFD ($n=9$).

Mean resting heart rate was not different between the two experimental diets (Table 5.3). Similarly, mean normalized HRV values for LF (reflecting sympathetic modulation) for supine and standing (Table 5.3), as well as the HF and LF-to-HF ratios were not different between trials (data not shown).

Table 5.2. Habitual dietary intake, dietary intake prior to the 200-km familiarization time-trial, and intake during the experimental dietary treatments.

| | Energy (MJ) | CHO | | | FAT | | | PROTEIN | | |
|---------------------|----------------|---------|----------|---------|---------|------------|---------|---------|---------|--------|
| | | g | g/kg | %E | g | g/kg | %E | g | g/kg | %E |
| Habitual | 12.9±2.6 | 359±82 | 4.5±1.3 | 50±10 | 112±43 | 1.4±0.5 | 33±10 | 129±35 | 1.6±0.3 | 17±4 |
| Pre-fam TT | 13.5±2.9 | 447±156 | 4.7±2.2 | 55±10 | 110±36 | 1.4±0.5 | 31±9 | 108±24 | 1.3±0.2 | 14±4 |
| Experimental | | | | | | | | | | |
| HFD | 12.6±1.1 | 138±13* | 1.7±0.1* | 18±0.5* | 221±20* | 2.8±0.2* | 67±0.9* | 120±11 | 1.5±0.1 | 15±0.9 |
| HCD | 12.6±1.2 | 510±45* | 6.4±0.4* | 68±1.0* | 60±5.3* | 0.75±0.05* | 18±0.6* | 105±9 | 1.3±0.1 | 14±0.8 |

Values are means ± standard deviations. Pre-fam TT, pre-familiarization 200km time-trial; CHO, carbohydrate; E, energy; HFD, high-fat diet; HCD, high carbohydrate diet. *Significant trial effect (p<0.001).

Table 5.3. Mean resting heart rate and heart rate variability in response to the HFD and HCD treatments (n=9).

| | Day 1 | Day 3 | Day 5 | Day 7 | Day 8 |
|--------------------|-----------|-----------|-----------|-----------|-----------|
| HR (bpm) | | | | | |
| HFD | 54±8 | 54±8 | 55±8 | 54±7 | 55±8 |
| HCD | 56±11 | 53±9 | 51±8 | 53±9 | 53±7 |
| LF supine | | | | | |
| HFD | 2.02±0.09 | 2.00±0.07 | 2.03±0.05 | 2.06±0.17 | 2.02±0.07 |
| HCD | 2.04±0.07 | 2.01±0.07 | 2.04±0.07 | 1.99±0.06 | 2.04±0.09 |
| LF standing | | | | | |
| HFD | 1.97±0.08 | 1.98±0.04 | 2.03±0.10 | 1.95±0.04 | 2.02±0.18 |
| HCD | 2.01±0.17 | 1.96±0.09 | 1.98±0.11 | 2.04±0.14 | 2.00±0.08 |

Values are means ± standard deviations; Heart rate variability values are expressed as the natural log [ln] for LF supine and LF standing; HR, heart rate; LF, low frequency; HFD, high-fat diet; HCD, high carbohydrate diet.

5.3.1.4 Physiological measurements during the constant-load cycle rides

Mean exercising variables, including RER, heart rate and RPE, measured at 15 minute intervals during the constant-load cycle on days 3, 5 and 7 are presented in Table 5.4. Mean exercising RER on days 3 and 5 was significantly lower on the HFD compared to the HCD ($p < 0.005$), indicating an increase in fat oxidation in response to the HFD. Mean exercising heart rate was higher in response to the HFD treatment during the three constant-load rides, although not significantly. RPE were not different between trials during the three training sessions.

Table 5.4. Mean exercising values during the constant-load cycle on days 3, 5 and 7 in response to the HFD and HCD treatments (n=9).

| | Day 3 | Day 5 | Day 7 |
|-----------------|-----------------------------|-----------------------------|-------------------------------|
| | 1-hr @ 55%W _{peak} | 1-hr @ 55%W _{peak} | 45 min @ 40%W _{peak} |
| RER | | | |
| HFD | 0.82±0.03* | 0.82±0.03* | Did not measure |
| HCD | 0.87±0.04* | 0.88±0.03* | |
| HR (bpm) | | | |
| HFD | 148±12 | 151±10 | 129±11 |
| HCD | 146±12 | 146±8 | 123±9 |
| RPE | | | |
| HFD | 13±1 | 13±1 | 9±1 |
| HCD | 14±2 | 12±1 | 9±2 |

Values are means ± standard deviations. RER, respiratory exchange ratio; HR, heart rate; RPE, rating of perceived exertion; HFD, high-fat diet; HCD, high carbohydrate diet; *p<0.005 for diet.

5.3.1.5 Metabolic and performance data during 200-km time-trial

Respiratory exchange ratio (RER) during the 200-km time-trial

Exercising RER at 55, 90, 140 and 170 km during the 200-km time-trial was significantly lower (p<0.05) following the HFD-CHO compared to the HCD- CHO diet, indicating increased fat oxidation during the 200-km time-trial following the HFD-CHO trial (Figure 5.6).

Total CHO and fat oxidation during the 200-km time-trial

Accordingly, fat oxidation increased significantly over the 200-km time-trial and tended to be higher in response to the HFD-CHO compared to the HCD-CHO

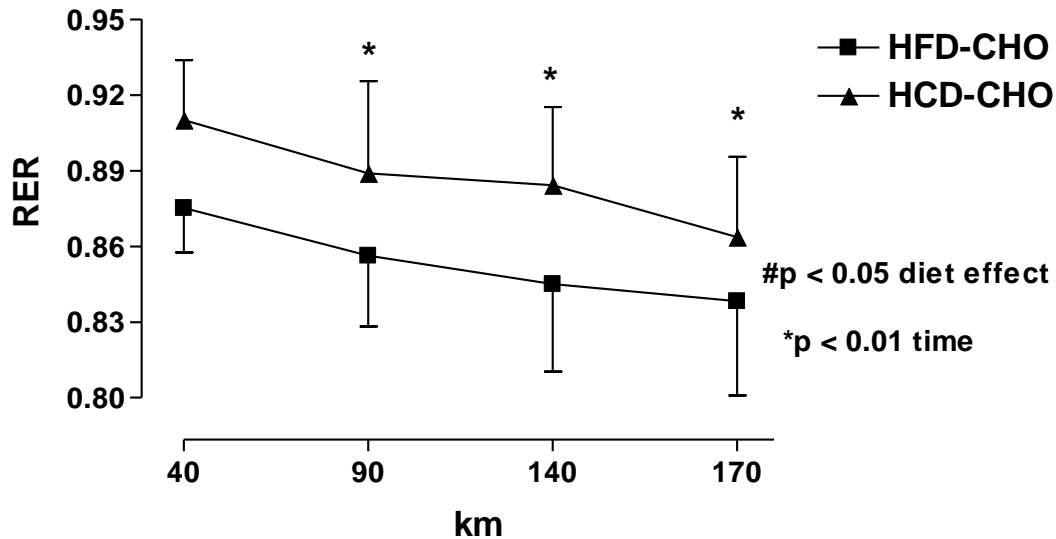


Figure 5.6. Exercising RER during the 200-km time-trial in response to the HFD-CHO and HCD-CHO dietary treatments ($n=8$).

diet ($p=0.086$, figure 5.7b). In contrast, total CHO oxidation tended to be lower in response to the HFD-CHO compared to the HCD-CHO ($p=0.063$, figure 5.7a) and decreased significantly over the 200-km time-trial.

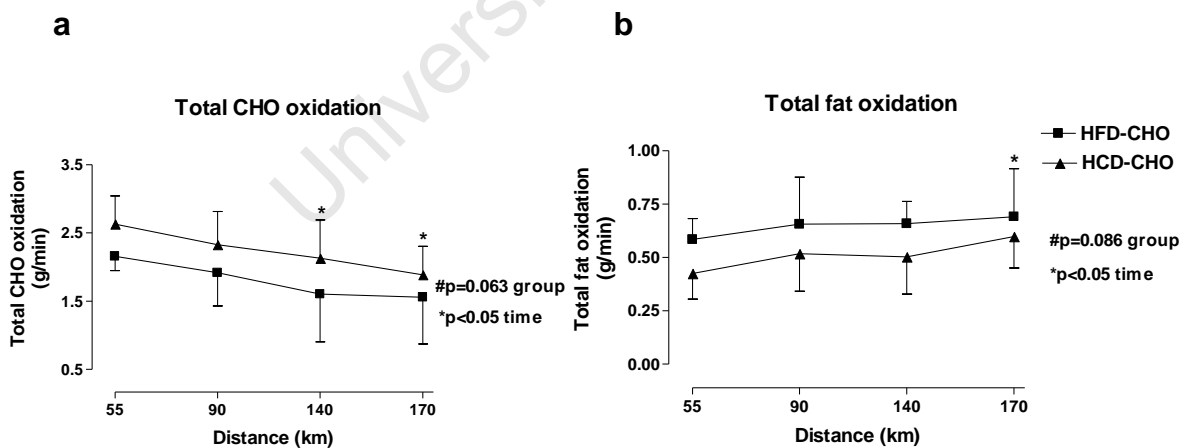


Figure 5.7. Total CHO oxidation (a) and fat oxidation (b) during the 200-km time-trial in response to the HFD-CHO and HCD-CHO dietary treatments ($n=8$).

Circulating hormone and substrate concentrations

Circulating glucose, lactate, FFA and catecholamine concentrations, measured at rest, 50, 100, 150 and 200 km are summarised in Table 5.5. Plasma glucose concentrations increased over time on both diets ($p < 0.005$) but were not different between the HFD-CHO and HCD-CHO diets, and all subjects remained euglycemic throughout the 200-km time-trial. Similarly plasma lactate, catecholamines (epinephrine and norepinephrine) and serum FFA concentrations increased significantly over the 200-km time-trial ($p < 0.05$), but were not different in response to the two diet interventions.

200-km Time-trial performance

Overall 200-km time-trial performance was not significantly different between trials ($p = 0.63$, Figure 5.8), but was 3 minutes 34 seconds slower on the HFD-CHO compared to the HCD-CHO diet.

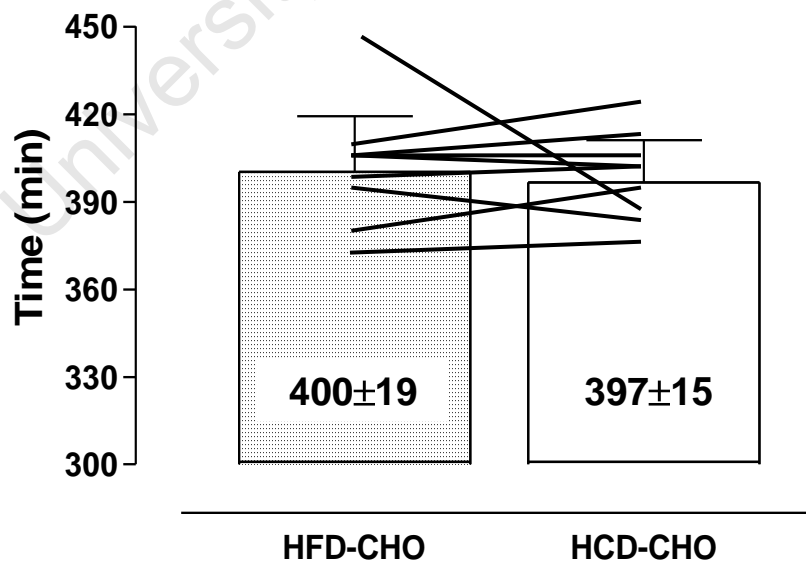


Figure 5.8. Overall 200-km time-trial performance in response to the HFD-CHO and HCD-CHO dietary treatments ($n=9$).

Table 5.5. Circulating blood hormone and substrate concentrations during the 200-km time-trial in response to the two diets.

| | 0 km | 50 km | 100 km | 150 km | 200 km | p-level |
|---------------------------------------|-----------|-----------|-----------|-----------|-----------|-------------|
| Plasma glucose (mmol/l) | | | | | | |
| HFD-CHO | 4.5±0.3 | 4.9±0.2 | 5.0±0.4 | 5.1±0.6 | 5.7±0.7 | <0.005 time |
| HCD-CHO | 4.3±0.3 | 4.6±0.1 | 4.9±0.4 | 5.1±0.8 | 5.4±0.5 | |
| Plasma lactate (mmol/l) | | | | | | |
| HFD-CHO | 1.13±0.43 | 2.48±0.99 | 2.05±0.69 | 1.38±0.13 | 2.22±0.96 | <0.001 time |
| HCD-CHO | 1.03±0.31 | 2.46±1.03 | 1.88±0.64 | 1.50±0.39 | 2.05±1.18 | |
| Serum FFA (mmol/l) | | | | | | |
| HFD-CHO | 0.20±0.18 | 0.20±0.08 | 0.48±0.25 | 0.58±0.20 | - | <0.001 time |
| HCD-CHO | 0.16±0.05 | 0.23±0.11 | 0.50±0.37 | 0.65±0.39 | - | |
| Plasma norepinephrine (nmol/l) | | | | | | |
| HFD-CHO | 0.91±0.53 | 5.21±2.5 | 4.81±3.66 | 6.83±5.60 | - | <0.005 time |
| HCD-CHO | 1.12±1.81 | 6.68±2.22 | 5.72±1.82 | 7.09±3.20 | - | |
| Plasma epinephrine (nmol/l) | | | | | | |
| HFD-CHO | 0.75±0.31 | 1.23±0.72 | 1.24±0.42 | 1.21±0.32 | - | <0.05 time |
| HCD-CHO | 0.42±0.10 | 0.60±0.15 | 1.85±0.98 | 1.19±0.26 | - | |

Values are means ± standard deviations. HFD-CHO, 6d high-fat diet + 1d carbohydrate-loading; HCD-CHO, 6d high carbohydrate diet + 1d carbohydrate-loading; FFA, free fatty acids.

Similarly, mean overall 200-km power output, speed and heart rate were not significantly different between trials (Table 5.6). When using magnitude based inferences, as described in Chapter 3, we found that there was only a 48% possibility that the mean difference in overall 200-km performance between the 2 diets had meaningful significance. Performance of 5 of the 9 subjects improved on the HFD-CHO compared to the HCD-CHO diet (Figure 5.8). However, one subject performed the 200-km time-trial 58 minutes slower following the HFD-CHO compared to the HCD-CHO diet. His performance during this trial was 40 minutes slower than the slowest subject, possibly skewing the data (total time: 400min 13sec vs. 394min 45sec with and without this subjects' data, respectively).

The mean exercising variables recorded during the 3 major sections of the 200-km time-trial (i.e. the first 100-km undulating hills, the 34-km climb section and the final 66-km downhill/home stretch) are summarized in Table 5.6. The 3 sections were examined independently as the profile of each section was so different (undulating profile vs. long climb vs. downhill) potentially eliciting a different physiological/metabolic response.

Mean time, power output and speed during the first 100-km of the time-trial were remarkably similar between diets for all subjects (Table 5.6). Although not significant, performance (time, power output and speed) improved on the climb section following the HFD-CHO vs. the HCD-CHO diet and was improved in 8 of the 9 subjects following the HFD-CHO compared to the HCD-CHO diet (Table 5.6). The remaining subject (potential outlier described above) performed the

climb 18 minutes slower following the HFD-CHO compared to the HCD-CHO. When his performance was excluded from the data set, climb performance was significantly improved in response to the HFD-CHO compared to the HCD-CHO (88.1 ± 5.0 vs. 92.4 ± 7.4 min, $p < 0.05$, respectively). Mean exercising heart rate on the climb was also higher following HFD-CHO vs. HCD-CHO diet (Table 5.6).

Table 5.6. Mean exercising values during the 200-km time-trial in response to the HFD-CHO and HCD-CHO treatments (n=9).

| | Overall | First 100-km | Climb | Last 66-km |
|-------------------------|----------|--------------|----------|------------|
| Time (min) | | | | |
| HFD-CHO | 400.2±19 | 187.3±5.0 | 90.1±8.0 | 128.3±19 |
| HCD-CHO | 396.6±15 | 186.8±5.0 | 91.9±7.0 | 122.6±17 |
| Power (W) | | | | |
| HFD-CHO | 191.3±21 | 208.3±12 | 192.9±26 | 170.4±32 |
| HCD-CHO | 194.0±18 | 206.8±18 | 186.1±20 | 181.8±20 |
| Speed (km/h) | | | | |
| HFD-CHO | 30.2±1.4 | 31.8±1.5 | 23.2±1.9 | 32.7±2.9 |
| HCD-CHO | 30.5±1.1 | 31.7±1.8 | 22.7±1.7 | 33.9±1.4 |
| Heart rate (bpm) | | | | |
| HFD-CHO | 154±9 | 154±12 | 157±11 | 151±12 |
| HCD-CHO | 152±6 | 151±8 | 153±7 | 154±5 |

Values are means ± standard deviations. HFD-CHO, 6d high-fat diet + 1d carbohydrate loading; HCD-CHO, 6d high carbohydrate diet + 1d carbohydrate loading.

In contrast to the climb performance, performance during the final 66-km was slower following the HFD-CHO compared to the HCD-CHO (Table 5.6). However, the one subject (potential outlier described above) performed the final 66-km 40 minutes slower on the HFD-CHO compared to the HCD-CHO. When

his data was excluded, performance times during the final 66-km were very similar in response to the HFD-CHO compared to the HCD-CHO (178.5 ± 21 vs. 180.4 ± 21 min, respectively).

Mean RPE recorded at 25-km intervals was not different between treatments and rose progressively over time ($p < 0.001$) (Figure 5.9). Despite a higher power output and heart rate (Table 5.6) on the climb section in response to the HFD-CHO compared to the HCD-CHO, RPE recorded at 125 km (~two thirds up the climb) was not different.

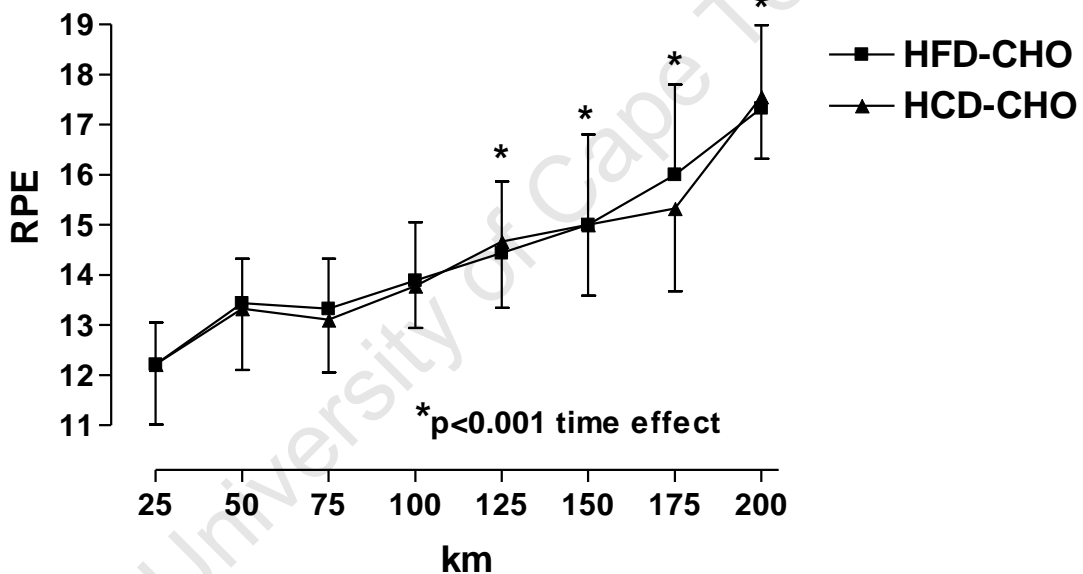


Figure 5.9. Mean RPE recorded at 25-km intervals during the 200-km TT in response to the two dietary treatments ($n=9$).

5.3.2 Responsiveness of the high-RER and low-RER groups to the HFD-CHO and HCD-CHO dietary interventions

The responsiveness of the high-RER and low-RER groups to a HFD-CHO and HCD-CHO are presented below. It is important to note that due to time constraints and the practical difficulties of conducting a study of this nature,

including the recruitment and selection of subjects, the sample size is limited ($n = 4$ and $n = 5$ for the low-RER and high-RER groups, respectively). Hence the analyses presented below represent a preliminary investigation.

5.3.2.1 Subject characteristics and dietary intake

The characteristics of the high-RER and low-RER groups are summarized in Table 5.7. Despite significant differences in mean fasting resting RER, mean age, weight, height and training status (VO_{2peak} and W_{peak}) were remarkably similar between the two groups (Table 5.7).

Table 5.7. Subject Characteristics

| Characteristic | High-RER (n=5) | Low-RER (n=4) |
|--------------------------|----------------|---------------|
| Resting RER | 0.87±0.03* | 0.82±0.02* |
| Age (years) | 32±3 | 32±3 |
| Weight (kg) | 78.8±8.9 | 83.3±12.7 |
| Height (m) | 1.86±0.05 | 1.82±0.02 |
| VO_{2peak} (ml/kg/min) | 55.9±4.8 | 54.9±6.8 |
| W_{peak} (W) | 363±46 | 366±21 |
| Power/weight (W/kg) | 4.6±0.4 | 4.5±0.5 |

Values are means ± standard deviations (SD). RER, respiratory exchange ratio; VO_{2peak} , peak oxygen uptake; W_{peak} , peak power output; W, watts. * $p < 0.05$ group.

Reported habitual dietary intake during the 3-day period prior to the familiarization time-trial for the RER groups is summarized in Table 5.8. Mean reported habitual energy intake was significantly higher ($p < 0.05$) in the low-RER compared to the high-RER group, mainly due to a higher fat intake ($p < 0.05$)

Table 5.8: Habitual training and pre-race dietary intake in the subjects with a high and low resting RER (n=9).

| Energy (MJ) | | CHO | | | FAT | | | PROTEIN | | |
|-------------------------------|-----------|---------|---------|---------|---------|----------|-----------|---------|---------|----------|
| | | g | g/kg | %E | g | g/kg | %E | g | g/kg | %E |
| Habitual training diet | | | | | | | | | | |
| High-RER | 11.4±2.4* | 347±99 | 4.4±1.3 | 55±10 | 82±33* | 1.1±0.5* | 27.5±6.5# | 116±5.0 | 1.5±0.1 | 17.5±3.7 |
| Low-RER | 14.7±1.2* | 375±64 | 4.6±1.4 | 44±7.0 | 148±18* | 1.8±0.1* | 40±2.0# | 145±52 | 1.7±0.5 | 16±5.1 |
| Habitual pre-race diet | | | | | | | | | | |
| High-RER | 13.1±3.6 | 483±173 | 6.2±2.5 | 62±6.4* | 86±29* | 1.1±0.4# | 25±7.0* | 106±23 | 1.4±0.3 | 13±3.2 |
| Low-RER | 13.9±2.3 | 401±141 | 5.0±1.9 | 48±9.9* | 140±19* | 1.7±0.4# | 39±6.0* | 109±30 | 1.3±0.2 | 13±6.2 |

Values are means ± standard deviations. CHO, carbohydrate; E, energy. *Significant group effect (p<0.05); #Tendency for a significant group effect (p=0.06). *#Tendency for a significant group effect (p=0.066)

(Table 5.8). Mean reported protein (g) and CHO (g) intakes were not different between the two groups (Table 5.8).

Reported dietary intake during the 3-day period prior to the familiarization time-trial for the RER groups is also summarized in Table 5.8. In contrast to the habitual diet, mean reported pre-race energy intake was not significantly different between the two groups. However the relative contribution (% of total energy) of CHO and fat to the diet was different ($p < 0.05$), with the low-RER group reporting a higher fat intake compared to the high-RER group ($p < 0.05$), and the high-RER group reporting a higher CHO intake compared to the low-RER group ($p < 0.05$) (Table 5.8). Mean reported protein intake was similar between the groups (Table 5.8).

Comparing the pre-race diet to the habitual training diet, it is interesting to note that the high-RER group adjusted their pre-race diet by increasing their CHO intake (136 g higher), resulting in a higher energy intake (1.7 MJ). In contrast the low-RER group did not alter their habitual diet in preparation for the 200-km time-trial.

5.3.2.2 Resting respiratory exchange ratio

The high-RER group had a higher resting RER compared to the low-RER group ($p < 0.05$) in response to both diets, however resting RER increased to a greater degree in the high-RER compared to the low-RER group in response to the HCD vs. the HFD ($p < 0.05$ diet x group, Figure 5.10).

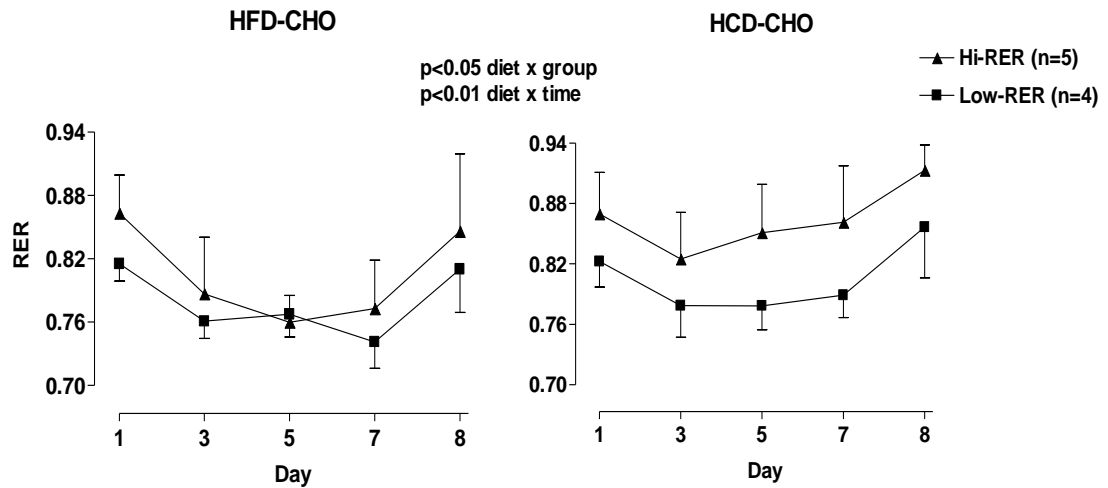


Figure 5.10. Resting RER over time in response to the HFD-CHO and HCD-CHO in the high-RER and low-RER groups.

5.3.2.3 RER, HR and RPE during constant-load training rides

Similar to the findings at rest, the high-RER group had a higher mean RER during the constant-load exercise on days 3 and 5 compared to the low-RER group in response to both diets (Figure 5.11). This was largely attributed to the higher RER in the high-RER compared to the low-RER group on days 3 and 5 in response to the HCD ($p < 0.05$ group \times diet, Figure 5.11).

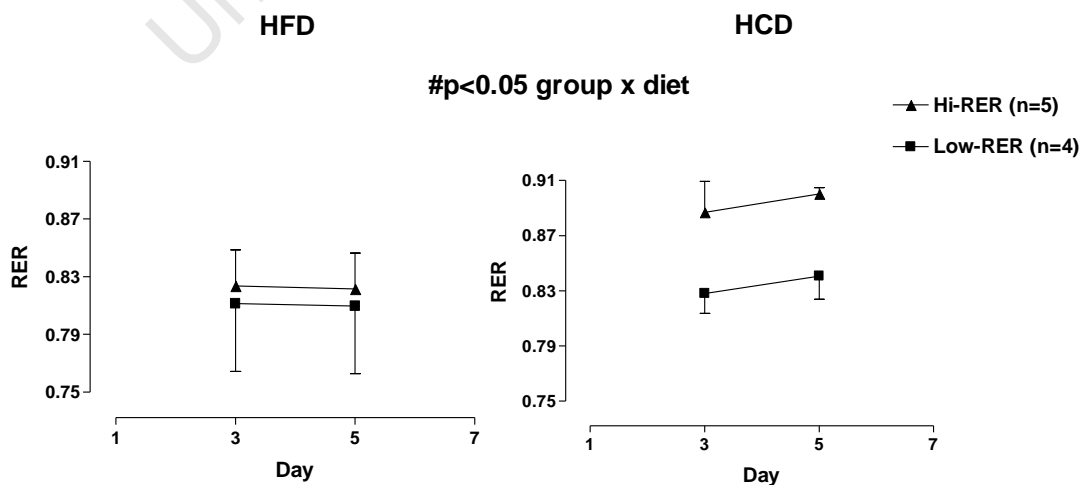


Figure 5.11. Mean exercising RER on days 3 and 5 in response to the HFD and HCD in the high-RER and low-RER groups.

Mean exercising heart rate and RPE were not different between trials or RER groups during the three training days (data not shown).

5.3.2.4 Metabolic and performance data during 200-km time-trial

Respiratory exchange ratio (RER) during the 200-km time-trial

In contrast to the resting and training RER in response to the HFD, exercising RER during the 200-km time-trial was lower following the HFD-CHO compared to the HCD-CHO diet ($p < 0.05$), but was not significantly different between the two RER groups (Figure 5.12).

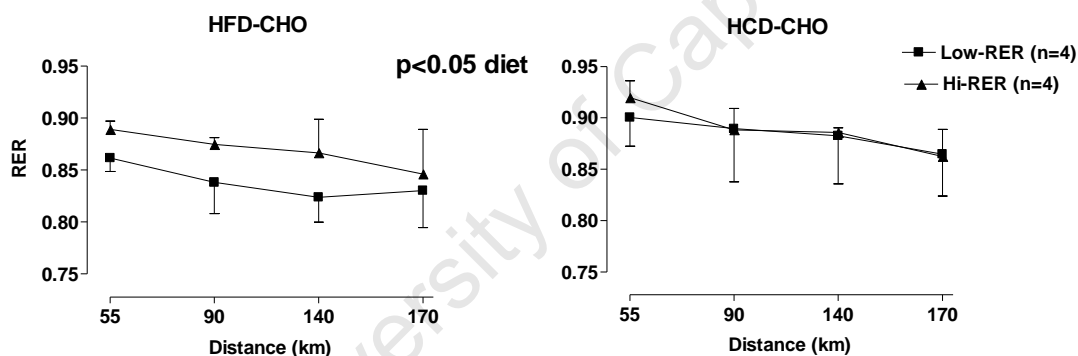


Figure 5.12. Exercising RER during the 200-km time-trial in response to the HFD-CHO and HCD-CHO dietary treatments in the high-RER and low-RER groups.

Total CHO and fat oxidation during the 200-km time-trial

Accordingly, total CHO oxidation tended to be lower on the HFD-CHO compared to the HCD-CHO diet ($p = 0.063$, Figure 5.13), but was not significantly different between the high-RER and low-RER groups.

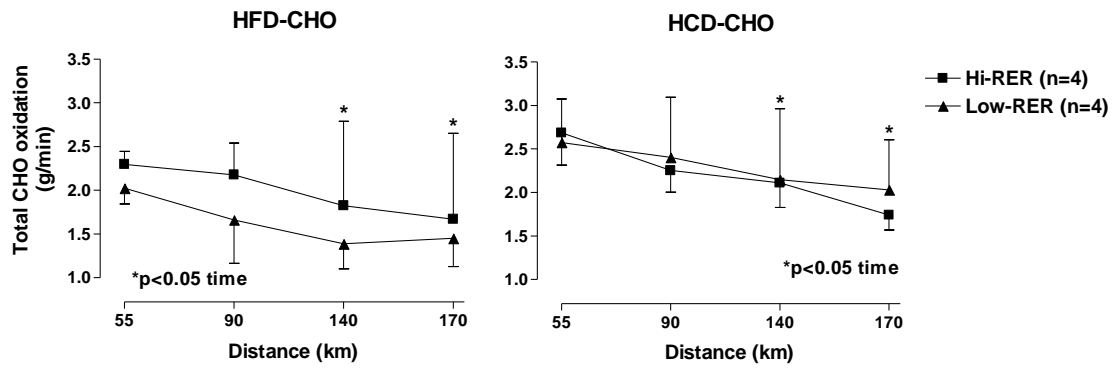


Figure 5.13. Total CHO oxidation during the 200-km time-trial in the high-RER and low-RER groups in response to the HFD-CHO and HCD-CHO dietary treatments. ($p=0.063$ diet effect)

In contrast, there were no significant differences in total fat oxidation between the HFD-CHO and HCD-CHO diets. However, mean total fat oxidation during the 200-km time-trial tended to be higher in the low-RER group compared to the high-RER group ($p=0.084$, Figure 5.14), largely due to higher rates of fat oxidation in response to the HFD-CHO. However, no interaction effect was demonstrated.

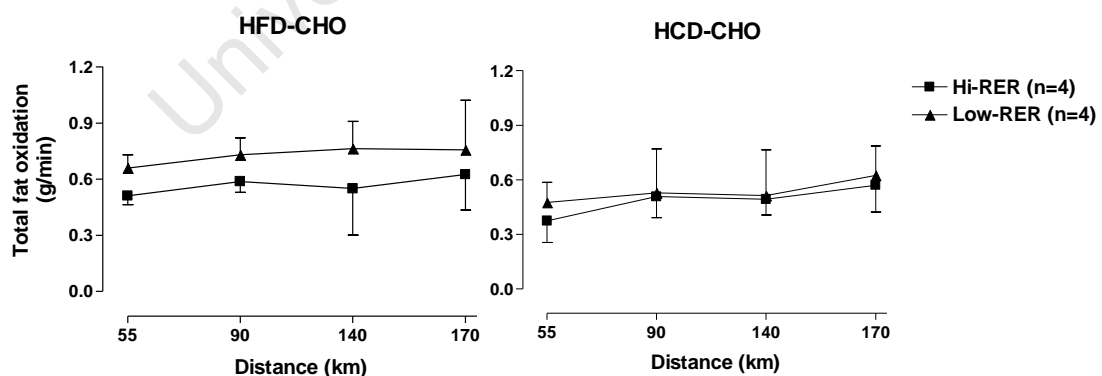


Figure 5.14. Total fat oxidation during the 200-km time-trial in the high-RER and low-RER groups in response to the HFD-CHO and HCD-CHO dietary treatments. ($p=0.084$ group effect)

Circulating hormone and substrate concentrations during the 200-km trial

Although not significant, overall mean serum FFA concentrations were higher in the low-RER compared to the high-RER group on both diets (Table 5.9). Mean plasma lactate concentrations were not significantly different between groups or trials. Similarly plasma glucose was also not different between groups or trials and all subjects maintained euglycemic (Table 5.9).

200-km Time-trial performance

Overall time-trial performance was not significantly different between groups or dietary interventions (Figure 5.15).

Table 5.9: Overall mean exercising blood concentrations during the 200-km time-trial in the high-RER and low-RER groups.

| | High-RER (n=5) | Low-RER (n=4) |
|--|----------------|---------------|
| Plasma glucose (mmol/l) | | |
| HFD-CHO | 5.1±0.25 | 5.3±0.37 |
| HCD-CHO | 4.9±0.12 | 4.8±0.24 |
| Plasma lactate (mmol/l) | | |
| HFD-CHO | 2.00±0.56 | 1.86±0.23 |
| HCD-CHO | 1.68±0.54 | 2.05±0.35 |
| Serum free fatty acids (mmol/l) | | |
| HFD-CHO | 0.39±0.17 | 0.50±0.17 |
| HCD-CHO | 0.39±0.23 | 0.53±0.16 |

Values are means ± standard deviations. HFD-CHO, high-fat diet; HCD-CHO, high carbohydrate diet.

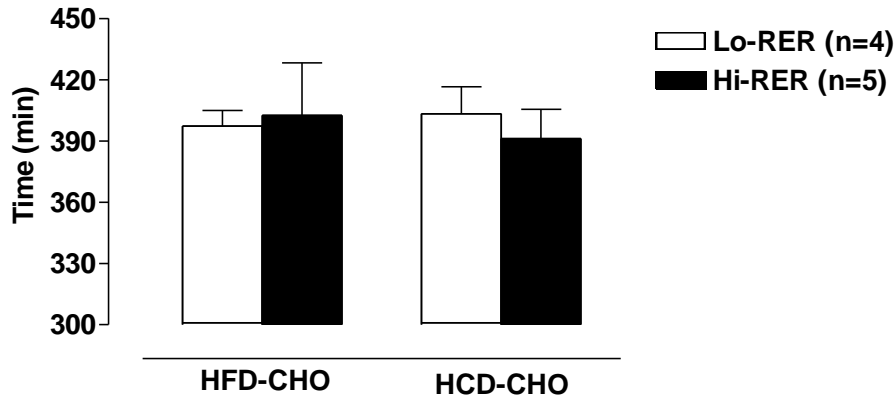


Figure 5.15. Overall 200-km time-trial performance in response to the HFD-CHO and HCD-CHO dietary treatments in the high-RER and low-RER groups.

However, although not significant ($p=0.284$ for diet \times group), mean 200-km performance in the low-RER group was faster (397.3 ± 8 vs. 403.3 ± 13 min) following the HFD-CHO compared to the HCD-CHO (Figure 5.15). In contrast, mean 200-km time-trial performance in the high-RER group was faster (391.2 ± 26 vs. 402.6 ± 14 min) following the HCD-CHO compared to the HFD-CHO. It is important to note that the subject who performed 58 minutes slower on the HFD-CHO compared to the HCD-CHO was in the high-RER group, and was possibly skewing the data. When he was excluded from analysis, mean overall performance for the high-RER group was almost identical in response to the two diets (392.2 ± 26 vs. 392.6 ± 14 min, for the HFD-CHO and HCD-CHO respectively).

When examining the 3 major sections of the 200-km time-trial individually, we failed to demonstrate a significant group \times diet interaction effect for any of the sections, possibly due to a small sample size. When we explored the two RER groups *independently*, mean time, power output (Figure 5.16) and speed during the first 100-km of the time-trial were remarkably similar in response to the two

dietary interventions in both RER groups. However, in the low-RER group, time, power output and speed improved significantly ($p < 0.05$) on the climb section following the HFD-CHO vs. the HCD-CHO diet (Table 5.10) without affecting performance during the final 66-km (Figure 5.16). Mean exercising heart rate on the climb was also significantly higher in the low-RER group following HFD-CHO vs. HCD-CHO diet ($p < 0.05$) (Table 5.10).

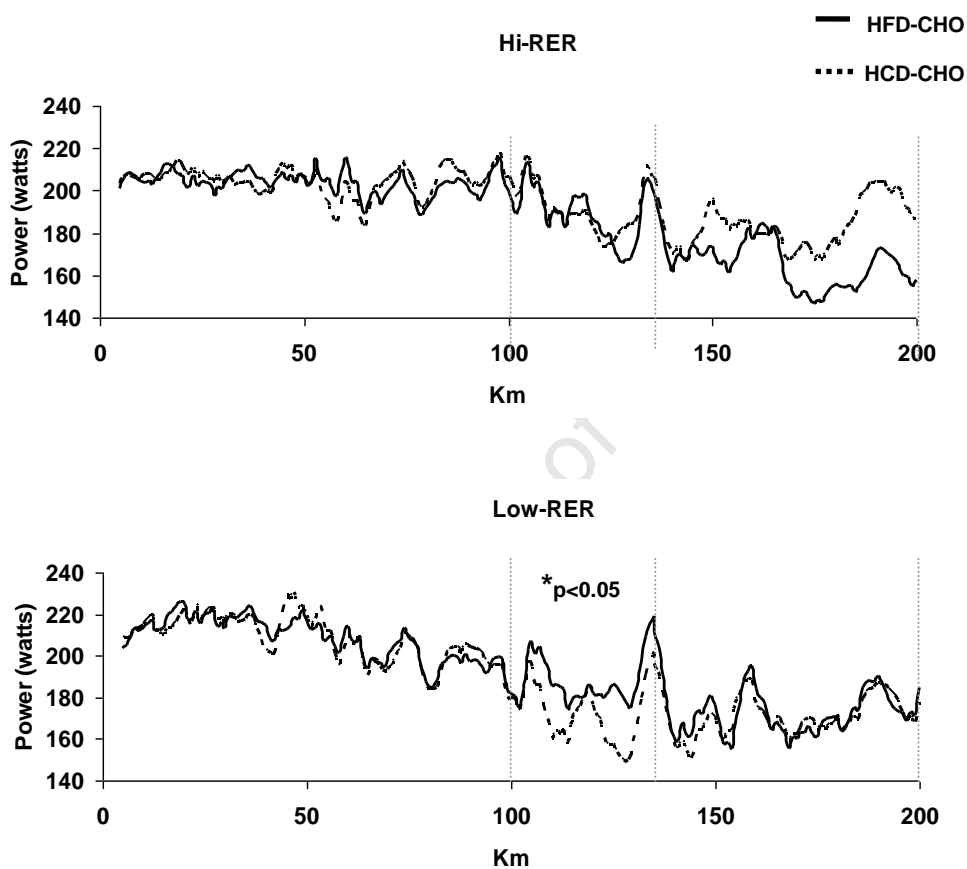


Figure 5.16. Continuous power output during the 200-km time-trial in response to the HFD-CHO and HCD-CHO dietary treatments in the Hi-RER ($n=5$) and low-RER ($n=4$) groups.

Table 5.10: Mean exercising values during the climb section in the Low-RER and High-RER (with and without the outlier) groups in response to the HFD-CHO and HCD-CHO treatments.

| | Lo-RER N=4 | Hi-RER n=5 | Hi-RER n=4# |
|-------------------------|---------------|---------------|----------------|
| Time (min) | | | |
| HFD-CHO | 90.3±6.0* | 90.0±10 | 86.0±3.9 |
| HCD-CHO | 96.5±7.0* | 88.2±5.0 | 88.3±5.7 |
| Power (watts) | | | |
| HFD-CHO | 194.2±7.0* | 191.9±37 | 204.7±26+ |
| HCD-CHO | 175.9±12* | 194.3±23 | 195.3±26+ |
| Speed (km/hour) | | | |
| HFD-CHO | 23.0±1.4* | 23.4±2.4 | 24.3±1.3 |
| HCD-CHO | 21.6±1.5* | 23.5±1.3 | 23.6±1.4 |
| Heart rate (bpm) | | | |
| HFD-CHO | 159±11* | 154±11 | 160±8 |
| HCD-CHO | 149±9* | 156±3 | 155±3 |

Values are means ± standard deviations. #Hi-RER group without the outlier; HFD-CHO, 6d high-fat diet + 1d carbohydrate loading; HCD-CHO, 6d high carbohydrate diet + 1d carbohydrate loading. *p<0.05; +p=0.079.

In contrast to the low-RER group, climb performance in the high-RER group was ~2 minutes *slower* following the HFD-CHO compared to the HCD-CHO with no difference in heart rate (Table 5.10). Performance during the final 66-km was 10 minutes slower in the high-RER group following the HFD-CHO compared to the HCD-CHO (135±25 vs. 125±23 min, p=0.235, respectively) (Figure 5.16) and was reflected by a lower heart rate during the final stretch following the HFD-CHO compared to the HCD-CHO diet (149±15 vs. 158±2 bpm, p=0.337, respectively).

Despite the large difference in performance time, the results were not statistically significant due to the aforementioned outlier who took 18 minutes longer to complete the climb, and 40 minutes longer to complete the final 66-km in response to the HFD-CHO compared to the HCD-CHO, possibly skewing the data. When his data was excluded, mean climb performance for the high-RER group was 2 minutes 15 seconds *faster* (Table 5.10) and mean performance during the final 66-km was 3.5 minutes slower (131.0 ± 26 vs. 127.5 ± 25 min, $p=0.370$, respectively) following the HFD-CHO compared to the HCD-CHO (Figure 5.17). Power output on the climb tended to be higher ($p=0.079$) in response to the HFD-CHO compared to the HCD-CHO when the outlier was excluded from the Hi-RER group (Table 5.10).

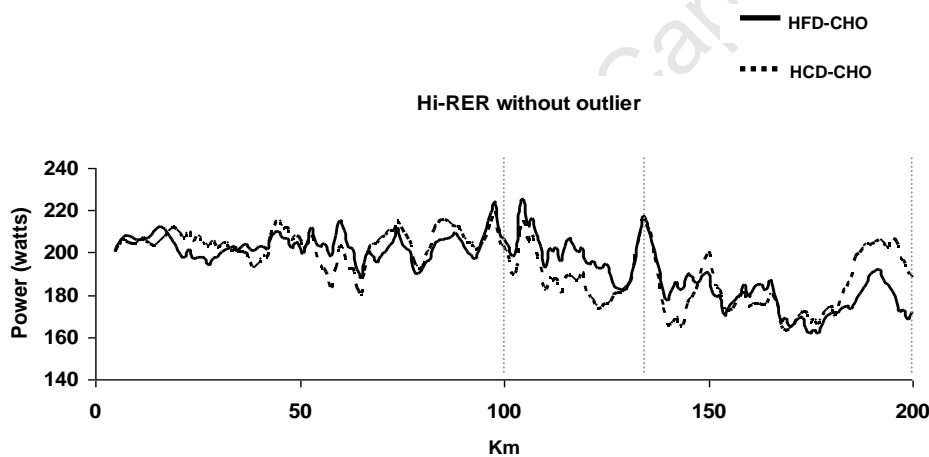


Figure 5.17. Continuous power output during the 200-km time-trial in response to the HFD-CHO and HCD-CHO dietary treatments in the Hi-RER group without the outlier.

Despite differences in power output, especially during the climb and the last downhill section in response to the two diets, mean RPE recorded at 25 km intervals rose progressively over time ($p<0.001$) in the high-RER and low-RER

groups but was not different between treatments or RER groups (data not shown).

5.4 DISCUSSION

In Chapter 4 we demonstrated that high-fat intake followed by CHO-loading (HFD-CHO) increased fat oxidation, but compromised high-intensity sprint performance possibly due to an increase in sympathetic nervous system activity and inability to oxidize CHO during very high intensities. As a result, the fifth and final study of this thesis explored the hypothesis that this particular dietary strategy may potentially enhance ultra-endurance exercise (>4-5 hours) that is typically undertaken at sub-maximal exercise intensities where very high rates of CHO oxidation are not necessarily required. This is the first study to examine the effects of fat-adaptation followed by CHO-loading on performance during self paced ultra-endurance (>5hrs) exercise that simulates a 'real-life' race situation.

The ingestion of a HFD for 6 days resulted in a shift in substrate metabolism towards a greater reliance on fat and a reduction in CHO oxidation. The increase in fat oxidation in the present study persisted on day 8 *despite* 1 day of CHO-loading on day 7, as demonstrated by the lower resting RER ($p < 0.001$, Figure 5.5) and exercising RER ($p < 0.05$, Figure 5.6), as well a trend toward higher rates of fat oxidation ($p = 0.086$, Figure 5.14) with the HFD-CHO compared to HCD-CHO. These findings are consistent with the findings in Chapter 4, as well a number of other similar published studies (26; 28; 33). As

mentioned in Chapter 4, the increase in fat oxidation with this dietary regime is unlikely due to low glycogen stores (192), but may rather be related to changes in insulin sensitivity (65), increased fatty acid uptake into the muscle (32), changes in skeletal muscle enzyme activities that favor fat oxidation (54; 65), and/or changes in skeletal muscle enzymes that reduce CHO oxidation (134; 175). Indeed, ingestion of a HFD-CHO has previously been shown to decrease CHO oxidation (26; 28; 33; 175) and reduce muscle glycogen utilization (26; 175) compared to the HCD-CHO dietary strategy.

Despite differences in substrate metabolism between the two diets, overall 200-km time-trial performance following the HFD-CHO and HCD-CHO dietary strategies was not significantly different. It is somewhat surprising that we did not show an increase in performance with the HFD-CHO as the exercise duration was sufficient to deplete muscle glycogen (~7 hours), promoting an increase reliance on fat. In addition, the mean overall exercise intensity during the 200-km time-trial (~53% W_{peak}) is associated with a greater reliance on fat (151) that was more likely to be met by increased rates of fat oxidation in response to the HFD-CHO compared to the HCD-CHO. Furthermore, the 200-km time-trial did not include very high intensity sprints that may be compromised by the HFD-CHO strategy as described in Chapter 4. Similarly, Carey et al. (33) also failed to demonstrate a performance benefit during a 1-hour time-trial following a more prolonged (4-hour) constant-load ride in response to a HFD-CHO compared to a HCD-CHO.

Proposed explanations for the inability to demonstrate a significant improvement in overall performance in response to the HFD-CHO strategy, despite increases in fat oxidation and a concomitant 'sparing' of muscle glycogen, include an increase in sympathetic nervous system activation, and an increase in effort perception associated with high fat feeding, as previously described in Chapter 4.

In addition to overall performance, we examined the 3 sections of the time-trial independently as the profile of each section was so different (undulating profile vs. long climb vs. downhill), possibly eliciting different results, as described in Chapter 4. Performance during the first 100-km was remarkably similar between trials (Table 5.6). However, when the energy demands of exercise increased during the climb section, power output was higher, although not significantly following the HFD-CHO compared to the HCD-CHO diet, and improved in 8 of the 9 subjects (Table 5.6). Despite a higher power output on the climb with the HFD-CHO compared to the HCD-CHO, mean reported RPE on the climb was similar for both treatments (14.4 ± 1.4 vs. 14.7 ± 1.3 for HFD-CHO and HCD-CHO diets, respectively) suggesting reduced effort perception for a given power output. This is in direct contrast to the results of 1-km sprints in Chapter 4, where a higher effort perception for a lower power output, in response to the HFD-CHO compared to the HCD-CHO was reported.

Despite the inability to demonstrate an overall performance effect, the present study demonstrated an individual variability in performance with 5 of the 9 subjects improving 200-km time-trial performance following the HFD-CHO

compared to the HCD-CHO diet. Carey et al. (33) also demonstrated an individual variability in performance with 5 of the 7 subjects improving performance in response to the HFD-CHO compared to the HCD-CHO. We proposed that the difference in performance in response to a HFD-CHO compared to a HCD-CHO could relate to the subjects RER phenotype as described in Chapter 3. Indeed, when we divided the subjects into a high-RER and low-RER phenotype, total fat oxidation during the 200-km time-trial tended to be higher in the low-RER compared to the high-RER group in response to a HFD-CHO vs. the HCD-CHO ($p=0.084$, Figure 5.14), demonstrating an increase ability to utilize fat in the low-RER group.

In Chapter 3, we demonstrated that RER phenotypes persisted during exercise despite 3 days of CHO-loading. Similarly, in the present study we demonstrated that exercising RER during the constant-load session in the high-RER group was higher compared to the low-RER group after 3 and 5 days of high CHO intake compared to high-fat intake ($p<0.05$ diet x group). We were surprised that there were no differences in RER between the high and low-RER groups during the exercise trial on day 8 in response to the HCD-CHO (Figure 5.12). Perhaps 7 days of high CHO intake compared to only 3 to 4 days of high CHO intake (Chapter 3) is long enough to 'reset' the RER phenotype. In contrast, 6 days of high-fat intake followed by 1-day of CHO-loading decreased exercising RER (Figure 5.12) and increased fat oxidation during the 200-km time-trial to a greater degree in the low-RER group compared to the high-RER group ($p<0.05$, Figure 5.14) despite the fact that both groups adapted similarly to the HFD (Figure 5.10). This data suggest that phenotypes not only persist during

exercise following short-term high CHO ingestion, but also after 6-days of fat-adaptation following 1 day of CHO-loading.

Indeed, the only distinguishing characteristic between the high-RER and low-RER groups were their habitual dietary intakes. The low-RER group consumed a higher fat diet compared to the high-RER group and did not alter their intake prior to the familiarization ride for which the subjects were instructed to prepare as they would normally do prior to an ultra-endurance race. In contrast the high-RER increased their CHO intake in preparation for the familiarization trial. Goedecke et al. (66) found that one of the most significant determinants for a fasting resting and exercising RER was dietary fat intake. As we did not take muscle biopsies during this trial, we were not able to determine if the 6-day dietary interventions changed the enzyme profile or glycogen content of the muscle, which have been previously shown to be associated with the RER phenotype.

Despite differences in fuel utilization in response to the HFD-CHO, overall 200-km time-trial performance was not significantly different between the RER groups. However, the low-RER group completed the time-trial ~6 minutes faster following the HFD-CHO compared to the HCD-CHO. In contrast, the high-RER group completed the time-trial ~10.5 minutes faster following the HCD-CHO compared to the HFD-CHO. In addition, climb performance was significantly higher ($p < 0.05$) in the low-RER group following the HFD-CHO compared to the HCD-CHO (Figure 5.12). The increased ability in the low-RER group to climb harder following the HFD-CHO compared to the HCD-CHO was also reflected

by a significantly higher heart rate ($p < 0.05$) on the climb following the HFD-CHO. In contrast, climb performance was 2 minutes slower in the high-RER group following the HFD-CHO. The climb section occurred ~3.5-4.5 hours into the ride, when muscle glycogen stores were presumably low (5). Although the HFD-CHO dietary strategy 'spared' muscle glycogen, we postulate that the increased performance on the climb with the HFD-CHO strategy, as demonstrated in the low-RER group might rather be due to an increased ability to utilize fat in order to meet the increased energy demands of maintaining power output whilst working against gravity.

Although the HFD-CHO strategy has been shown to compromise high-intensity sprint performance ($>90\% W_{peak}$), we propose that this strategy might be beneficial for ultra-endurance events, especially for athletes with a low-RER phenotype where the advantage obtained during the moderate intensity periods and during long climbs where an increase ability to oxidize fat is required to meet the high energy requirements, supersedes the decrement in performance during short high-intensity sprint sections (e.g. during a short breakaway, uphill surge and sprint to the finish line). The low-RER group completed the climb section 6 minutes 15 seconds faster following the HFD-CHO compared to the HCD-CHO diet, which might very well compensate for a few seconds lost during brief high intensity spurts elsewhere during the trial. In 'real race' situations, performances of elite athletes are separated by small margins and minute and second differences can distinguish between first and second place. In fact, the time difference between the first and second team during the actual 200-km Double Century cycle race in 2006 was 4 minutes and 44 seconds.

In conclusion, ingestion of a HFD for 6 days, followed by 1 day of CHO-loading, increased fat oxidation during ultra-endurance exercise without significantly affecting overall 200-km performance. Despite 6 days of fat-adaptation followed by 1 day of CHO-loading, the RER phenotypes persisted as demonstrated by a lower RER and higher rates of total fat oxidation in the low-RER compared to the high-RER group during the 200-km time-trial. Despite differences in fuel utilization, overall performance was not significantly different between the RER groups, however, the low-RER group tended to perform better on the HFD-CHO compared to the HCD-CHO, whereas the high-RER group tended to perform better following the HCD-CHO compared to the HFD-CHO. In addition, the preliminary evidence suggests that the HFD-CHO strategy might improve performance when glycogen levels are presumably low and the energy demands of exercise are increased (i.e. during climbs), especially in athletes with a low-RER phenotype that are able to utilize more fat during exercise. However, the sample size in the present study was very small and the data was presented as preliminary data and should therefore be interpreted with caution. Further research is required to explore the effect of a HFD-CHO compared to a HFD-CHO on ultra-endurance events in athletes with different RER phenotypes.

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CHAPTER 6

Summary and Conclusions

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Carbohydrate-loading (CHO-loading) is generally recommended prior to prolonged (>90 minutes) exercise to maximize muscle glycogen stores and enhance endurance performance. However, the body's endogenous CHO stores are limited, therefore a dietary strategy that would not only increase CHO availability but also 'spare' muscle glycogen during exercise may be more beneficial during ultra-endurance exercise. Indeed, 5-6 days of fat-loading followed by 1 day of CHO-loading has been shown to increase fat oxidation and 'spare' muscle glycogen during prolonged exercise. However, the effectiveness of a high fat diet followed by CHO-loading on exercise performance has not been tested in self-paced endurance and ultra-endurance events.

There is limited data on the habitual nutritional intakes in cyclists prior to ultra-endurance events. We demonstrated that some cyclists competing in endurance events habitually select diets which are different in macronutrient content than those diets typically recommended for endurance events. Moreover, not all athletes respond to these typical dietary recommendations, such as CHO-loading, in the same manner. Indeed, when athletes were categorized according to their fasting, resting respiratory exchange ratio (RER) into "high" and "low" RER groups, rates of CHO oxidation were higher in the high RER group, compared to the low RER group, during prolonged (3 hours) moderate intensity ($55\% W_{\text{peak}}$) exercise in the fasted state (64) and even when CHO was ingested prior to and during exercise (45). However, the changes in substrate utilization during exercise in response to CHO-loading and other pre-event nutritional strategies in athletes with a low and high RER phenotype have not been examined.

Therefore, the aims of this thesis were: (1) to examine the habitual nutritional intakes of sub-elite male cyclists before and during an ultra-endurance event; (2) to investigate the effects of different dietary strategies aimed at increasing CHO availability and 'sparing' muscle glycogen (e.g. CHO-loading and fat-adaptation followed by CHO-loading), on substrate utilization and exercise performance during simulated endurance (>90 minutes) and ultra-endurance (>4-5 hours) exercise; and (3) to investigate the individual responsiveness of athletes with different RER phenotypes to these dietary strategies.

In the first study of this thesis, a field study was conducted to characterize the habitual pre-race nutritional intake and the nutrition practices while racing, in a sample of sub-elite male cyclists competing in a 210-km 1-day ultra-endurance cycle race. The majority of cyclists (62%) reported the ingestion of a moderate CHO diet (4-6 g CHO/kg body mass) during the 3-day period prior to the race. Although a CHO-loading diet (7-10 g CHO/kg) is typically recommended prior to endurance exercise, only 57% of cyclists indicated that they CHO-loaded prior to the 210-km cycle race. Furthermore, less than 25% of these cyclists achieved the recommended CHO intake over the 3-day period prior to the race, demonstrating a discrepancy between perceived and actual intakes of carbohydrates. Although mean reported CHO intake varied between the cyclists and ranged from 3.7-11.3 g/kg over the 3-day period prior to the race, 210-km race performance was not correlated to pre-race CHO intake after covarying for training status. It seems that a CHO-loading diet does not necessarily provide an additional performance benefit during an ultra-endurance cycle race

compared to a moderate CHO diet which was representative of the habitual pre-race diet of the majority of cyclists in this study.

Indeed, in Chapter 3, it was demonstrated that the ingestion of a high CHO diet (HCD) vs. a moderate CHO diet (MCD) significantly increased pre-exercise muscle glycogen levels ($p < 0.05$), but only tended ($p = 0.096$) to improve short time-trial (~16 min) performance following 2.5-hours of moderate intensity, constant-load exercise in well-trained male cyclists. In Chapter 3 we also examined, for the first time, the responsiveness of athletes with a high or low RER phenotype to both high and moderate CHO diets. The HCD resulted in higher total rates of CHO oxidation during moderate intensity exercise in the high-RER group compared to the low-RER group ($p < 0.05$). However, despite differences in substrate utilization, time-trial performances were not different between the two RER groups in response to the two diets. The novel findings in this chapter suggest that RER phenotypes are maintained during prolonged exercise despite CHO-loading. Further studies are required to explore the responsiveness of these RER phenotypes to alternative nutritional strategies.

Chapter 4 examined the potential of an alternative dietary strategy that not only increases CHO availability but also 'spares' muscle glycogen during exercise. It was hypothesized that 6 days of high fat intake followed by 1 day of CHO-loading would sufficiently spare endogenous glycogen stores, such that performance during a 100-km time-trial, interspersed with alternating 1-km and 4-km sprints would be enhanced. The effect of this dietary strategy has not previously been tested in a 'real life' race situation. The HFD-CHO increased fat

oxidation during rest and exercise, but in contrast to the working hypotheses, overall 100-km time-trial performance was not different between the HFD-CHO compared to the HCD-CHO. However, high intensity 1-km sprint performance was compromised following the HFD-CHO compared to the HCD-CHO ($p < 0.05$), possibly due to an increase in sympathetic activation and effort perception associated with high fat intake, and an inability to oxidize available glycogen. As a result, the final study tested the hypothesis that a HFD-CHO compared to a HCD-CHO may enhance 200-km ultra-endurance time-trial performance that is typically undertaken at sub-maximal exercise intensities where very high rates of carbohydrate oxidation are not necessarily required, and where high rates of fat oxidation and/or muscle glycogen 'sparing' may also be beneficial to performance. Similar to the findings in Chapter 4, the HFD-CHO increased fat oxidation during rest and exercise and 'spared' muscle glycogen, but without affecting overall 200-km time-trial performance. The profile of the 200-km time-trial replicated the 'double century' cycle race which included flats sections, climbs and downhill sections. The majority of cyclists (89%) improved climb performance on the HFD-CHO compared to the HCD-CHO. Furthermore, there was an individual variability in response, with 5 of the 9 subjects improving performance on the HFD-CHO compared to the HCD-CHO.

The final study also provided the first preliminary data on the responsiveness of athletes with high or low RER phenotypes to a HFD-CHO compared to a HCD-CHO dietary strategy. The HFD-CHO tended to increase total fat oxidation rates during the 200-km time-trial in the low-RER compared to the high-RER group ($p = 0.084$). In contrast, there were no differences in substrate utilization in

response to the HCD-CHO between the 2 RER groups. Preliminary data from this study again suggested that RER phenotypes persisted during exercise in response to the HFD-CHO. However, this was not the case for the HCD-CHO where there were no differences in substrate oxidation between the RER phenotypes. Perhaps 7 days of high CHO intake (compared to only 3 days, Chapter 3) is long enough to 'reset' the RER phenotype during exercise. Despite differences in substrate utilization during the 200-km time-trial between the high and low RER groups in response to the HFD-CHO, overall 200-km time-trial performance was not different. As this was only a preliminary investigation due to the small sample size and logistical challenges involved (n=4 and 5 for the low and high RER groups, respectively) a significant performance effect was not anticipated. However, it is of interest to note that the low-RER group completed the 200-km time-trial ~6 minutes faster following the HFD-CHO and significantly improved climb performance following the HFD-CHO compared to the HCD-CHO ($p<0.05$). In contrast, the high-RER group completed the 200-km time-trial ~10.5 minutes faster following the HCD-CHO compared to the HFD-CHO.

There are a few limitations and strengths that warrant mentioning. As with all studies of this nature, a small sample size is a major limitation, especially when exploring the RER phenotypes in Chapter 5 where the sample size was only 4 and 5 for the low and high RER groups, respectively. However, the majority of studies in the literature that explore the effect of a fat-adaptation, CHO-loading dietary strategy on exercise performance reported similarly limited sample sizes between 5-9 subjects (26; 28; 33; 90). Due to time constraints and the practical

difficulties of recruiting subjects and conducting dietary intervention studies of this nature, the sample size is often small. However, race performances of elite athletes are separated by small margins which are often indiscernible and not possible to detect with null-hypothesis testing, even with a bigger sample size.

A further limitation of this thesis was quantifying the habitual *training* and *pre-race* dietary intakes of the subjects (using household measures instead of food scales), with the aim of characterizing the habitual pre-race nutritional intakes, devising experimental diets that match the energy content of habitual training diets, and making comparisons between the habitual training and pre-race diets in the high and low RER groups. A recent review from Burke et al. (27) showed that most surveys in athletic populations used a 3- to 4-day food diary with the quantification of intake described by household measures. Household measures were used for practical reasons and, in part, due to limited facilities (requirement of ~50 food scales in Chapter 2). However, all the diets were analyzed by the same registered dietician using the same dietary program.

A strength of this thesis was the strict dietary and training control in Chapters 3 to 5. The dietician who analyzed the diets, also devised the experimental diets and personally prepared and packaged the food. Furthermore, all the experimental diets were blinded, and although the subjects could distinguish that the high fat and high CHO diets were different, they were unaware of the dietary composition, thereby minimizing the placebo effect. Moreover, the experimental diets were prescribed in a randomized cross-over controlled fashion and training during the dietary periods was controlled. Subjects

completed supervised, structured training sessions in the laboratory during the 8-day dietary periods in Chapters 4 and 5 and recorded a training diary during the 3-day dietary period in Chapter 3. Therefore any difference in performance could not be attributed to differences in training or energy expenditure in the days prior to the experimental trial. Another strength of this thesis, was the simulation of 'real-life' race situations to explore differences between the high fat and high CHO dietary strategies. The 100-km time-trial that was employed in Chapter 4 is a standardized laboratory based performance protocol [between-test CV = 0.93 (95%CI 0.79 to 0.89); within-subject CV = 1.7% (95%CI 1.1 to 2.5%) (160)]. Although the 200-km time-trial profile in Chapter 5 is not standardized, the profile simulated the 210-km "double century" race in Chapter 2. The race real race profile was recorded with a GPS and downloaded onto an indoor cycle ergometer. Differences in metabolic response to the different sections could therefore be explored.

Direct measurement of differences in muscle glycogen utilization and enzyme activities in response to the different dietary interventions, especially in the two phenotype groups could have provided valuable information on how the different RER groups adapted and responded to different dietary strategies. However, inclusion of muscle biopsies in the trials would have increased the already high subject burden, compromising subject recruitment and retention.

Finally, in Chapter 5 there was no pre-screening of the subjects to select those with distinct low and high RER phenotypes as in Chapter 3. However, this was

not possible given the difficulty in recruiting subjects for a study of this extreme nature.

In summary, this thesis demonstrated that the majority of sub-elite cyclists that participated in an ultra-endurance cycle race ingested a moderate CHO diet and failed to meet recommended pre-race CHO intakes, even the majority of those who attempted to CHO-load prior to an ultra-endurance event. Although a HCD significantly increased pre-exercise muscle glycogen levels compared to a MCD, the HCD only tended to improve short time-trial performance following 2.5-hours of moderate intensity constant-load exercise compared to the MCD. This thesis further demonstrated that 6 days of high fat intake followed by 1 day of CHO-loading, increased fat oxidation during rest and exercise, but did not alter overall simulated 100-km or 200-km time-trial performance. However, the HFD-CHO strategy compromised high intensity 1-km sprint performance during a 100-km time-trial in all subjects, but improved prolonged climb performance during the 200-km time-trial in the majority (89%) of subjects. In addition, there were individual differences in 100-km and 200-km time-trial performance in response to the different dietary strategies, suggesting individuality in response to the dietary strategies. Indeed, this thesis demonstrated for the first time, that cyclists with high or low RER phenotypes responded differently to a high CHO diet compared to a moderate CHO diet and a HFD-CHO. Despite differences in substrate utilization between the two RER groups in response to two dietary strategies, short-duration time-trial performance following 2.5-hours of constant-load exercise and 200-km ultra-endurance time-trial performance were not significantly different between RER groups or diets. However, the preliminary

evidence suggests that the HFD-CHO strategy might be beneficial for athletes with a low-RER phenotype participating in ultra-endurance events where an increase ability to utilize fat may enable the athlete to meet the increased energy demands of exercise (i.e. during the climbs). Further research is required to explore the efficacy of different dietary interventions on endurance and ultra-endurance exercise performance in athletes with high or low RER phenotypes. These studies will provide scientific evidence for presenting individualized nutritional recommendations for athletes with specific RER phenotypes.

Given the large variability in substrate utilization and performance in athletes to specific dietary recommendations, it seems logical that the prescription of individualized diets to suit the specific metabolic profile of the athlete is warranted. This thesis for the first time provides evidence that the RER phenotype is robust and that athletes with a high and low RER phenotype respond differently to pre-race dietary interventions. Therefore, future research should focus on investigating the efficacy of different dietary interventions on endurance and ultra-endurance exercise performance in athletes with a high or low RER. Further, this research should test the efficacy of these interventions in athletes of different abilities.

CHAPTER 7

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