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Metabolic Flexibility and Endurance Performance

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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all of the sources of information which have been used in the thesis.

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September 2017

FOREWORD

The research topic outlined in this thesis began as a result of my love of science, the human body and personal involvement in endurance sports as an athlete and coach.

After completing my honours degree in sport and exercise I took a year off formal work and study in an attempt to become a professional triathlete. Despite training exorbitant volumes during this time, I struggled with weight maintenance while following what I thought at the time was the ideal endurance athlete diet—high carbohydrate low fat. In an attempt to learn more about metabolism, I self-experimented with a low carbohydrate diet. The results I experienced were extremely positive in the way of performance and weight loss and in line with other anecdotal reports.

In order to learn more about what I, and others, were experiencing with a low carbohydrate diet I decided side-line my athletic career and focus on continuing my education. The studies I have undertaken during my PhD candidacy represent research that I have personally wanted to learn from in a practical sense. While some aspects of the study design are not in line with the traditional or common research practices for carbohydrate intakes, they represent my want to learn directly from what athletes are willing and able to do in the “real world”.

When reading this thesis, it’s important to understand that I took responsibility for the entire body work reported here and did so with limited funding. The study design, participant recruitment, data collection, analysis both statistical and biochemically as well as

interpretation and reporting were completed by me personally and overseen by my supervisors.

I thank you for taking the time read my work and hope you enjoy the story.

William J O'Connor

PhD Candidate

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There are many people that have helped to the completion of this thesis. The few people I would like to acknowledge personally have made huge contributions of personal time and resources.

Firstly, to my Mum and Dad, I would not be the man I am today without the environment you provided me growing up. Challenging my beliefs and allowing me to make mistakes has given me the ideal framework for academia and research.

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Dr Matthew Barnes, your love of early mornings, drawing blood and your proximity of residence made you one the best supervisors a PhD student could ask for. Your willingness to answer questions, give feedback and generally offer an interest in what I was doing made you an extremely helpful and enjoyable supervisor to work with.

To my fellow PhD researchers (A.K.A “The PhD Crew”); Joe Lei, Matt Miller and Emma Crum, I have enjoyed your company and input throughout the highs and lows of PhD life and I wish you all the best for your future careers.

To my partner in crime, Emma Crum, without you this PhD would have been a far longer, harder process.

ABSTRACTS

This thesis examined the sex-specific biochemical, physiological and physical performance responses of highly-trained endurance athletes to chronic moderate and low carbohydrate (CHO) training diets. In addition, a novel exogenous ketone supplement was studied to examine its effects on participants' physiology and performance during the two contrasting diets.

STUDY ONE:

This study was designed to test whether adaptation to a low CHO diet affects physical capacity during prolonged exercise. Thirteen highly-trained endurance athletes (eight males, VO_{2max} = 66.0 ± 9.5 ml/kg/min; five females, VO_{2max} = 50.6 ± 8.4 ml/kg/min) consumed a moderate (>5 g CHO/kg/day) or low (<2 g CHO/kg/day) CHO training diet for four weeks, in a randomised cross-over design. Performance was measured, after a 24 h moderate CHO "loading" regime, through a self-paced time trial to complete a fixed workload, equivalent to five hours at a workload calculated to elicit 55% VO_{2max}. Although time-to-complete was not significantly different between diets, the average absolute (watts) and relative (W/kg) power outputs were significantly better on the low CHO diet (p = 0.03 and 0.02 respectively). Both sexes responded similarly in terms of performance, whilst only women significantly improved body composition when CHO was restricted (p = 0.02). It was concluded that when CHO is restricted during training, trained endurance athletes show improved ultra-endurance performance relative to their body mass.

STUDY TWO:

This study was designed to test the sex specific response to a low CHO diet during fasted endurance exercise. The participants and dietary restrictions were the same as outlined in Study One. Physiological measures were collected before, during and after a two-hour ride at a fixed power output, equivalent to 60 % VO_{2max}. The ride was undertaken after an overnight (>12 hours) fast and completed at three points throughout each dietary intervention (baseline, week two, week four). As expected there were a significant main effect of diet and time on substrate oxidation rates during fasted exercise ($p < 0.05$). The low CHO diet resulted in lower CHO oxidation and higher fat oxidation (FATox) in both sexes throughout the exercise. The degree of 'adaptation' to low CHO intake increased from baseline to week four, with significant interactions between trial and diet ($p < 0.05$). There was a sex specific negative correlation between the rate of CHO oxidation and perceived exertion (RPE) at the end of the fasted exercise ($p = 0.001$). Women consistently had a higher RPE at the end of the exercise ($p = 0.04$). These data show that both men and women can increase their rates of FATox, in a time-dependent manner, when CHO is restricted in the training diet.

STUDY THREE:

This study was designed to examine the differences in the blood metabolome of highly-trained male endurance athletes (VO_{2max} = 66.0 ± 9.5 ml/kg/min) who each underwent two contrasting dietary interventions, in a randomised crossover design as follows: four weeks moderate (>5 g CHO/kg/day) or low (<2 g CHO/kg/day) CHO. Exercise training was controlled during both conditions. Fasting venous blood samples were collected before and after exercise at 60% VO_{2max} and the plasma metabolome was analysed using 700 Hz H^1

nuclear magnetic resonance (NMR) spectroscopy. Unsupervised (PCA) and supervised (PLSA-DA & OPLS-DA) multivariate statistical analysis models failed to statistically separate the sample groups in regards to the dietary intervention. However, both methods of supervised discriminant analysis (PLS-DA and OPLS-DA) could separate groups based on time (i.e. pre–post exercise). The variable influence on projection (VIP) was used to identify the individual metabolites causing the group separation within the discriminant analysis. Metabolites were analysed using two-way ANOVA and paired t-tests, with the only significant difference being the blood glucose response to exercise at the end of each dietary intervention (p = 0.006). In conclusion, neither the resting nor exercising metabolome is significantly influenced by the CHO content of the diet. This indicates that endurance-trained individuals possess the metabolic flexibility to counter changes in dietary CHO availability and maintain a normal circulating metabolic profile.

STUDY FOUR:

The aim of this case study was two-fold: to test the effectiveness of a proposed study, and to explore the validity of reports which have claimed that ingesting a ketone supplement can improve endurance performance. One highly-trained male triathlete (VO_{2max} = 73.0 ml/kg/min) completed four time-to-exhaustion (TTE) cycling bouts, each preceded by two hours of cycling at 60% VO_{2max} (power = 213 W). The exercise bouts were completed in a crossover design as follows: ketogenic diet (<1.5 g CHO/kg/day) and regular (non-ketogenic) sports drink (K), ketogenic diet with ketone-containing drink (K+KS), high CHO diet (>5 g CHO/kg/day) and regular sports drink (CHO), moderate CHO diet and ketone-containing drink (CHO+KS). Ketosis was confirmed with sustained resting blood β –hydroxybutyrate (β-HB) levels of >0.2 mM. Ketone supplementation was associated with better performance

following both dietary interventions, with CHO+KS being better than K+KS (12:54 minutes vs 13:32 minutes, respectively). Ketone supplementation resulted in higher [β-HB] during exercise relative to the sports drink (0.63 & 0.78 mM vs 0.20 & 0.25 mM, respectively). VO_2 and blood lactate did not noticeably differ during the fixed intensity ride, but differed greatly during the TTE, with VO_2 beginning higher on the high CHO diet.

The results from this study show the potential benefits of ingesting a ketone supplement on endurance performance and suggest that the moderate CHO status of the individual may have an additive effect. Based on these results, it was suggested that a full scientific study be carried out to further test the effectiveness of ketone supplementation on endurance performance.

STUDY FIVE:

The aim of this study was to test the effects of ingesting a ketone supplement on endurance performance in two different metabolic states, induced by dietary interventions. Six well-trained male endurance athletes (age: 29 ± 9 yrs, mass: 74.1 ± 7.7 kg, VO_{2max}: 64.1 ± 5.8 ml/kg/min) underwent a randomised, double-blinded, placebo-controlled protocol, consisting of two dietary interventions, completed as a cross-over design. Following each dietary intervention, a performance session was carried out, during which, participants drank either a ketone-containing (KS) or placebo (PLB) drink. Thus, the performance session was carried out a total of six times; habitual diet (BASE1, BASE2), moderate-CHO diet + PLB (PLB+CHO), moderate-CHO diet + KS (KS+CHO), ketogenic diet + PLB (PLB+K), ketogenic diet + KS (KS+K). Physiological measures were taken during each performance

session, which consisted of a 40-minute fixed intensity ride, followed by a self-paced time trial (TT), to complete a fixed workload equivalent to 20 minutes at 75% VO_{2max}.
There were no main effects or interactions between diet and KS on TT performance or body mass. The KS significantly increased the beta-hydroxybutyrate concentration [β-HB] in the blood at rest and during exercise (peak = 1.1 mM) (p = 0.001). The KS caused an attenuated blood lactate response during the TT compared to baseline and PLB. The respiratory exchange ratio (RER) was significantly lower on the ketogenic diet at rest and throughout fixed intensity exercise but did not differ during the TT.

It is concluded that the circulating [β-HB] attained were not high enough to significantly contribute to muscular energy provision via oxidative phosphorylation and that future research into ketone supplements and exercise performance should ensure that a minimum of 2 mM [β-HB] is obtained. Further, the CHO status of the individual can be largely ignored as supplementation appears to be equally effective irrespective of the CHO status.

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LIST OF ABBREVIATIONS

The following abbreviations have been used within this manuscript;

°C: Degrees Celsius
AA: Amino Acid
AcAc: Acetoacetate
ACSM: American College of Sports Medicine
ADP: Adenosine Diphosphate
AMP: Adenosine Monophosphate
ANOVA: Analysis of Variance
ATP: Adenosine Triphosphate
BCAA: Branch Chain Amino Acids
BF%: Body Fat Percentage
BIA: Biological Impedance Analyser
BM: Body Mass
bpm: Beats per Minute
cAMP: Cyclic Adenosine Monophosphate
CHO: Carbohydrate
CHOox: Carbohydrate Oxidation
CNS: Central Nervous System
D_2O: Deuterium Oxide
DFTMP: Difluorotrimethylsilanylphosphonic Acid
DNA: Deoxyribonucleic Acid
DSS: 4,4-Dimethyl-4-silapentane-1-sulfonic acid
ETC: Electron Transport Chain
FATox: Fat Oxidation
FFA: Free Fatty Acids
FRU: Fructose
g/day: Grams per Day
g/min: Gram per Minute
g/mL: Grams per Millilitre
g/mol: Grams per Mole
g: Gram
G6P: Glucose 6-phosphate
GABA: Glutamic Acid
GG: Greenhouse–Geisser
GI: Gastrointestinal
GLU: Glucose
GLUT-4: Glucose Transporter 4
GLUT-5: Glucose Transporter 5

GSH: Glutathione (reduced)
GSSG: Glutathione (oxidised)
H+: Hydrogen Ion
H_2O: Water
MC: Moderate Carbohydrate
LC: Low Carbohydrate
HPLC-MS: High-Performance Liquid Chromatography-Mass Spectrometry
HR: Heart Rate
hr: Hour
HSQC: 2D Heteronuclear Single Quantum Coherence
Hz: Hertz
IMP: Inosine Monophosphate
IMTG: Intramyocellular Triglyceride
kCal/g: Kilocalories per Gram
kCal: Kilocalorie
KE: Ketone Monoester
kg: Kilogram
kHz: Kilohertz
kJ: Kilojoule
KS: Ketone Supplement
Lac: Lactate
LCFA: Long Chain Fatty Acids
LC-MS: Liquid Chromatography-Mass Spectrometry
LV: Latent Variables
MALT: Maltodextrin
MCT: Monocarboxylate Transporters
MeOH: Methanol
mg/Kg: Milligram per Kilogram
Mhz: Megahertz
min: Minute
mL: Millilitres
MS: Mass Spectroscopy
ms: Milliseconds
NADPH: Nicotinamide Adenine Dinucleotide Phosphate
N: Nitrogen
NMR: Nuclear Magnetic Resonance
NOESY: 1D Nuclear Overhauser Effect Spectroscopy
O_2: Oxygen
O_2-•: Superoxide
OOA: Oxaloacetate
OPLS-DA: Orthangonal Partial Least Squares Discriminant Analysis
PC: Principle Component
PCA: Principle Component Analysis

PCr: Phosphocreatine
PDH: Pyruvate Dehydrogenase
PDHa: Pyruvate Dehydrogenase Active
PDHb: Pyruvate Dehydrogenase Inactive
PDHc: Pyruvate Dehydrogenase Complex
Pi: Inorganic Phosphate
PLB: Placebo
PLS-DA: Partial Least Squares Discriminant Analysis
PO_4: Phosphate
POMS: Profile of Mood State
ppm: Parts per Million
PRO: Protein
RER: Respiratory Exchange Ratio
RNA: Ribonucleic Acid
ROS: Reactive Oxygen Species
RPE: Rating of Perceived Exertion
rpm: Revolutions per Minute
RQ: Respiratory Quotient
SD: Standard Deviation
SGLT1: Sodium-Dependent Glucose Cotransporter 1
SpD: Sports Drink
SW: Spectral Width
t: Time
TAG: Triacyl Glyceride
TCA: Tricarboxylic Acid
TCOSY: 2D Total Correlation Spectroscopy
TMD: Total Mood Disturbance
TSP: Trimethylsilylpropanoic Acid
TT: Time Trial
TTE: Time to Exhaustion
VIP: Variable Importance on Projection
VLDL: Very Low Density Lipoprotein
VO_2max: Maximal Oxygen Uptake
W/kg: Watts per Kilogram
W: Watts
Wk: Weeks
β-HB: Beta-Hydroxybutyrate
µL: Microlitres
ϒ-HB: Gamma Hydroxybutyrate

CHAPTER 1. INTRODUCTION

Following the seminal work by Jonas Bergström, Hermansen, Hultman, and Saltin (1967) which conclusively showed that a high carbohydrate (CHO) ingestion can improve physical work capacity, much research has focused on how to further increase the body's ability to store and ingest CHO, in order to improve exercise performance. In comparison, much less effort has focused on finding ways to increase the ability of the skeletal muscles to burn fatty acids during exercise, and subsequently reduce the rate of CHO utilisation. In line with this discrepancy, in the discipline of sport performance nutrition, few studies have examined the relationship between high CHO and low CHO diets, under the context of "metabolic flexibility".

Metabolic flexibility describes the ability of a cell or tissue to regulate fuel selection when energy demands call for a sudden change in metabolic turnover. Metabolic flexibility of skeletal muscle is the hypothesised ideal state for an endurance athlete, enabling both optimal fatty acid and/or CHO oxidation. Additionally, metabolic flexibility is characterised by a reliance on fatty acid oxidation at submaximal exercise intensities, which can allow preservation of muscle and liver glycogen. A low CHO diet is said to be the most effective means of stimulating a favourable adaptation away from a metabolically inflexible system that is constantly reliant on CHO oxidation for low–moderate intensity exercise. However, the downfall of low CHO intake is the depletion of muscle glycogen, which is required for bouts of high intensity exercise in training and racing; this has subsequently lead to a recommendation against this training strategy by sport nutrition practitioners. Yet, with the recent development of supplemental ketones, the door remains ajar in regard to achieving a

scenario whereby fatty acid utilisation is up-regulated to a maximum without compromise of glycogen storage or glycolysis.

This thesis was therefore designed to work through the broader concept of low CHO endurance performance. Firstly, the thesis aimed to understand how a long term (four week), low CHO diet compared to a moderate CHO diet of the same duration, in terms of endurance cycling performance, body composition and physiological responses. Further, the response to the two diets was compared between sexes. To add further clarity to the biochemical responses of participants of both sexes, a novel analytical technique—outlined in the third chapter—was applied to the major study that makes up the first two research chapters. Moving on from broader research into the long-term response of a low CHO diet, the fourth and fifth chapters detail the investigation of a novel exogenous supplement that claims to offer the hypothesised endurance performance benefits of a low CHO diet. The supplement is trialled following both a low and a moderate CHO diet, in order understand its application and interaction with the two major macronutrients of interest—CHO and fat.

CHAPTER 2. REVIEW OF LITERATURE

2. 1. ADAPTATIONS TO ENDURANCE EXERCISE

When an individual begins an exercise program, a number of physiological changes take place to allow for the continuation of exercise and progression of fitness. For many years, it was thought that an increase in endurance exercise capacity was exclusively the result of cardiovascular adaptations which, by increasing the capacity to deliver oxygen (O_2) to the working muscles, were said to reduce the reliance upon anaerobic metabolism and associated early fatigue (Briggs, 1920). We now know that increased endurance is the cumulative effect of many adaptations and increasing the capacity to deliver O_2 is not the sole contributor. In the context of this thesis, particular note should be given to the adaptive changes that the skeletal muscle undergoes, which improve its ability to oxidise fatty acids (Molé, Oscai, & Holloszy, 1971) and ketone bodies (Winder, Baldwin, & Holloszy, 1974) in addition to CHO. Underlying these increases in cellular respiratory capacity are increases in the levels of the enzymes responsible for β-oxidation of long-chain fatty acids (Baldwin, Klinkerfuss, Terjung, Mole, & Holloszy, 1972; Molé et al., 1971), ketone body oxidation (Winder et al., 1974) and those of the tricarboxylic acid (TCA) cycle (J. O. Holloszy, Oscai, Don, & Mole, 1970).

2. 2 BIOCHEMISTRY OF ENDURANCE EXERCISE

2. 2. 1 Overview

As mentioned in the introduction, the most important exercise-related physiological adaptations, in the context of this thesis, are those pertaining to the ATP-generating biochemical pathways of skeletal muscle. It is however, worth acknowledging that the biochemical systems outlined and the actions of the cardiovascular system are not entirely distinct. For example, an increase in the oxidative capacity of the muscle is not effective without an improved capacity to deliver O_2 (Hepple, Hagen, & Krause, 2002). Additionally, increased blood volume may enhance fatty acid release from adipose tissue through enabling a more even distribution of blood flow during intense exercise (Achten & Jeukendrup, 2004).

It is also worth noting that, based on physiological and histochemical characteristics, skeletal muscle fibres have been classified as two (I & Π) or three (I, Πa & Πb) different types (Buchthal & Schmalbruch, 1970). Despite older (colour) and newer (immunohistochemical) methods being available, the majority of recent historical data describing athletes of different sports and levels of fitness use this histochemical classification (D. L. Costill et al., 1976). However, it must be realised that within the same fibre type, in the same muscle, there is a wide spectrum of various enzyme activities specifically relating to substrate utilisation (Lowry et al., 1978). As stated by Henneman's size principle, under external increasing load, motor units are recruited from smallest to largest, and always in the same order. Accordingly, type I (slow-twitch), low-force, fatigue-resistant muscle fibres are activated before type Π (fast-twitch), high-force, less fatigue-resistant muscle fibres (Henneman, Somjen, & Carpenter, 1965). While the majority of individuals are said to have roughly 50% type I and 50% type Π fibres, competitive endurance athletes tend to have a higher percentage of type I

fibres, while sprinters have a higher percentage of type Π fibres (Gollnick, Armstrong, Saubert, Piehl, & Saltin, 1972). In this respect, theories applied to the biochemical adaptation of skeletal muscle to a particular nutritional intervention may be applicable for one exercise-related muscle recruitment pattern/intensity or genetic phenotype while being completely out of context in another, i.e. low CHO intakes for sprinters vs. marathon runners.

2. 2. 2 Regulation

The energy yielding cellular biochemical pathways of mammalian skeletal muscle are supported by three main substrates, which are differentiated as the macronutrients—fat, CHO and protein. The dominant biochemical pathways used within the myocyte to convert the potential energy of these macronutrients into mechanical energy for muscular work are glycolysis, β-oxidation, and the tricarboxylic acid (TCA) cycle, which provide reduced co-enzymes (NADH + $FADH_2$) for oxidative phosphorylation within the electron transport chain (ETC) and thus oxidatively generate ATP. Glycolysis and the TCA cycle also provide small amounts of ATP internally (substrate level phosphorylation) (J O Holloszy & Booth, 1976). Sub-pathways exist outside of the skeletal muscle—in the liver and kidneys—ensuring a consistent supply of one or more macronutrients to the myocytes (Figure 2-1). Each of the major pathways mentioned, operate in synergy in healthy people, and are fundamentally controlled by the requirement for ATP at the cellular level. The rates of ATP production and degradation during exercise are influenced by (1) intrinsic exercise-related factors, including intensity, duration, and the relationship between the two, and (2) metabolic factors including, enzymatic capacities, rates of substrate delivery, and the activity of substrate cycles (Spriet & Watt, 2003). Manipulating whole-body substrate availability can alter rates of substrate flux through the various control points of these pathways, making it possible to induce adaptations

favouring metabolism of one or another nutrient, the effect of which can then influence endurance exercise capacity (J. S. Volek, Noakes, & Phinney, 2015). Outlined within the following chapters are the specifics of the macronutrient pathways, products, reactants and control factors.

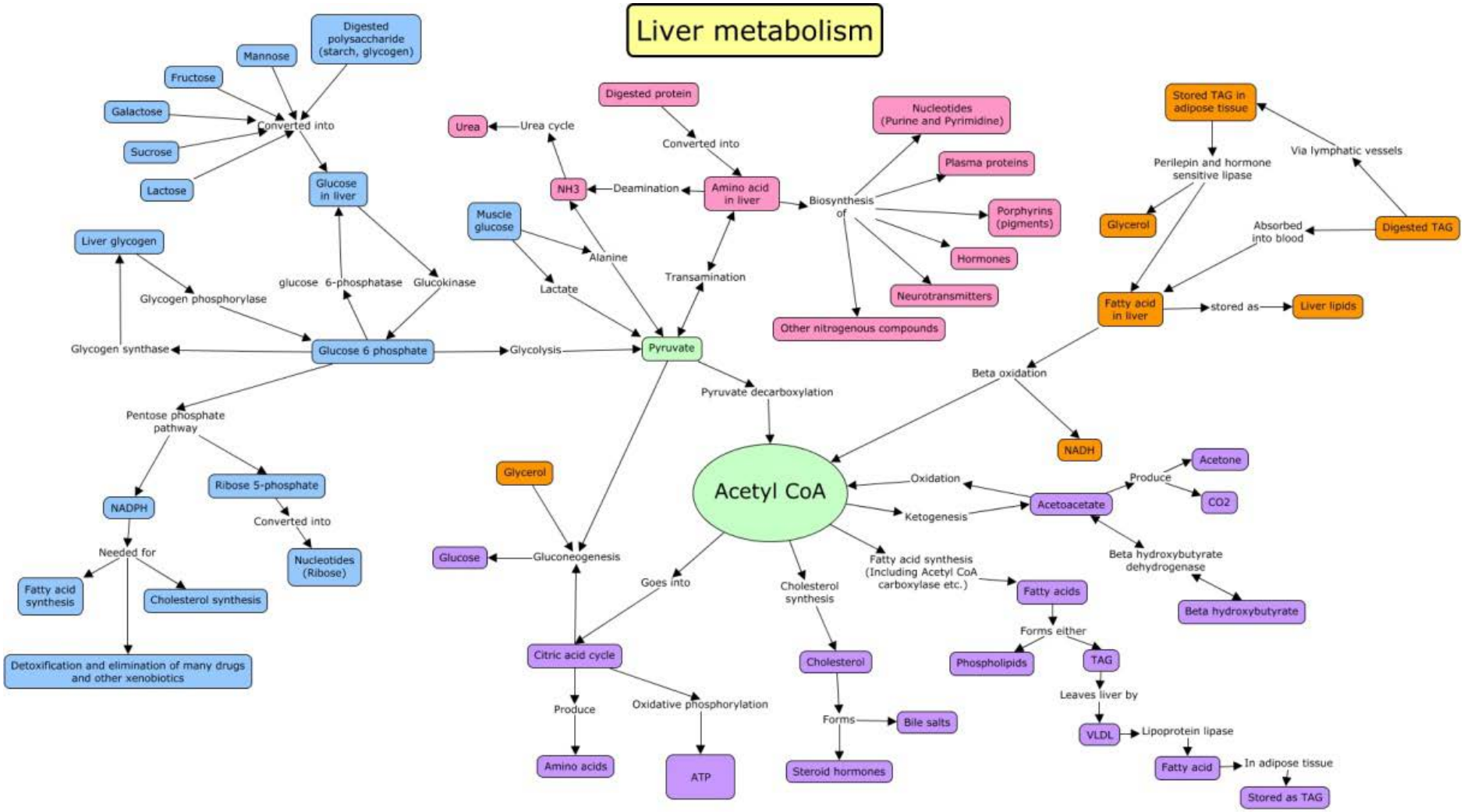


Figure 2-1. Comprehensive overview of the metabolic pathways of the liver (Cañas et al., 2004).

2. 3. CARBOHYDRATE METABOLISM

At the whole-body level and in the post-absorptive state, CHO oxidation (from plasma glucose and muscle glycogen) accounts for 10–15% of the total energy utilised during low intensity aerobic exercise (<40%VO_{2max}). This increases progressively to ~70–80% during exercise of ~85% VO_{2max}, eventually accounting for almost all of the energy consumption at exercise intensities of >100% VO_{2max} (Romijn et al., 1993). The extent of plasma glucose muscle uptake during exercise is dependent upon the availability of glucose (blood flow and blood glucose concentration) and the permeability of the muscle membrane to glucose (activation of sarcolemmal glucose transporters). The latter is heavily influenced by both the process of contraction itself and the concomitant levels of circulating insulin. At a tissue level, the rate of muscle glycogen utilisation is positively related to contractile intensity and duration, and as such, low muscle glycogen concentrations are associated with impaired endurance exercise performance (Hermansen, Hultman, & Saltin, 1967). The relationship between muscle glycogen and endurance performance has resulted in the widespread practice of using high CHO diet regimens to increase pre-exercise glycogen levels (CHO loading) (Academy of Nutrition and Dietetics, 2016). However, this association has been extrapolated, to the point that a high muscle glycogen concentration has erroneously become a surrogate for predicting both exercise performance (Hawley, Schabort, Noakes, & Dennis, 1997), and the extent of recovery from exhaustive exercise (Hawley, Burke, Phillips, & Spriet, 2011).

2. 3. 1 At Rest

At rest, in the post-absorptive state, CHO metabolism contributes very little to the overall resting metabolic rate (RMR) (Andres, Cader, & Zierler, 1956). However, CHO intake, as a

single load or with a mixed meal, stimulates CHO oxidation and promotes glucose storage as glycogen and triglyceride (Godsland, Crook, Walton, Wynn, & Oliver, 1992). CHO storage primarily occurs through the actions of insulin, which promotes the uptake of glucose in muscle and free fatty acids (FFA), and triacyl glycerol (TAG) in adipose tissue. Insulin, at the same time, inhibits both the release of glucose and fatty acids from liver stores (Godsland et al., 1992) and adipose tissue, subsequently reducing the rate of whole-body fatty acid oxidation. In broad terms, these effects become more prominent with increasing CHO volumes and greater CHO (i.e., monomer vs. polymer); the combination of volume and type is often represented by glycaemic load (Salmerón et al., 1997).

The human brain is, under normal CHO status, reliant on circulating glucose, as it lacks the ability to store substrate (Kety, 1957). It thus requires a continuous supply of glucose from the blood, adequate blood flow and a glucose concentration of at least 4 mM at all times. At rest, the brain consumes ~110–145 g of glucose daily, which corresponds to an energy input of ~420 kcal (1760 kJ), accounting for ~60% of the utilisation of glucose by the whole body in the resting state (Owen et al., 1967). In the case of prolonged starvation or low CHO intake, the brain can partly offset its reliance upon glucose through the uptake and oxidation of ketones produced in the liver and kidney or supplied exogenously (George F. Cahill, 2006; Hashim & VanItallie, 2014) (see 2.8 Ketosis).

The liver is an essential support for blood glucose concentration, enabling continual supply to the brain, muscle, and other peripheral organs. In the CHO-fed state, the liver removes up to two-thirds of glucose from the blood and converts the majority of it into glycogen with liver specific enzymes glucokinase and glycogen synthase Π. Excess glucose is metabolised to acetyl CoA to form fatty acids, cholesterol, and bile salts (Figure 2-1). Unlike skeletal muscle,

in the post absorptive or fasted state, the liver can release glucose into the blood by breaking down glycogen or synthesising it from non-glycolytic precursors—lactate and alanine released into the circulation from muscle, glycerol from adipose tissue lipolysis, or glucogenic amino acids from the diet (George F. Cahill, 2006). The kidney is also capable of gluconeogensis, but this is generally in the latter stages of starvation (George F. Cahill, 2006).

2. 3. 2 Exercise Intensity

Glycogen storage allows the muscle to be much less reliant on the hormonal control of hepatic glucose production for which glucagon and catecholamines (epinephrine, dopamine, and norepinephrine) are required for stimulation, and insulin for suppression. That the muscle is not completely reliant on an external supply of glucose, is important for the brain during exercise, as glycolysis provides 50–100% of the energy required for muscular contraction in the post-absorptive state, and if this were to be taken from the circulating pool of glucose, hypoglycaemia would quickly occur. The degree of reliance on glycolysis is dependent on the muscle fibre type recruited which, as described by the size principle, varies according to the intrinsic factors mentioned at beginning of this section (Lowry et al., 1978).

Table 2-1. Fuel reserves in a typical 70 kg man with 15% body fat.

	Available energy in kCal (kJ)		
Organ	**Glucose or glycogen**	**Triacylglycerols**	**Mobilisable proteins**
Blood	60 (250)	45 (200)	0 (0)
Liver	400 (1700)	450 (2000)	400 (1700)
Brain	8 (30)	0 (0)	0 (0)
Muscle	1,200 (5000)	450 (2000)	24,000 (100,000)
Adipose tissue	80 (330)	94,000 (376,000)	40 (170)

Source: (G. F. Cahill, 1976)

2. 3. 3 Prolonged Exercise

In the context of this thesis, prolonged exercise is defined as exercise lasting over two hours in duration, such as marathon running (42.2 km) or century bike racing (100 miles). Self-paced exercise of this duration, in thermoneutral conditions, is considered to be limited, in a broad sense, by the metabolic capacity of the muscle (i.e., glycogen availability), heat storage capacity, cardio-respiratory capacity, and the capacity of the central nervous system (CNS) (Tucker & Noakes, 2009). However, it is important to note that during self-paced exercise, even when one or more of these limiting factors have been exceeded (i.e., muscle glycogen depletion), exercise can continue, albeit at a much-reduced rate. As an example, if a cyclist completing a self-paced 100 km ride exceeded the capacity of their 'system' (encompassing

physiological, biochemical, neurological and biomechanical systems) during the initial stages of the ride, the time to complete the 100 km would be longer than if they followed an even pacing strategy (i.e., had started at an intensity below the limit of their 'system' and as such, avoided slowing down).

Understanding the ways that skeletal muscle can compensate for the declining muscle glycogen availability associated with prolonged exercise may help in designing strategies to attenuate this performance-compromising decline. When muscle glycogen begins to decline, a large portion of hepatic glycogen is mobilised and an increasing fraction of the splanchnic glucose output is derived from gluconeogenesis (Webster et al., 2016). Although glucose and insulin delivery to the contracting skeletal muscle are increased during exercise, as a consequence of the large increase in muscle blood flow, it is local factors within the muscle, such as increased activation of glucose membrane transporters (GLUT4) (Kristiansen, Hargreaves, & Richter, 1997) and activation/inhibition of the rate limiting glycolytic and oxidative enzymes, that play the major role in glycolytic regulation (Katz, Sahlin, & Broberg, 1991; Zinker, Lacy, Bracy, & Wasserman, 1993).

2. 3. 3. 1 Carbohydrate Supplementation

"Beyond an individual athlete's genetic trainability and optimised training program, perhaps the largest single determinant of ensuring optimal performance during prolonged endurance events is through the intake of CHO and fluid" (Stellingwerff & Cox, 2014).

As early as 1932, Dill, *et. al.* reported that feeding a laboratory dog 20 grams (g) of CHO every hour during prolonged exercise maintained blood glucose levels and enabled the animal

to run at a sustained exercise intensity for at least 13 hours without fatiguing. Through the reintroduction of the muscle biopsy procedure in the 1960s, the emphasis shifted from blood glucose towards muscle glycogen as the major source of CHO during exercise (Bergstrom & Hultman, 1966). Furthermore, glucose uptake by skeletal muscle during exercise was reported to be minimal (J. Bergström & Hultman, 1967), and the intravenous infusion of glucose at high rates was found to have little effect on the rate of muscle glycogenolysis (Hultman, 1967). It therefore became established that muscle glycogen was the primary CHO source during exercise, and that blood-borne glucose contributed little to the CHO needs of muscle during moderate or intense exercise. Despite these earlier views, however, substantial evidence began to accumulate indicating that blood glucose was in fact an important source of CHO energy during exercise (A. R. Coggan & Coyle, 1987, 1989; Andrew. R. Coggan & Coyle, 1991). The seminal work of Coggan and Coyle in the late 1980s–early 90s (A. R. Coggan & Coyle, 1987, 1989; Andrew. R. Coggan & Coyle, 1991; A. R. Coggan, Kohrt, Spina, Bier, & Holloszy, 1990) conclusively showed that blood glucose does become the dominant CHO energy source during prolonged exercise, and that, as originally suggested by Dill *et al*. (1932), CHO ingestion can improve endurance performance, primarily by maintaining the availability and oxidation of CHO.

Subsequent to the initial findings suggesting the importance of CHO ingestion on prolonged exercise performance, two major mechanisms have emerged: (1) mental/cognitive stimulation of the central nervous system by oral exposure of CHO during shorter exercise durations (e.g.,<1 hr) when muscle glycogen stores are not limiting; and (2) direct contribution of CHO energy and exogenous CHO oxidation during muscle glycogen limiting exercise situations (e.g., >2 hr) (Stellingwerff & Cox, 2014).

In 2004, research was published showing that a CHO mouthwash (swirling 25 mL of a 6% CHO beverage around in the mouth for 10 s, every 7.5 minutes) significantly improved time trial (TT) performance by 3% (J. M. Carter, Jeukendrup, & Jones, 2004). Taken together with several other performance studies (Stellingwerff & Cox, 2014), the data strongly suggests that in short-duration exercise situations (<1 hr), CHO absorption and oxidation appear completely irrelevant to enhancing performance (J. M. Carter, Jeukendrup, Mann, & Jones, 2004), so long as there is adequate oral exposure to CHO.

It is scientifically accepted that the intake of CHO can significantly improve prolonged endurance capacity and performance (Temesi, Johnson, Raymond, Burdon, & O'Connor, 2011). Collective data has clearly shown a positive dose–response between increasing CHO intake rates and subsequent CHOox (J. W. Smith et al., 2010) and performance (J. W. Smith et al., 2013; Watson, Shirreffs, & Maughan, 2012), with an upper limit of 90 g CHO/hr during moderate intensity exercise. It is thought that the rate limiting step to exogenous CHOox is at the level of the GI tract due to the intestinal CHO transport mechanisms—specifically the activity of the sodium-dependent glucose cotransporter 1 (SGLT1) transporter for glucose and the GLUT-5 transporter for fructose (A. Jeukendrup & Jentjens, 2000).

The GI-related limitations of CHO ingestion have been partially reduced with the introduction of multi-transportable CHO; glucose:fructose (GLU:FRU) or maltodextrin:fructose (MALT:FRU). Multi-transportable CHOs utilise both the SGLT1 and GLUT5 intestinal transporters and enable high CHO ingestion rates (>60 to 90 g/hr) resulting in 20% to 50% higher CHOox and increasing prolonged endurance performance compared with isocaloric glucose (Currell & Jeukendrup, 2008; O'Brien, Stannard, Clarke, & Rowlands, 2013; Triplett, Doyle, Rupp, & Benardot, 2010).

2. 4. FAT METABOLISM

The amount of energy stored in the form of fat represents 92–98% of a normal weight, healthy individual's endogenously stored energy, while CHO contributes only about 2–8% (Table 2-1). Body fat, referring primarily to TAG, an ester of three fatty acid chains, and the alcohol glycerol, is commonly thought of as being at the bottom of an oxidative hierarchy of fuel selection; its rate of oxidation at the whole-body level is largely governed by the presence or absence of CHO (Acheson et al., 1984; Sidossis, Gastaldelli, Klein, & Wolfe, 1997).

Figure 2-2. Transportation of fatty acids across the inner mitochondrial membrane facilitated by carnitine palmitoyl transferase.(Abo Alrob & Lopaschuk, 2014).

2. 4. 1 At Rest

After an overnight fast, most energy needs at rest, are provided by oxidising fatty acids liberated from adipose tissue TAGs through the action of hormone-sensitive lipase (HSL). Adipose tissue lipolytic activity is regulated by the balance between hormones that stimulate (primarily catecholamines) and inhibit (primarily insulin) HSL (Holm, 2003). Upon stimulation, HSL hydrolyses TAGs to glycerol and three fatty acids, which, when released into the circulation, bind to albumin for transport in the blood. Glycerol can be re-esterified into TAGs or used as a gluconeogenic precursor in the liver. At rest, the amount of fatty acids released from adipose tissue typically exceeds the amount oxidised; the fatty acid rate of appearance into plasma is approximately twice the rate of fatty acid oxidation (Wolfe, Klein, Carraro, & Weber, 1990). Therefore, a large portion of fatty acids liberated by lipolysis of adipose tissue TAGs are re-esterified back into TAGs, principally by the liver.

2. 4. 1. 1 Intramyocellular Triglyceride (IMTG)

Once FFA enters the muscle cell, it can be either oxidised or stored as TAG in lipid droplets known as intramuscular or intramyocellular triglyceride (Newsholme, 1986; van Loon, 2004). Intramyocellular triglyceride (IMTG) resides within the plasma of muscle fibres, between myofibrils, in close proximity to the mitochondria, and thus provides a readily available source of fatty acids for β-oxidation (A. E. Jeukendrup, 2002).

If being metabolised, these IMTG stores must first be broken down into glycerol and FFA (Sherman, Costill, Fink, & Miller, 1981), transported to the surface of the outer mitochondria membrane and subsequently hydrolysed to fatty acyl carnitine (Watt, Heigenhauser, O'Neill,

& Spriet, 2003) (Figure 2-2). The underlying mechanisms of heightened muscular fat oxidation can be attributed to an increase in circulating FFA and/or IMTG levels (McClelland, 2004).

Past research has provided variable opinions regarding the contribution of IMTG towards energy production and endurance performance. Research utilising isotope methods has identified enhanced TAG oxidation (Saris, Schrauwen, Wagenmakers, Westerterp, & van Marken Lichtenbelt, 2000) and human muscle biopsy studies have reported a reduction in IMTG stores post-exercise (Zderic, Davidson, Schenk, Byerley, & Coyle, 2004). These results suggest there is a relevant contribution from IMTG stores, while others have found no such change in IMTG post-exercise (Helge, Wulff, & Kiens, 1998). The variation observed may be a result of methodological and experimental limitations.

2. 4. 2. Exercise Intensity

In the post-absorptive state, the estimated relative contribution of plasma fatty acid and IMTGs to total whole-body fat oxidation during exercise at rest and low, moderate, and high intensities (40%, 55%, and 75% VO_{2max}, respectively) is shown in Figure 2-3.

Figure 2-3. Energy expenditure as a function of exercise intensity (expressed as a percentage of maximal workload capacity). The relative contribution of plasma glucose, muscle glycogen, plasma free fatty acid (FFA), and triacylglycerol (TG) sources (sum of muscle- plus lipoprotein-derived TG) to total energy expenditure are illustrated, as described in the legend. (van Loon, 2004).

During low intensity exercise, it is likely that most of the oxidised fatty acids come from plasma fatty acid released from adipose tissue as a result of a higher [glucagon]:[insulin] and enhanced muscle blood flow (Ahlborg, Felig, Hagenfeldt, Hendler, & Wahren, 1974; Romijn

et al., 1993). Total fat oxidation and IMTG contribution increase to maximal values during sustained moderate intensity exercise (~65% VO_{2max}). Plasma fatty acid uptake is equal to or lesser than the rate of fat oxidation during this period (Kanaley, Cryer, & Jensen, 1993; Martin et al., 1993; Romijn et al., 1993) and after one–two hours of moderate-intensity exercise, IMTG can account for 60–75% of the total amount of fat oxidised (Carlson, Ekelund, & Froberg, 1971; Froberg & Mossfeldt, 1971). After two hours of exercise, the rate of plasma fatty acid uptake becomes greater than the rate of fatty acid oxidation, suggesting that fatty acids released into plasma from adipose tissue can supply all fatty acids used by active muscles (Horowitz & Klein, 2000). Thus, as exercise duration increases, it is likely that the relative contribution of IMTGs to total whole-body fat oxidation declines and the contribution from plasma fatty acid increases, similar to the mechanism of muscle glycogen and blood glucose, described in 'Carbohydrate Metabolism' (Johnson et al., 2003).

As exercise intensity increases beyond that sustainable for prolonged durations (>70% VO_{2max}), the contribution of fatty acid oxidation diminishes. It has been shown that the rate of whole-body fatty acid oxidation, during high intensity exercise, can be significantly increased when the concentration of fatty acids is artificially increased via infusion (Romijn, Coyle, Sidossis, Zhang, & Wolfe, 1995). Yet, despite the large increase in fatty acid oxidation, the rates are still below those reported during moderate intensity exercise (Romijn et al., 1993) (Figure 2-3).

Inside the myocyte, the suppression of fatty acid oxidation during high-intensity exercise may be influenced by increased glycogenolysis, recruitment of fast glycolytic muscle fibres and reduced blood flow to adipose tissue. The high rate of muscle glycogenolysis during high-intensity exercise increases the amount of acetyl–CoA derived from glycogen, increasing

malonyl–CoA concentrations in muscle (Elayan & Winder, 1991) and thus reducing the transport of long chain fatty acids (LCFA) into the mitochondria (Figure 2-7)

2. 4. 2. 1. Crossover Concept

According to the "crossover concept" the pattern of substrate utilisation in an individual fibre at any point in time depends on the crossover between the exercise intensity induced responses (increased CHO utilisation) and the endurance training induced responses (increased fatty acid oxidation) (Brooks & Mercier, 1994). The crossover point is identified as the workload at which energy derived from oxidation of CHO-based fuels predominates over that derived from lipids (Figure 2-4). However, two major issues arise when using the crossover concept to directly represent training status. Firstly, the cross-over is more representative of CHO status than training status. During a prolonged low CHO diet, the crossover point will shift to the right or fail to occur at all; this occurs independent of physical fitness. The same can be said for a highly trained endurance athlete who habitually consumes a diet high in CHO (70–80% energy intake), which may cause a leftward shift in the cross-over point; this is associated with de-training.

Figure 2-4. Diagrammatic representation of the crossover concept.(Brooks & Mercier, 1994)

Secondly, the crossover point does not take into account the absolute energy expenditure required at the same relative intensity. A. R. Coggan (1997) tested the crossover concept between trained and untrained subjects, cycling for 30 minutes, at the same relative intensity (80% VO_{2max}). The overall rate of CHO oxidation during exercise was approximately 25% higher in the trained subjects, as a result of their higher absolute energy expenditure.

2. 5. PROTEIN METABOLISM

Although muscle proteins and constituent amino acids (AAs) can contribute to cellular energy metabolism, their contribution is small, even during periods of prolonged starvation (Felig, Owen, Wahren, & Cahill, 1969). Protein-based energy provision during moderate intensity exercise in the post absorptive state is estimated at 5–15%, while energy-compromised states,

such as glycogen depletion and starvation, demand higher contributions (Horton, Pagliassotti, Hobbs, & Hill, 1998; Wagenmakers et al., 1991).

Skeletal muscle has many mechanisms to limit protein catabolism (Ahlborg et al., 1974). Using data mainly from competitive marathon races, Haralambie and Berg (1976) demonstrated that after ~70 minutes, serum urea increased nearly linearly with exercise duration. Moreover, they also observed a fall in serum amino nitrogen (N), beginning at the same duration of exercise (70 minutes), suggesting that these changes occurred at a time when both liver and muscle glycogen would have been considerably depleted (D. L. Costill, Gollnick, Jansson, Saltin, & Stein, 1973; Vissing, Wallace, & Galbo, 1989). It may be that, in terms of substrate supply to working skeletal muscle, the situation during prolonged exercise is similar to short-term starvation. The metabolic events of starvation appear to have two phases (G. F. Cahill, 1976; Saudek & Felig, 1976); the initial response (1–7 days), consisting of an attempt to maintain hepatic glucose output, largely by increasing gluconeogenesis, and the secondary response (>7 days), which is concerned with minimising protein catabolism.

Support for the existence of a parallel between prolonged exercise and starvation can be found in the data published by Gontzea, Sutzesco, and Dumitrache (1962) In their study, 10 to 12 subjects were in positive N balance while sedentary. On the first day of exercise, with a mean energy cost of 4990 kJ/day (1192 kcal), the N balance of all 12 became negative (increased N loss), in spite of an adequate food intake. This N loss continued to increase, reaching a peak at days three and four, and then gradually began to decrease, reaching close to zero N balance by 11 to 12 days of exercise (90% lower than days two to four). Of particular interest is this pattern of initial increased protein catabolism followed by protein conservation, because of its similarity to the time course of events during starvation. This situation of compromising total-

body CHO stores, could therefore lead to increased protein breakdown, in order to supply gluconeogenic precursors to the liver.

It appears that in times of stress, skeletal muscle metabolism possesses the ability to catabolise muscle, in order to provide energy-yielding substrates. However, almost all catabolic stimulating pathways are tightly regulated, with multiple inhibitory mechanisms. It would also appear that type Π muscles degrade before type I (Lemon & Nagle, 1981). This may be due to the lower insulin sensitivity of these muscles to the anti-proteolytic properties of insulin.

2. 6. SEX

There is a common perception that women may be relatively more suited to endurance exercise than men because of a greater ability to use fatty acids as energy substrates during exercise. This notion is supported by several studies that have found some index of fat metabolism to be greater in women than in men at comparable exercise intensities (Horton et al., 1998; M. A. Tarnopolsky et al., 2001). Conversely, several other studies have failed to show a gender difference in substrate metabolism (D. Costill, Fink, Getchell, Ivy, & Witzmann, 1979; Wallace et al., 1980). It has been suggested that the difference in substrate metabolism between men and women during exercise decreases as training status increases (Ruby & Robergs, 1994), but not all data are consistent with this explanation. Changes in body fat percentage result in altered lipolytic sensitivity, which makes individuals with low body fat better able to mobilise fatty acids than might be anticipated otherwise (Romijn, Coyle, Sidossis, Rosenblatt, & Wolfe, 2000). This suggests that trained, lean males may be

able to oxidise fatty acids during exercise at higher rates than females, which may be indicative of improved metabolic flexibility.

Another often overlooked contributor to the sex-related metabolic differences is hormonal status, and the influence that menstruation can have on the hormonal profile of women. Throughout ovulatory menstrual cycles, women are exposed to continuously-changing female steroid hormone profiles. Research investigating large fluctuations in female steroid hormones (such as during pregnancy, menopause and hormone administration) has shown that both oestrogen and progesterone cause many physiological effects, including changes in the thermoregulatory, respiratory and renal systems (see review Lebrun (1993)). These secondary effects of oestrogen and progesterone and their interaction may in turn influence exercise performance. The research literature is equivocal concerning the effects of the smaller menstrual cycle hormone fluctuations on exercise performance. Most research suggests that VO_2 , heart rate (HR) and rating of perceived exertion (RPE) responses to submaximal, steady-state exercise are not affected by the menstrual cycle (de Jonge, 2003). However, several studies report a higher cardiovascular strain during moderate exercise in the mid-luteal phase (Dombovy, Bonekat, Williams, & Staats, 1987; Nicklas, Hackney, & Sharp, 1989). Nevertheless, according to a review by de Jong (2003), time to exhaustion at submaximal exercise intensities shows no change over the menstrual cycle.

2. 7. METABOLIC FLEXIBILITY

It is Kelly et al. 1999 who are given the credit for coining the term “metabolic flexibility”. They describe how leg muscle of lean, healthy individuals can aptly switch from predominant fatty acid oxidation and low CHO use in the fasted state, to suppression of fatty acid oxidation and elevation in glucose oxidation with insulin stimulation. This group also demonstrated how this switch is delayed or compromised in individuals with lifestyle-related metabolic disorders, such as obesity and type Π diabetes. The cause of this latter “inflexibility” in fuel selection to changes in substrate availability has since been extensively studied, and broadly revolves around a state of insulin resistance to glucose sensitive tissues.

The most important predictor of metabolic inflexibility is physical inactivity, which down-regulates recruitment of GLUT4, plasma catecholamine release and insulin sensitivity (Blanc et al., 1998). These factors are associated with plasma fatty acid clearance, a lower mitochondrial oxidative capacity and increased malonyl–CoA activity in muscle, resulting in the development of hypertriglyceridemia. Since the term ‘metabolic flexibility’ essentially describes the capacity of an individual to alter fuel selection to best suit substrate availability, we can also apply this to other physiological conditions, aside from those associated with obesity and diabetes, where changes in fuel availability occur—examples may include exercise, starvation, macronutrient controlled diets (e.g. low CHO, low fat, high protein), and disease states.

2. 7. 1. Exercise Context

Metabolic flexibility describes what could be considered as the ideal state for endurance athletes. A metabolic flexible cell or tissue possesses the means to protect itself against the three main contributors of CHO-related fatigue: maintenance of euglycemia, glycogen depletion and maintenance of high rates of CHO oxidation. As an example, D. L. Costill, Bowers, Branam, and Sparks (1971) had trained runners complete 10 mile (16.1 km) runs (80% VO_{2max}) on successive days with moderate CHO consumption post-exercise (50% of energy intake). Results showed that athletes were capable of completing the run on the third day at glycogen concentrations lower than the end of the run on the first day. To allow for the continuation of exercise, the rate of whole-body CHO oxidation was reduced as a result of increased circulating FFAs. Therefore, without the capacity to increase fat oxidation to compensate for reduced CHO availability, an athlete would need to reduce their work rate in order to complete their event or training session. Support for this was shown in a low CHO intake study by Rauch, Hawley, Noakes, and Dennis (1998), in which subjects became fatigued before the end of the prolonged cycling test (6 hours at 55% VO_{2max}) because their maximal rates of fatty acid oxidation were unable to compensate for the declining rates of CHO oxidation.

Metabolic flexibility can be attributed to a high degree of insulin sensitivity for glucose disposal (Goodpaster, He, Watkins, & Kelley, 2001) and a high reliance upon fatty acid oxidation by skeletal muscle during physical activity (Romijn et al., 1993). The skeletal muscle of trained endurance athletes is markedly insulin-sensitive and has a high oxidative capacity, despite having elevated lipid content (Goodpaster et al., 2001). Thus, the capacity for fatty acid oxidation is an important mediator of the association between excess muscle

lipid accumulation and insulin resistance. The capacity to oxidise lipid is closely tied to the overall mitochondrial content of the skeletal muscle. Thus, trained individuals can facilitate a high throughput of metabolic substrates, storage of fat, CHO and to a lesser extent protein.

2. 7. 2. Fat Adaptation and Carbohydrate Restoration

As outlined in the previous section *(*Metabolic Flexibility*)*, there is a need for endurance athletes to oxidise fatty acids at high rates, in order to compensate for the reduced glucose supply associated with prolonged exercise. Nutritional strategies to enhance fatty acid metabolism in a hope of improving endurance performance have typically seen equivocal results due, in part, to reduced CHO availability (Louise M Burke et al., 2000).

To circumvent the problem of reduced pre-competition glycogen storage, associated with low CHO intake, ‘‘dietary periodisation’’ was developed. The dietary periodisation model involved fat adaptation followed by CHO restoration, to allow for optimisation of both fat oxidation and pre-event glycogen storage (Louise M Burke et al., 2000)(Figure 2-5).

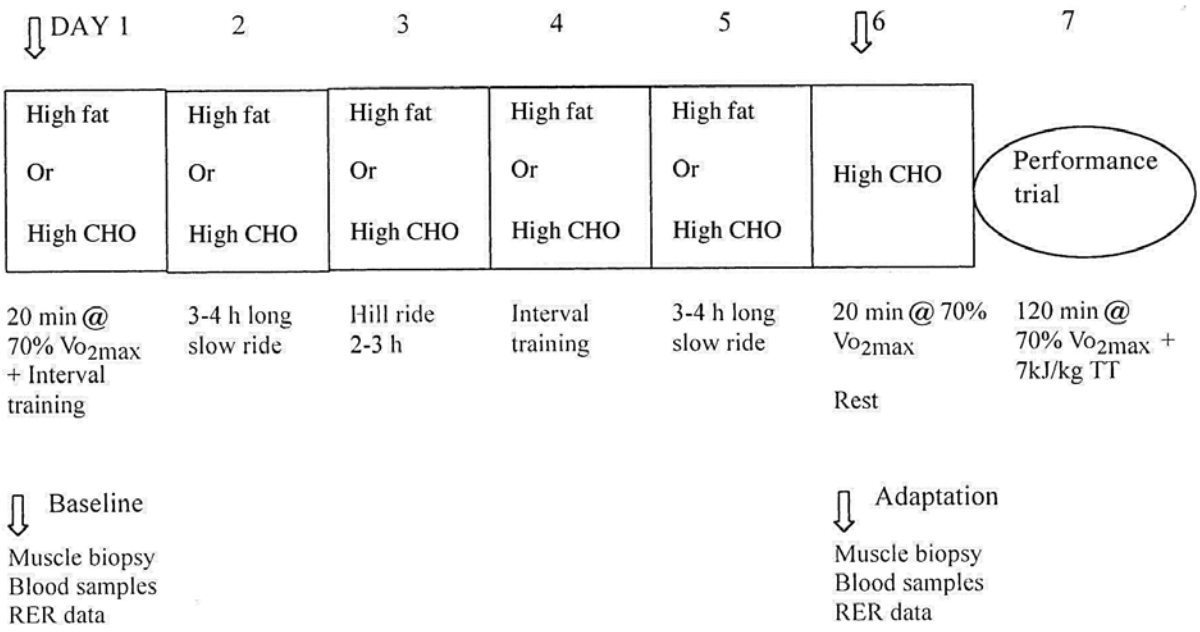


Figure 2-5. Example of carbohydrate depletion and restoration protocol, taken from (Louise M Burke et al., 2000).

In practice, this model has been successful when compared with an isoenergetic CHO diet over the same duration. Both fat adaptation and the combined ‘‘dietary periodisation’’ of fat adaptation–CHO restoration increase whole-body rates of fat oxidation and attenuate the rate of muscle glycogen utilisation during subsequent exercise (Louise M Burke et al., 2000; Carey et al., 2001; Lambert, Speechly, Dennis, & Noakes, 1994; S. D. Phinney, Bistrian, Evans, Gervino, & Blackburn, 1983). An additional benefit of dietary periodisation’’ is that it results in a super-compensation of muscle glycogen, in which starting concentrations are greater than with a standard high CHO intake (Hawley et al., 1997). However, with super-compensation of muscle glycogen comes a reduced capacity to store IMTG (Johnson, Stannard, & Thompson, 2004). At low intensities, where large glycolytic reserves within large motor units are not required, it may indeed be advantageous to minimise glycogen storage, to

enable higher IMTG storage and fatty acid oxidation (Yeo, Carey, Burke, Spriet, & Hawley, 2011).

2. 7. 3. Low CHO Intake and Pyruvate Dehydrogenase Activity

The pyruvate dehydrogenase complex (PDHc) is the primary regulator of whole-body oxidative CHO metabolism and is introduced in 'Carbohydrate Metabolism'. Fat adaptation is said to have profound effects on the regulation of PDH (Stellingwerff et al., 2006). It has been shown that low CHO diets rapidly down-regulate the proportion of the PDH protein in the active form (PDHa), at rest. This is accomplished by rapid up-regulation of the enzyme PDH kinase (PDK), which moves PDH to the inactive form (St. Amand, Spriet, Jones, & Heigenhauser, 2000). It is believed that the reduction in circulating insulin concentration, and the increased FFA levels during the high-fat diet, rapidly induce these changes (Putman et al., 1993). It has been shown that, during exercise, following fat adaptation and CHO restoration, PDH activation is reduced at rest and over a range of exercise intensities (Yeo et al., 2011). Accordingly, CHO oxidation is reduced, and, along with other changes affecting muscle oxidative phosphorylation, glycogenolysis is suppressed and glycogen subsequently spared.

Although, the suppression of flux through PDH spares glycogen, which, as discussed, is a contributing factor to fatigue during prolonged exercise, it may have a negative effect on high-intensity exercise. The persistence of down-regulated PDH activity following fat-adaptation strategies, even with CHO restoration, suggests that such protocols may not be advisable in circumstances in which exercise of a sustained higher intensity is required (L. Havemann et al., 2006) . Along with storage of IMTG, this creates a potential balancing act whereby fuel utilisation pathways and substrate storage need to be a maximised in-line with

the demands of the exercise bout. This, in essence, describes the potential benefits of an athlete possessing a high degree of metabolic flexibility; they are able to perform optimally across a range of metabolic demands.

2. 8. KETOSIS

2. 8. 1. Overview

Ketone bodies are three water-soluble molecules—acetoacetate, beta-hydroxybutyrate (β-HB)—and their spontaneous breakdown product, acetone. β-HB is technically not a ketone due to its carboxyl (-COOH) group, but is referred to in human metabolism as a ketone or ketone body. Ketone bodies are produced by the liver during periods of low food intake (i.e., fasting and starvation), low CHO diets, prolonged intense exercise, and in untreated (or inadequately treated) type 1 diabetes mellitus (Kanikarla-Marie & Jain, 2016). Ketosis is a metabolic state of elevated blood ketones (acetoacetate, β-hydroxybutyrate) above 0.2 mM/L (Robinson & Williamson, 1980). Ketones can be used by the brain and multiple other tissues when the glucose supply is inadequate. However, they are often considered in a negative light due to the related and familiar-sounding diabetic condition, ketoacidosis, which is caused by a decreased blood pH, due to uncontrolled hyperketonaemia.

2. 8. 2. Ketone Metabolism

Ketones are synthesised in the liver from the pivotal metabolite acetyl–CoA. Since acetyl–CoA is not only a product of both β-oxidation and glycolysis, but is also produced by the transamination of amino acids, the body can manufacture ketones from all three macronutrients–protein, fat and CHO. Because ketones bodies are small water-soluble molecules, they are readily absorbed by the extra-hepatic tissues, and converted into acetyl–CoA, which then enters the TCA cycle and is oxidised in the mitochondria for energy.

Figure 2-6. Bihormonal model for the control of ketogenesis. The model proposes that the mobilisation of free fatty acids from adipose tissue to the liver results primarily from insulin deficiency. The activation of hepatic fatty acid oxidation and ketogenesis comes about through elevation of the [Glucagon]:[Insulin] ratio. (McGarry & Foster, 1980)

Studies indicate that accelerated hepatic ketogenesis during starvation is a result of both enhanced activity of the enzymatic systems involved in ketone body production and an increased FFA load. In low CHO states, elevation of the [glucagon]: [insulin] suppresses the synthesis of malonyl–CoA and lipogenesis within hepatocytes with concomitant activation of fatty acid oxidation and ketogenesis. Simultaneously, an increase in hepatic carnitine and fatty

acyl–CoA contents further enhance β-oxidation and the production of ketone bodies (McGarry & Foster, 1980).

In the CHO fed state (low [glucagon]: [insulin]), the direction of fatty acid metabolism in the liver is primarily towards synthesis. Under these conditions, fatty acid oxidation is inhibited because of the high tissue level of malonyl–CoA, which acts as a potent inhibitor of the initial step in the oxidative sequence (Fat Metabolism) (Figure 2-7). As such, cellular regulatory mechanisms, influenced by the circulating [glucagon]: [insulin], provide one of the key adaptations which allows for improved whole-body metabolic flexibility.

Figure 2-7. Regulatory interactions between the pathways of fatty acid synthesis and oxidation in liver. In the fed state, malonyl–CoA levels are high, assuring rapid fatty acid synthesis and suppression of fatty acid oxidation (through inhibition of carnitine acyltransferase I). Malonyl–CoA concentrations may be lower due to glucagon excess or high tissue levels of free fatty acyl–CoA. In both cases, the net result is cessation of lipogenesis and activation of fatty acid oxidation and ketogenesis. (McGarry & Foster, 1980)

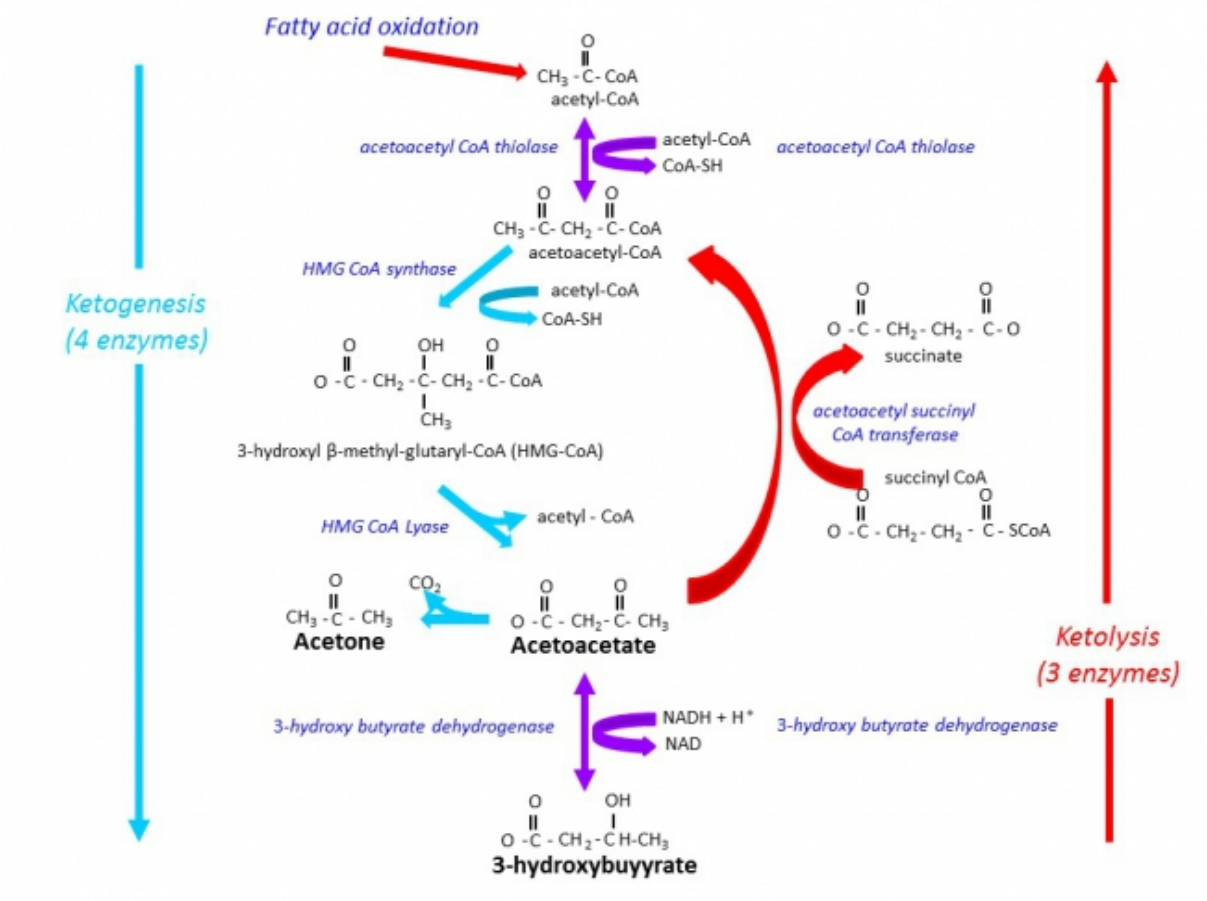


Figure 2-8. Ketogenesis and ketolysis pathways. Fatty acid oxidation in liver mitochondria generates acetyl–CoA. Under conditions of low glucose availability, the acetyl–CoA cannot be oxidised via the TCA cycle because, in the liver, the oxaloacetate required for the first step is unavailable, due to being redirected to gluconeogenesis. Consequently, the acetyl–CoA is converted into ketone bodies. (Ward, 2015)

2. 8. 3. Metabolic Efficiency

An important metabolic characteristic of ketones is that they bypass the rate-limiting enzyme complex, PDH (outlined in "Carbohydrate metabolism"). β-HB can enter the mitochondria, be converted into acetoacetate, and enter the TCA Cycle directly, between succinyl–CoA and succinate, (Figure 2-8) without regulation from rate-limiting enzymes.

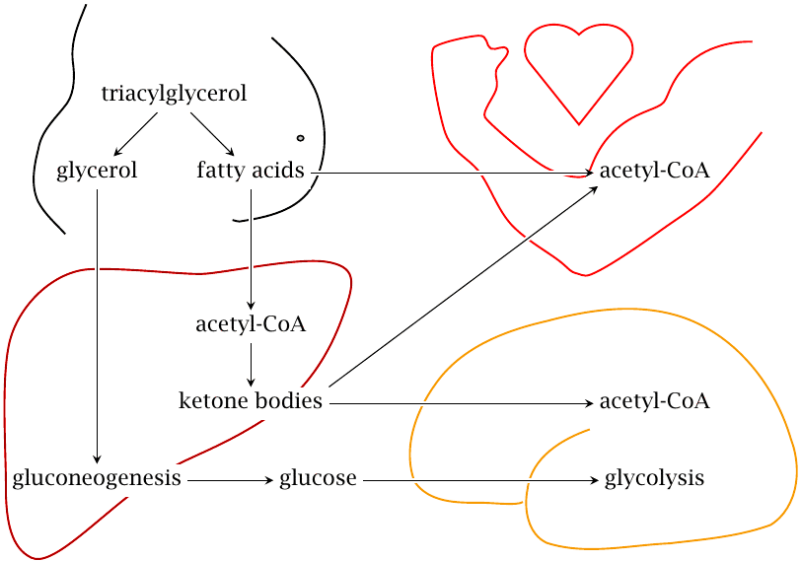


Figure 2-9. Simplistic representation of the relationship between stored adipose triacyl glycerol (TGA), fatty acids, glucose, ketone bodies and acetyl–CoA production (Michael Palmer).

Late last century, ketones were shown to improve the metabolic efficiency of perfused rat hearts compared to glucose (G) and glucose + insulin (GI) (Sato et al., 1995). This was due to two major shifts in substrate utilisation—the reactants NADH/NAD+ becoming more reduced and the reactants CoQ/$CoQH_2$ becoming more oxidised. These changes, taken together, widen the energetic gap between redox states and translate to a higher (more negative) delta G (ΔG, Gibbs free energy), which results in greater ATP production per unit of carbon. Compared to

GI, β-HB was able to "mimic" the effect of additional insulin (increased acetyl–CoA), without the complex cascade of events brought on by additional insulin (e.g., decreased lipolysis), while avoiding the rate-limiting PDH.

In an exercise context, this could allow for reduced reliance on substrate-level phosphorylation (glycolysis) and subsequent disruption to cellular homeostasis from related increases in cytosolic $[H]^+$.

2. 8. 4. Protein Sparing

Being a major glucose consumer during contraction, when faced with low CHO intake, skeletal muscle provides competition with the muscle and brain for remaining blood glucose. At the same time, hepatic gluconeogenesis from amino acid precursors supports blood glucose supply, though this process must be minimised to protect functional tissue (Felig, Marliss, Pozefsky, & Cahill, 1970). Ketosis can protect muscle protein by inducing hyperalaninaemia, which results from the lack of need for gluconeogenetic substrates to produce glucose for the brain, since the brain can use ketones (Paoli et al., 2012; Sherwin, Hendler, & Felig, 1975).

To orchestrate the appropriate response, basal insulin levels are maintained, even during starvation, to minimise protein breakdown, yet during prolonged low CHO intake, resistance to both insulin (Mansell & Macdonald, 1990b) and contraction-mediated glucose uptake, develops in muscle, to limit its glucose demand (Knapik et al., 1988; Rowlands, Johnson, Thomson, Chapman, & Stannard, 2009). Despite this, the antiproteolytic effects of insulin on muscle are maintained (Fryburg, Barrett, Louard, & Gelfand, 1990). A severe restriction in

CHO is, as discussed earlier, related to a reduction in PDHa, which could be due, in part, to reduced insulin-mediated stimulus from both PDH and GLUT4. Ketones then provide a means of buffering the reduced GLUT4 and PDH activity by providing a direct supply of acetyl–CoA.

2. 8. 5. Ketone Supplements

The presence of elevated blood ketones appears to be advantageous for the metabolic system during endurance exercise performance. However, a major limiting factor in the process of elevating blood ketones, starvation or prolonged low CHO intake, is the need for CHO, even at low intensities (<60% VO_{2max}). What is therefore needed, is the ability to store glycogen, while maintaining high concentrations of β-HB. Yet, as discussed, the ingestion of a large bolus of CHO alters the [malonyl–CoA] in the hepatic tissue, signalling fatty acid synthesis, re-esterification of TAG and the elimination of β-HB synthesis. However, in the last few years, a potential solution to this problem has emerged: supplemental ketones. It is hypothesised that the ingestion of an exogenous ketone supplement could raise ketones to concentrations previously only achieved during starvation, or severe CHO restriction (Hashim & VanItallie, 2014). In theory, this would allow for CHO ingestion without impacting the benefits of elevated [β-HB].

Two ketone esters have been reported in published literature: R,S-1,3-butanediol acetoacetate diester (Kesl et al., 2016) and (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (Clarke et al., 2012; Pete J Cox et al., 2016). Acute ingestion of either ester can result in short-term (~0.5 to 6 hours) nutritional ketosis, indicated by [β-HB] >1 mM (Clarke et al., 2012;

Kesl et al., 2016). Nutritional ketosis is therefore achieved without the impracticality of prolonged fasting or ketogenic dieting.

Only two studies have convincingly examined the relationship between exercise and ketone metabolism with an exogenous source rather than prolonged fasting or low CHO intake (Pete J Cox et al., 2016; Françoise Fery & Balasse, 1988). In the former study (Françoise Fery & Balasse, 1988), the infusion of sodium AcAc after an overnight fast achieved a ketone body concentration of ~6 mM (β-HB ~3.5 mM, AcAc ~2.5 mM) at the onset of 2 hours of exercise at ~52% VO_{2max}. Subsequently, Pete J Cox et al. (2016) completed a series of experiments in which the ingestion of the *(R)*-3-hydroxybutyl *(R)*-3-hydroxybutyrate ketone monoester resulted in acute ketosis, indicated by [β-HB] of ~3 mM after 10 minutes and rising to ~6 mM at 30 minutes post-ingestion. Both studies found evidence of contraction-dependent disposal of β-HB during exercise. Moreover, based on expired air analysis, adjusted for oxidation of ketone bodies, β-HB oxidation contributed 2–18% of the O_2 consumption required for energy provision. The rise in plasma [lactate] was blunted, relative to its concentration during fasted exercise, and following the ingestion of an isocaloric CHO drink. Ingestion of the exogenous ketones inhibited glycolytic metabolism, spared of muscle glycogen, reduced deamination of branched-chain amino acids, and increased reliance on IMTG during exercise (Pete J Cox et al., 2016). Notably, AcAc did not change during exercise, whereas β-HB declined throughout and was reduced by ~2 mM at its completion. Taken together, these data suggest that achieving acute nutritional ketosis through the consumption of exogenous ketones has dramatic effects on skeletal muscle metabolism during exercise, and can confer a performance benefit to endurance athletes.

The positive findings notwithstanding, potential adverse effects should be considered for any performance aid prior to adoption. Side effects of ketone body ingestion have been reported in humans (Clarke et al., 2012). specifically, in a repeated-dose design over five days, adverse effects, such as flatulence, nausea, diarrhoea, and dizziness were reported in five out of 24 participants, at doses ranging from 420 to 1071 mg/kg.

2. 8. 6. Reactive Oxygen Species

An antioxidant can be defined, in simple terms, as anything that inhibits or prevents the oxidation of a susceptible substrate. They are employed to defend against reactive oxygen species (ROS). ROS are free radicals (possess unpaired electrons), whose accumulation can damage biological substrates, leading to a variety of pathological changes (Vollaard, Shearman, & Cooper, 2005). ROS are typically produced in mitochondria, as electrons leak from the ETC and react with O_2 to form superoxide anion ($O_2^{-\bullet}$). This process occurs more frequently during endurance exercise, due to increased O_2 flux through the ETC and subsequent release of free electrons (Ji, 1999). The human body has endogenous antioxidants, the main one being glutathione (GSH), which is reduced to GSSG on absorption of ROS-based free electrons. Ketogenic diets, or at least high [β-HB], up-regulate GSSG biosynthesis, enhance mitochondrial antioxidant status, and protect mitochondrial DNA from oxidant-induced damage (Jarrett, Milder, Liang, & Patel, 2008). In this regard, ketogenic diets or ketone supplementation may provide alternative benefits to exercise performance via improved mitochondrial stability, through reduced ROS degradation.

2. 8. 7. Neurological influence

A ketogenic diet leads to alterations in the metabolism of brain amino acids, most importantly glutamic acid, the major excitatory neurotransmitter GABA (Yudkoff, Daikhin, Nissim, Lazarow, & Nissim, 2001). In the adult brain, $GABA_a$ receptors, upon activation, hyperpolarise the neural membrane and inhibit action potentials. Being that epilepsy is a disorder of the central nervous system, characterised by a hyper-excitable neuronal network, $GABA_a$ receptor agonists (activators) are well known to suppress seizure activity. However,

the same can be replicated by a ketogenic diet and/or exogenous ketone supplementation (Veech, 2004; Yudkoff et al., 2001). This is because β-HB, like its isomer ϒ-HB, acts as weak partial agonist for $GABA_b$ receptors (Brown, 2007).

In an exercise context, GABAergic agonists have been shown to improve performance in a running time to exhaustion protocol, in both untrained and trained rats (Abdelmalki, Merino, Bonneau, Bigard, & Guezennec, 1997).These findings suggest that fatigue during prolonged exercise can be influenced by high [β-HB] acting on the neural network, in addition to muscular and hepatic substrate metabolism.

2. 9. METABOLOMICS

2. 9. 1. Overview

Metabolomics, in the wider context of bioanalytical techniques, is a relatively new cousin to genomics and proteomics. Specifically, metabolomics involves the rapid throughput characterisation of small molecule metabolites found in an organism. Since the metabolome (complete set of small-molecule metabolites) is closely tied to the genotype of an organism, its physiology, and its environment (what the organism eats or breathes), metabolomics offers a unique opportunity to look at genotype-phenotype as well as genotype-envirotype relationships. The introduction of metabolomics has now allowed the combination of what was once a series of tests, extractions, and preparations into one relative simple and partially non-invasive process.

Currently, the method is being applied in many biological studies ranging from carbon–nitrogen interactions in plants, to the development of personal metabolomics as the next

generation of nutritional assessment in humans. However, as yet, there are only a handful of instances where metabolomics has been applied to sport and exercise (Harker, Coulson, Fairweather, Taylor, & Daykin, 2006; Kuhl et al., 2008; O'Connor, Olivecrona, Edwards, & Stannard, 2015; Pechlivanis et al., 2010; Yan et al., 2009).

2. 9. 2 High-resolution NMR spectra

An NMR spectrum comprises a horizontal axis recording the 'chemical shift' values, with a vertical axis that records the signal intensity for each of many thousands of those chemical shifts (Figure 2-10). For any given metabolite, every distinct hydrogen atom or group of equivalent hydrogen atoms (for instance, the three hydrogens in a methyl group) gives rise to an NMR signal at a chemical shift that is characteristic of the chemical environment in which that hydrogen sits. The signal can be a singlet or a multiplet; the multiple structures arise from interactions with neighbouring hydrogen atoms and generally the multiplicity increases with the number of 'near-neighbour' hydrogens. The area of the NMR signal is directly proportional to the concentration of the parent molecule in the biofluid mixture. This is the basis for the use of NMR as an analytical technique and is also the fundamental reason why data analysis based upon linear algebra methods is so effective. The main caution with this method is that all metabolites present in the biofluids are detected simultaneously, because the chemical shift range for hydrogen is rather limited, so there is inevitably some signal overlap. The water signal itself is massive compared with signals of the metabolites and has to be suppressed in order to allow detection and quantification of those much weaker signals (Kemsley et al., 2007). However, the suppression of the water signal can sometimes remove signals from nearby metabolites.

Figure 2-10. 600 MHz ^{1}H NMR spectra of control human blood plasma: The spectrum shows signals of low molecular weight metabolites as well as larger molecules, such as lipoproteins. The large molecules give rise to much broader resonances in both the aromatic and aliphatic areas, due to their fast relaxation properties in the NMR experiment. The signal intensities of low molecular weight compounds from 6–9.5 ppm are low in this type of acquisition. LDL, low density lipoprotein; VLDL, very low density lipoprotein. (Beckonert et al., 2007)

Assignment of peaks to respective metabolites can be achieved with reference to the comprehensive table established by J. K. Nicholson, Foxall, Spraul, Farrant, and Lindon

(1995) (Appendix D Table 14-28) and variations thereof, either manually or using specific analytical software.

2. 9. 3. Pattern Recognition Analysis

2. 9. 3. 1. Principle Component Analysis (PCA)

Principle component analysis (PCA) is considered a primary tool in metabolomics, used to reduce the dimensionality of a multivariate dataset, and thus helping to better understand possible differences between classes. It is an unsupervised method; hence the clustering or separation of samples is purely due to similarities or differences, respectively, among all the samples.

In nutritional intervention studies, PCA is applied to identify groupings of spectra between the different diet classes—in this instance low CHO vs high CHO. During the course of PCA, it is possible to calculate a score vector for each sample for a given principal component (factor vector). Score vectors provide the principle component (PC) composition related to a specific sample, while the loading vectors provide the sample composition related to the variables (small segment of the spectra). Since score vectors account for the highest variance in the different spectra, the first two calculated scores are used to describe similarities between spectra (Bradley Worley & Robert Powers, 2013).

2. 9. 3. 2. Supervised Pattern Recognition

While the unsupervised nature of the PCA algorithm provides a means to achieve unbiased dimensionality reduction, its application only reveals group structure when the within-group

variation is sufficiently less than the between-group variation. Therefore, supervised forms of discriminant analysis, such as Partial Least Squares (PLS-DA), which rely on the class membership of each observation, are also commonly applied in metabolic fingerprinting experiments (Wold, Sjöström, & Eriksson, 2001).

PLS-DA is used to optimise separation between different groups of samples, which is accomplished by linking two data matrices X (i.e., raw data) and Y (i.e., groups and class membership). This approach aims to maximise the covariance between the independent variables X (sample readings) and the corresponding dependent variable Y (classes, groups) of highly multidimensional data by finding a linear subspace of the explanatory variables. This new subspace permits the prediction of the Y variable, based on a reduced number of factors (PLS components, or latent variables). These factors describe the behaviour of dependent variables (Y) and they span the subspace onto which the independent variables (X) are projected (Gromski et al., 2015). The main advantage of this PLS-DA approach is the availability and handling of highly collinear and noisy data, which are very common outputs from metabolomics experiments (Want & Masson, 2011). In addition, this provides several statistics such as loading weight, variable importance on projection (VIP) and regression coefficient.

OPLS is an extension to the supervised PLS regression method. OPLS uses information in the Y matrix to decompose the X matrix into blocks of structured variation correlated to and orthogonal to Y, respectively. OPLS can, analogously to PLS-DA, be used for discrimination (OPLS-DA) with then main benefit over PLS-DA thus lying in the ability of OPLS-DA to separate predictive from non-predictive (orthogonal) variation (Fiehn et al., 2007). This advantage can be demonstrated using a simple two-class scenario based on spectral data as

shown (Figure 2-11). The corresponding loading vectors (t1 and t2) contain a mixture of both the discriminatory properties as well as the non-discriminatory properties that are mainly confounded with the direction of t2. In the same example, OPLS-DA effectively separates the discriminatory direction in t1 from the Y-orthogonal direction to 1, making the corresponding predictive loading vector t1 straightforward to interpret (Figure 2-11). The parts of the spectra responsible for the remaining variation can be identified from the Y-orthogonal t1 loading vector, which is mainly related to high within-class variance(Bylesjö et al., 2006).

Figure 2-11. A geometrical illustration of the difference between the PLS-DA and OPLS-DA models. In the left panel, the PLS components cannot separate the between-class variation from the within-class variation, and the resulting PLS component loadings mixes both types of variations. In the right panel, the OPLS components are able to separate these two different variations. Component 1 (t1p) is the predictive component and displays the between-class ([blue circles], [yellow squares]) variation of the samples. The corresponding loading profile can be used for identifying variables important for the class separation. Component 2 (t2o) is the Y - orthogonal component and models the within group (within-class) variation (Trygg, Holmes, & Lundstedt, 2007)

2. 9. 3. 3. Model Validation

A permutation test is the calculation of goodness of fit (R2) and the predictive ability of the model (Q2). Permutation testing can only be applied to PLS but results can be inferred to OPLS-DA since the parameters are the same. The information extracted from permutation testing gives an indication of whether the model can be considered statistically valid or not (Stumpe, Engel, Steinweg, & Marschner, 2012).

The R2 value can vary from 0 to 1, where 1 indicates a model with a perfect fit. If the Q2 value is greater than 0.5, the model is considered to have good predictability and if it is higher than 0.9 and less than 1.0, an excellent predictability. Model over-fit is indicated if the original models, R2 and Q2, have values that differ by more than 0.2. This is because adding PLS components (latent variables) to the model optimises its parameters, causing R2 and Q2 to increase towards 1. However at a certain point, the two variables will diverge, with Q2 trending toward 0 as the model becomes over-fitted (Broadhurst & Kell, 2006). For Q2, all values must be below the original model's Q2 value, but R2 only requires the majority of values to be below the original R2 value. Additional requirements have been proposed by Bradley Worley and Robert Powers (2013), which state that Q2 must be below -0.2 and R2 be below 0.3. Thus, R2 values should not exceed 0.3–0.4 and Q2 should not exceed 0.05 (Eriksson, 2006).

2. 9. 4. Biofluid Metabolomics

In body fluids, metabolites are in dynamic equilibrium with those inside cells and tissues, and consequently, abnormal cellular processes in tissues of the whole organism following a toxic

or metabolic insult will be reflected in an altered biofluid composition (Lindon, Nicholson, Holmes, & Everett, 2000). In all cases, the analytical problem usually involves the detection of ‘‘trace’’ amounts of analytes in a very complex matrix of potential interferences. It is, therefore, critical to choose a suitable analytical technique for the class of analyte of interest in the particular biomatrix—which could be blood, plasma, urine, bile, or organ samples. High resolution NMR spectroscopy, namely ^{1}H NMR, is appropriate for investigating abnormal body fluid compositions, because a wide range of metabolites can be detected simultaneously with minimal sample preparation and ‘‘without prejudice,’’ and hence, many cellular biochemical can be probed (Lindon et al., 2000).

By analysing the metabolite content and concentration of a biofluid using NMR or liquid chromatography MS, the metabolic profile of a subject can be generated to provide a ‘whole-body snapshot’. A comparison of these snapshots before and after dietary or exercise interventions may highlight metabolite(s) that are altered due to such stimuli. This in turn, may lead to the identification of a reference biomarker and ultimately to a greater understanding of biochemical pathways (Kemsley et al., 2007). The disadvantage of NMR-based metabolomics is the overlap of spectral peaks. That is, the chemical shifts (ppm) of one metabolite may overlap with that of another metabolite and as a result the metabolite which exists in a lower concentration becomes masked to the researcher.

2. 9. 5. Nutritional Metabolomics

Nutritional metabolomics has emerged from the metabolomics field in the hope of identifying novel correlations between dietary patterns and health. However, like the other applications of metabolomics outlined, the study of nutritional interventions has presented challenges due to

individual variability in complex metabolic pathways and digestion, as well as identification of novel metabolites (Lodge, 2010).

Deo et al. (2010) illustrates the important contribution of nutritional metabolomics to the goal of complex biosystems models. Deo et al. (2010) showed that considerable additional information about an individual could be obtained by applying nutritional metabolomics methods, alongside an oral glucose tolerance test. Using the unbiased metabolomics approach, active modules of metabolites were identified and grouped according to System A and System L, as described in “Pattern Recognition Analysis” (Deo et al., 2010). The data illustrated an important conceptual advance in nutritional metabolomics, namely, transitioning from a mono-dimensional view of metabolism involving only substrate clearance or enzymatic conversions, to a multi-dimensional view in which biochemical reactions occur in different subcellular compartments, linked by transport systems. In such multidimensional models, movement between compartments can compensate for changes within compartments. Because volumes, concentrations and enzyme contents of compartments differ, the response of a complex system is often not a simple function. Hence, the use of nutritional metabolomics in integrated biosystems research represents a critical advance in addressing complex issues of diet and health, or in the case of this thesis, diet and exercise

CHAPTER 3. HYPOTHESES

3. 1. HYPOTHESIS 1;

Chronically reducing an individual's dietary carbohydrate (CHO) intake during training will increase their maximal rate of fatty acid oxidation during subsequent exercise compared to a chronic, moderate CHO diet. The increased reliance on fatty acid oxidation will increase metabolic flexibility, spare glycogen and thereby increase endurance exercise capacity.

3. 2. HYPOTHESIS 2;

When compared to a standard moderate CHO diet, a chronic low CHO training diet will allow for an increase in fatty acid oxidation and a reduction in CHO oxidation during submaximal, fasted exercise, which will be more pronounced in men than women.

3. 3. HYPOTHESIS 3;

A low CHO diet will increase concentrations of β-oxidative related metabolites, such as fatty acids, ketone bodies, glycerol and triglycerides, at rest and during exercise. In contrast, a moderate CHO diet will result in higher concentrations of glycolytic metabolites, such as glucose, lactate, alanine and pyruvate.

3. 4. HYPOTHESIS 4

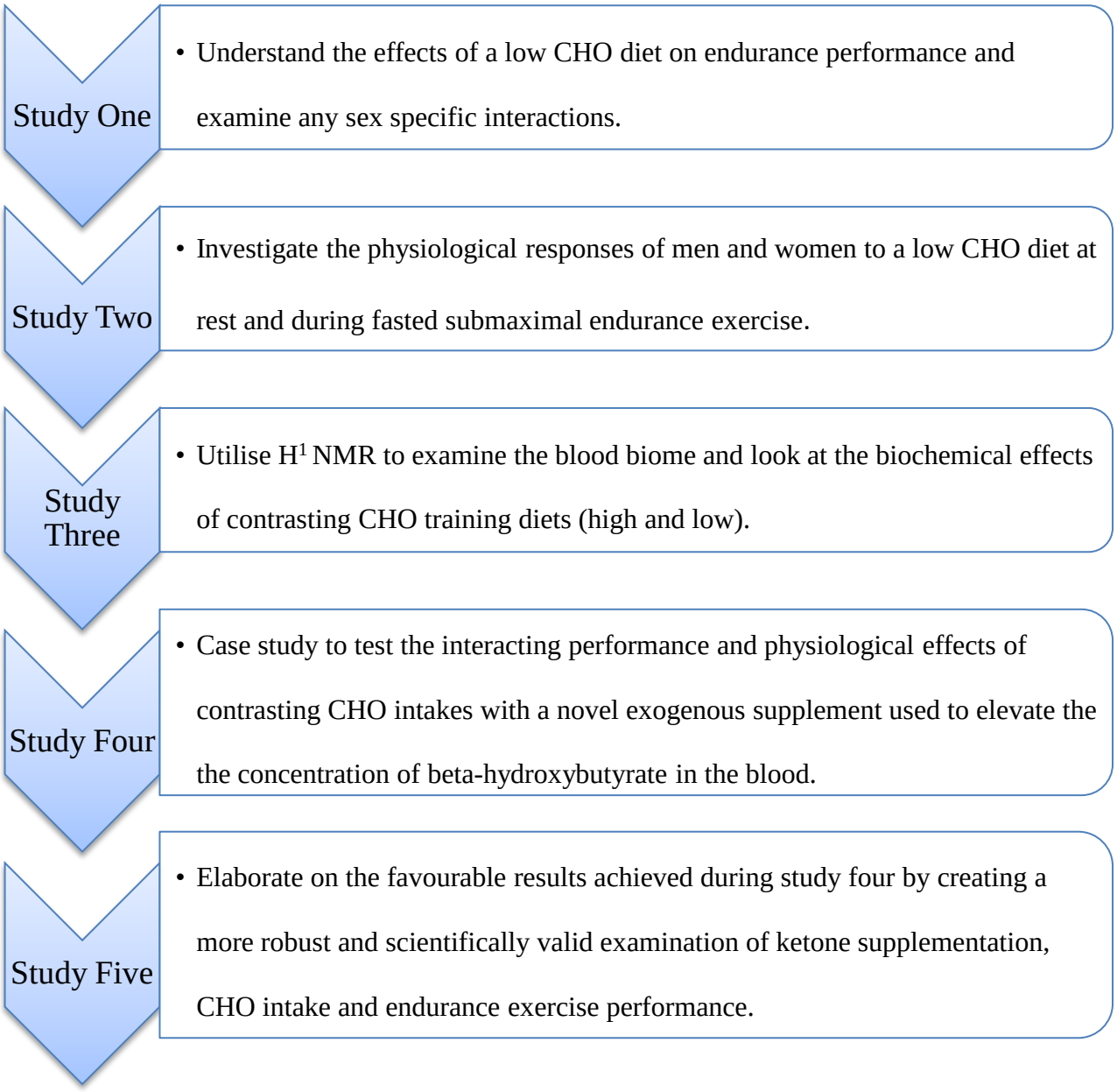
Through ingesting the ketone precursor, 1, 3-butanediol as a drink, it is possible to raise blood ketones to a level that the body has only previously encountered during periods of starvation or severe CHO restriction. The resulting elevated concentrations of beta-hydroxybutyrate [β-HB] will increase the availability of non-glycolytic substrates, thereby lowering CHO utilisation and enhancing endurance exercise performance. In addition, it is thought that cells which are adapted to a low CHO environment (i.e., nutritional ketosis) may respond better to utilising ketones, than those adapted to CHO abundance (moderate CHO).

3. 5. HYPOTHESIS 5;

Ingesting a supplement that could artificially elevate blood [β-HB] in the presence of CHO supplementation will allow for simultaneous benefits from both ketosis and CHO supplementation. In addition, it is thought that cells that are adapted to a low CHO environment (i.e. nutritional ketosis) may respond better to utilising ketones than those adapted to CHO abundance (moderate CHO). It is expected that these two factors will work together to result in improved endurance exercise performance.

CHAPTER 4. THESIS OUTLINE

4. 1. TIMELINE



4. 2. STUDY DESIGN (CHAPTERS 6–8)

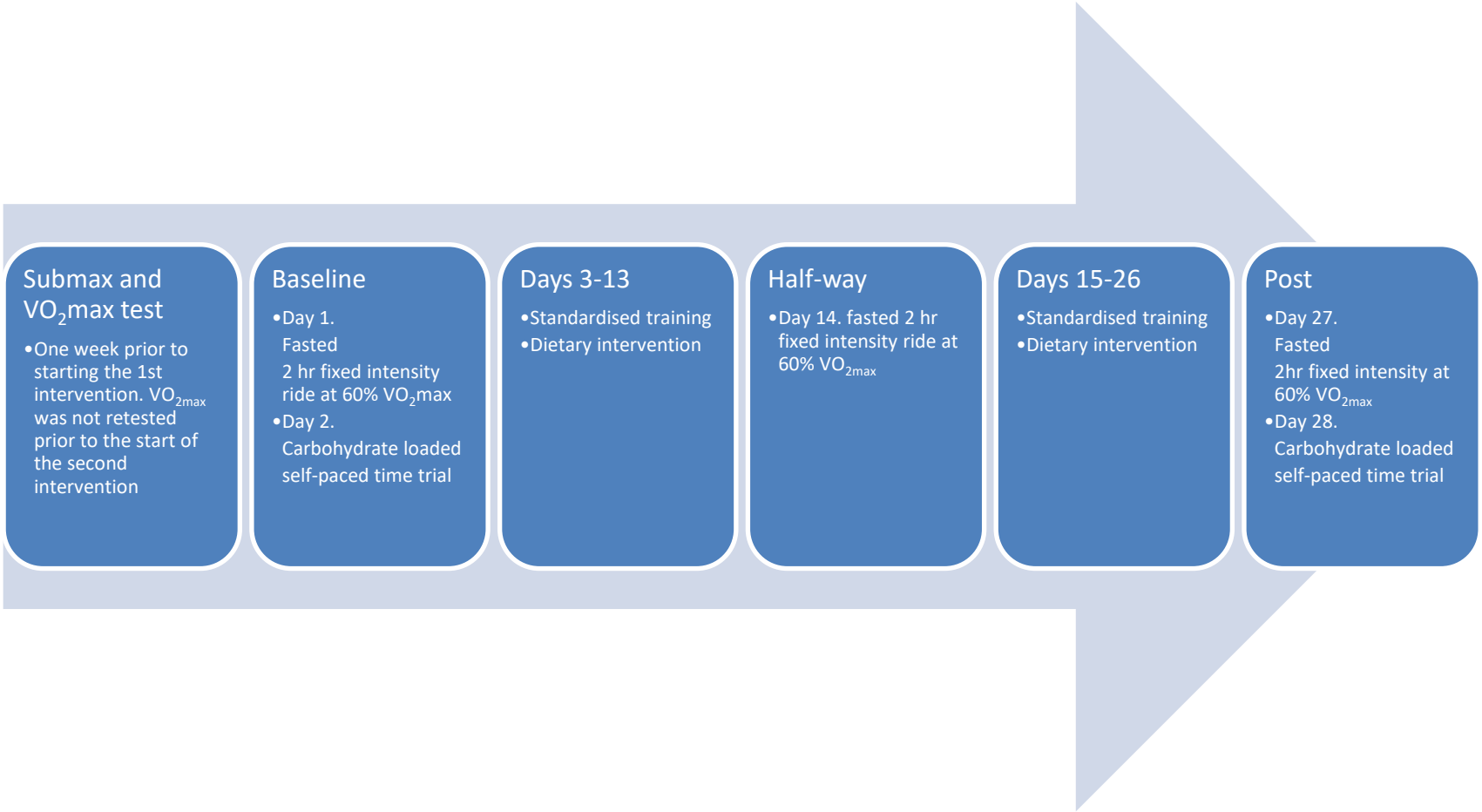


Figure 4-1. Timeline of exercise tests and diet interventions used for research chapters 6–7

4. 2. 1. Studies One—Three

The first three research chapters (6–8) are drawn from parts of a larger protocol, as outlined in Figure 4-1. The protocol was designed to firstly test the performance response of endurance-trained athletes to contrasting CHO intakes. The means of testing the performance response was a cycling time trial (TT), in which the participants attempted to complete a fixed workload (measured in kJ) equivalent to the amount of work they would do if they cycled at a power output representative of 55% of their VO_{2max} for 5 hr (i.e., (160 W x 3.6 kJ) x 5 hr = 2880 kJ total workload). The TT was completed inside, on an electronically-braked cycle ergometer (Excalibur Sport, Lode BV, The Netherlands).

The TT was completed on three occasions: baseline, at the completion of the first intervention and at the completion of the second intervention. The reason for not incorporating another baseline test at the beginning of the second intervention was due to participant recruitment; the complete study was extremely time consuming for both participant and researcher. In particular, each TT session required a full day, when taking into account the breakfast and pre- and post-exercise measures. It is acknowledged that the lack of a secondary baseline may have resulted in the participants being in a different state of fitness and therefore may not be comparable with the initial baseline. However, the participants underwent the same training load prior to both post intervention performance TTs, and the main comparative measure was between each post intervention TT, rather than the performance change compared to baseline.

In addition to the performance measures, the protocol was designed to incorporate a test that allowed a comparison between physiological measures across all tests and participants. The performance TT could not be used as a comparative test because each participant would have

completed the TT at different relative intensities and for differing durations. Thus, a fasted two hour fixed intensity (60% VO_{2max}) cycle was incorporated. An intensity of 60% VO_{2max} was selected to ensure a steady state metabolism and sufficient contribution of both energy yielding pathways—CHO and fat. The ride was completed fasted, the day before the CHO-loaded TT, to allow a comparison of RER values in CHO-depleted and loaded states. This comparison allowed for an analysis of the metabolic responses (measured via RER) to chronic low CHO intake followed by a CHO load. It was hypothesised that the increase in glycogen stores could stimulate an up-regulation of glycolysis and subsequent down-regulation of fatty acid oxidation in the day following the CHO load.

A potential weakness of the protocol was the lack of VO_{2max} retesting prior to the second intervention. Consequently, if participants had gained or lost aerobic capacity, during the washout period, they may have been exercising at different relative intensities during each intervention. The reasoning behind not retesting VO_{2max} was that it would not allow for a direct comparison between TTs, because each TT would have been completed over a different total workload.

CHAPTER 5. COMMON METHODOLOGY

5. 1. VO_{2MAX} AND SUBMAXIMAL EXERCISE TESTS.

5. 1. 1. Submax

Submaximal and VO_{2max} cycle exercise tests were completed prior to the beginning of each study. Participants cycled at four submaximal seven-minute workloads (men: 100, 150, 200, 250 W, women: 100, 125, 150 175 W), on an electronically-braked cycle ergometer (Excalibur Sport, Lode BV, The Netherlands). During the last minute of each workload, ventilatory gases were collected into Douglas bags via a breathing apparatus. These were analysed for oxygen (O_2) and carbon dioxide (CO_2) concentrations, using a zirconia cell O_2 analyser (AEI Technologies Inc, Pittsburgh, USA), and an infra-red based CO_2 analyser, respectively (AEI Technologies Inc, Pittsburgh, USA). Volume measurement was made with a Harvard dry gas meter (Harvard, UK). Calculations of the rate of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were made at Standard Temperature Pressure Dry (STPD). A breath-by-breath system was used for study four ("Ketone Supplementation: Case Study") (K4 b^2, COSMED s. r. l., Rome, Italy). A breath-by-breath system measures exhaled VO_2 and VCO_2 instantaneously at the mouth via sensors integrated into a turbine that is fixed to a breathing apparatus. This system was used during the case study to test its application compared to the Douglas bag method used in all other studies.

5. 1. 2. VO_{2max}

Five minutes after the final submaximal workload, VO_{2max} was measured using a 'ramp protocol' (men: 25 W/min, starting at 150 W; women: 20 W/min starting at 100W) until volitional fatigue. Respiratory gases were collected via repeated (~40 seconds) Douglas bags after the respiratory compensation point was identified. Douglas bags were analysed as above. Attainment of VO_{2max} was confirmed with an RER ≥1.1.

A linear relationship was drawn between steady state O_2 consumption and workload as previously described (Figure 5-1)(Conway, Orr, & Stannard, 2003). After VO_{2max} was identified, a percentage of this figure was put into a linear equation (Figure 5-1) to calculate the power output (W) predicted to produce the desired % VO_{2max}.

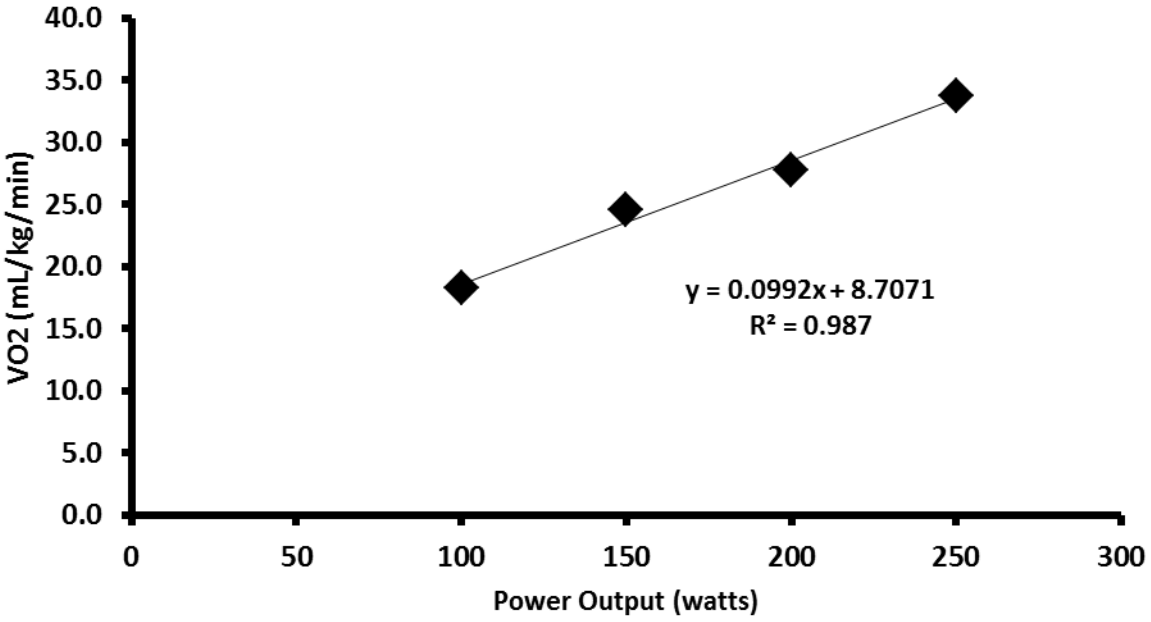


Figure 5-1 Linear relationship between relative VO_2 (mL/kg/min) and submaximal power output. Black diamonds represent data collection points.

5. 2. RESTING METABOLIC ASSESMENT

Resting metabolism was assessed by collecting respiratory gases, using the Douglas bag method, as outlined in the previous two sections. Prior to measurement, participants were seated for five minutes to allow resting homeostasis to be achieved before respiratory gases were collected (for a further five minutes).

Resting metabolic measurements were taken at two-time points—on arrival to the laboratory prior to the beginning of any testing procedure or blood collection (to avoid stress related influences), and again, post-exercise, at least ten minutes after the completion of physical testing and blood collection. Calculations of fat and CHO oxidation rates were determined using the Weir equation (Weir, 1949). Although, this may not have been completely representative of resting metabolism, it did allow for an insight into the metabolic situation at rest, post-exercise.

5. 3. BODY COMPOSITION

Body composition was evaluated using a portable Bioelectrical Impedance Analyser (BIA, InBody 230, InBody®, USA). The InBody® 230 BIA was selected due to its practicality and low cost of implementation. Research reviewed prior to selection of the BIA suggested that its accuracy strongly correlated ($r = 0.94–0.99$) with that of traditionally-used, dual-energy X-ray absorptiometry (DXA) (Karelis, Chamberland, Aubertin-Leheudre, & Duval, 2013).

Body composition, body fat mass, skeletal muscle mass, total body water and total body mass were measured at the following time points: after an overnight fast, before each two hour

fixed intensity ride and at the half-way point during each four-week intervention. Participants were instructed to arrive hydrated and to wear the same cycling shorts and top, to ensure consistency between measurement points. However, beyond these instructions, no further protocols were put in place to ensure hydration status was similar between or within participants; thus, it is acknowledged that the participants' hydration statuses may have differed between measurement points.

5. 4. STANDARDISED TRAINING

To minimise training-related variation in physical performance, training was standardised during dietary interventions. Training sessions were prescribed so that each participant completed the same total workload per session. This was based off the participants' preliminary VO_{2max} data and consisted of four sessions per week—three interval sessions and one long aerobic session. The interval sessions were completed on electronically-braked cycle ergometers (Wahoo kickr ®, Wahoo Fitness LLC, Atlanta, GA, USA), and heart rate (HR) was used to monitor intensity for the long aerobic ride, which was done outdoors. There was a slight variation in supplemental training for each athlete (e.g., running) which was replicated in the second intervention, to avoid within-subject variation of training loads.

Figure 5-2. Participants completing a standardised training session.

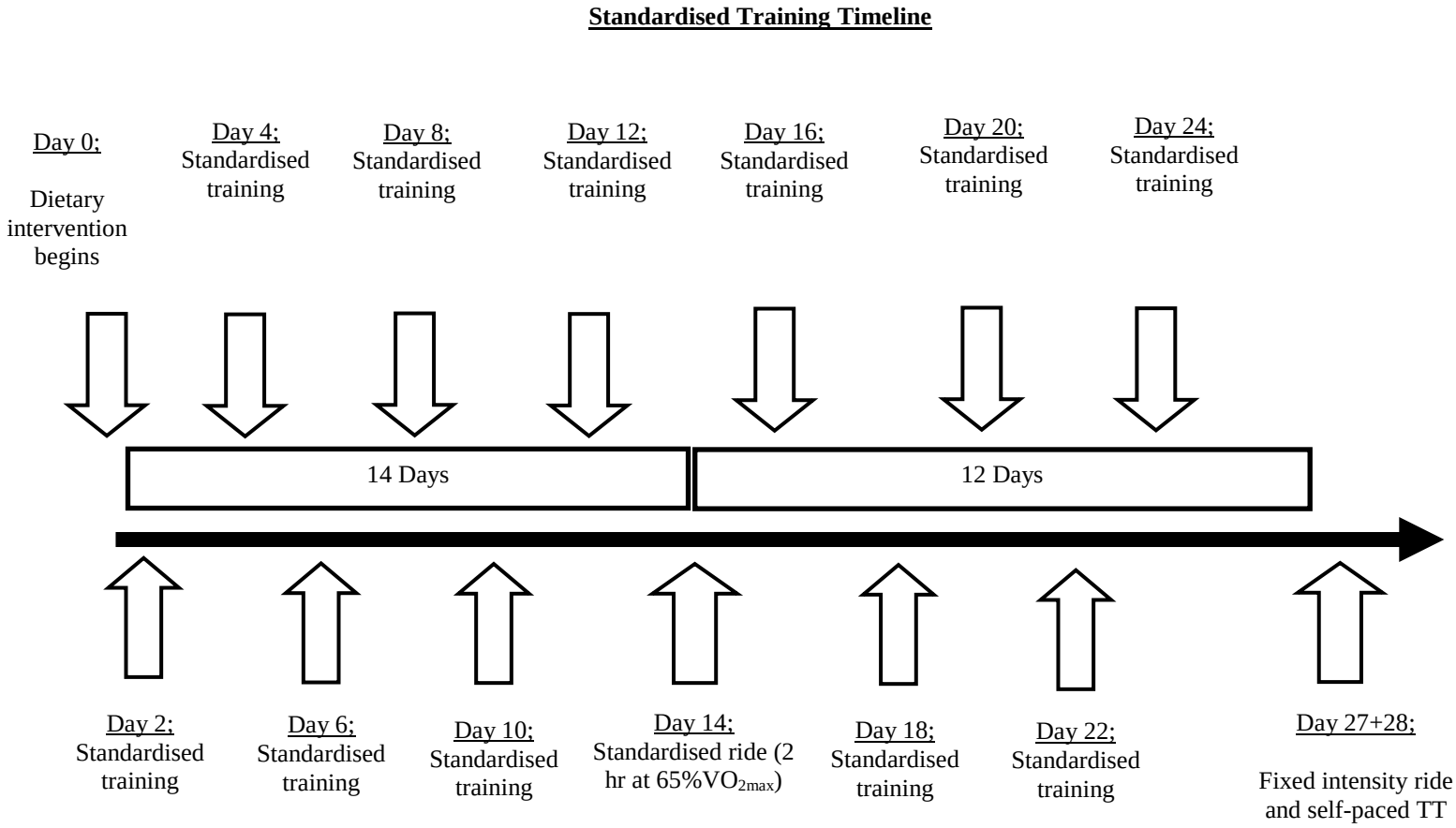


Figure 5-3. Timeline of the standardised training that was applied to each dietary intervention.

5. 5. DIETARY CONTROL

Composition of daily food intake was prescribed and monitored using "MyFitnessPal®" online and mobile software (MyFitnessPal®, Inc. 525 Brannan Street, San Francisco). MyFitnessPal® was selected due to the ease of use of the platform compare to more traditional, and potentially more reliable, diet diaries such as FoodWorks, Easy Diet Diary or Research Food Diary. Because MyFitnessPal® operates via a user-generated database, there is potential for the nutrient profile of foods to be recorded inaccurately. Thus, to avoid any misreporting, the researcher personally checked the participants' diaries every two–three days to ensure foods were being recorded accurately.

In both diets, protein intake was fixed at 15–20% of total energy intake. There were no caloric or other restrictions, to allow the diets to be as natural as possible. During the moderate CHO diet a minimum of 50% of total energy came from CHO and total CHO in grams was at least 5 g/kg/day; this was based off the common endurance athlete's diet (van Erp-Baart, Saris, Binkhorst, Vos, & Elvers, 1989). During the low CHO diet, CHO intake was restricted to <2 g/kg/day; this value selected because it has previously been shown to achieve a significant lowering of the participant's habitual CHO intake (J. W. Helge, 2002)

On initial consultation with the participants, a list of food/meal likes and dislikes, as well as allergies, was compiled, to ensure that the diets were not too far removed from habitual. A sufficient amount of natural and whole foods were provided to ensure adequate micronutrient consumption occurred (e.g., iron, selenium, calcium).

Before the low CHO intervention, the participants were given a copy of the book "The 30-Day Low-Carb Diet Solution" (Eades & Eades, 2010). The introductory chapters pertaining to the "benefits" of a low CHO diet were not included to avoid inducing a bias. The book was selected based on the large array of diet information and recipes it contains.

At the beginning of both interventions, a food parcel was provided for the participants to help educate them in regards to what foods were high and/or low in CHO.

The researcher had personal contact with all participants a minimum of three times per week, and was available via phone and email to answer any queries that the participants had in regards to the diet. In addition, the researcher was able to keep track of the participants' diets by regularly checking their data on MyFitnessPal®.

5. 6. BLOOD COLLECTION DURING THE TWO-HOUR SUBMAXIMAL RIDE

An indwelling cannula was placed in the antecubital vein, after resting expired respiratory gas analysis and prior to the onset of exercise (time = 0 min). The area was disinfected with alcohol before a 20 gauge 1.1 x 30 mm intravascular catheter (BD Insyte, Becton Dickson Infusion Therapy Systems Inc, USA) was inserted into the median cubical vein and 15 cm extension immediately attached (Alaris Products, Carefusion, USA). A 10-mL sample was gently extracted and allocated into three vacutainers as follows: 4 mL lithium heparin, 3 mL EDTA and 3 mL oxaloacetate (BD, Belliver Industrial estate, UK). Samples were immediately placed in a refrigerated centrifuge and allowed to sit for ten minutes at 4^{o}C before being spun at 2500 rpm for twelve minutes. The serum was extracted, placed in 1.5 mL Eppendorf tubes and stored at -80^{o}C until analysis.

The indwelling line was kept patent by injecting 3 mL of saline solution (Demo S.A. Pharmaceutical Industry, Greece) every 20 minutes. A 2-mL sample was taken and discarded before each blood draw or saline solution injection, to avoid clotting or sample contamination. After the last blood draw, the cannula was removed and covered with sterile plaster.

5. 7. MISSING VALUES

There was only one instance where equipment failure resulted in missing values needing to be filled (Study 1/2, participant 13, week 2, moderate CHO, time point 120 minutes, VO_2 and associated variables). This was done to enable the repeated measures ANOVA analysis to be performed, and in this case a single imputation method was used to replace the missing value. The method used was the average group change (AGC).

CHAPTER 6. CHRONIC CARBOHYDRATE RESTRICTION AND ULTRA-ENDURANCE PERFORMANCE

6. 1. INTRODUCTION

Underlying the plethora of physiological, biochemical, and psychological changes that occur when an individual begins an endurance training program there are some fundamental adaptations that are considered most important in improving endurance exercise performance.

At the skeletal muscle level, these include, but are not limited to, increases in capillarisation and oxygen (O_2) transport, mitochondrial (volume) density and associated increased capacities for lipid oxidation and other energy conversion pathways, muscle fibre-type shifting (type IIx → type I), and adrenergic stimulation (Baldwin et al., 1972; Gollnick et al., 1972; John O. Holloszy, 1967; J. O. Holloszy et al., 1970; Molé et al., 1971; Morgan, Cobb, Short, Ross, & Gunn, 1971).

Of these adaptations, an increase in the capacity of muscle to take up and utilise fatty acids, thereby supporting energetic requirements and preserving muscle and liver glycogen, is well described (Bergman et al., 1999; S. L. Carter, Rennie, & Tarnopolsky, 2001; A. R. Coggan et al., 1990; Gollnick et al., 1972; Henriksson & Reitman, 1977; John O. Holloszy, 1967; J. O. Holloszy & Coyle, 1984; J. O. Holloszy et al., 1970; Hurley et al., 1986; Molé et al., 1971). The increased ability of skeletal muscle to take up fatty acids also presents an opportunity for increased lipid storage within the myocyte when the uptake rate is greater than the rate of

oxidation, as shown by greater intramyocellular triglyceride (IMTG) concentrations in athletes vs sedentary adults (Hoppeler, Lüthi, Claassen, Weibel, & Howald, 1973). Whilst training does not necessarily result in greater fatty acid utilisation rates during a single exercise bout (which is also affected by a number of other signals such as muscle glycogen content), the capacity for fatty acid utilisation is improved, enabling continuation of work when muscle glycogen levels and circulating glucose levels decline. This is one reason why an endurance-trained person can exercise for longer than an untrained person at a given exercise intensity.

This training-induced increased potential to utilise fatty acids can be accelerated when the CHO content of the training diet is limited. That is, low CHO training diets, or fasted training, produce an increased capacity for fatty acid utilisation during subsequent exercise compared to when CHO is consumed before or during exercise training (Stannard, Buckley, Edge, & Thompson, 2010). This effect persists even when CHO is provided in significant quantities in the 24 hours prior to the test (Louise M Burke et al., 2000). However, improvements in endurance performance with low CHO diets are not clear. In part, this may be due to methodical flaws (Louise M Burke et al., 2000) or because the performance tests employed have not been sufficiently long enough to engender a limitation based on CHO depletion. In fact, only a handful of low CHO studies have utilised exercise tests of three or more hours, with each citing equal or better performance when compared to a high CHO diet (Carey et al., 2001; Lize Havemann, Goedecke, Noakes, & Lambert, 2007; L. Havemann et al., 2006; Lambert et al., 2001; S. D. Phinney et al., 1983; Rowlands & Hopkins, 2002); though due to the demanding nature of such protocols and necessary length of intervention periods, most of these studies are under-powered. However, despite Erlenbusch and colleagues' meta-analytical evidence outlining much the same heterogeneity in high and low

CHO trials, and specifically endorsing that a high-CHO diet is difficult to support (Erlenbusch, Haub, Munoz, MacConnie, & Stillwell, 2005), high CHO diets are still a standard recommendation for endurance athletes around the globe (Academy of Nutrition and Dietetics, 2016).

A number of studies have observed that females demonstrate greater fatty acid utilisation and less CHO and protein metabolism than equally trained and nourished males, at submaximal intensities (Horton et al., 1998; Knechtle et al., 2004; L. J. Tarnopolsky, MacDougall, Atkinson, Tarnopolsky, & Sutton, 1990). Enzymes specific to fatty acid utilisation within the skeletal muscle of women also respond to a lesser extent than those in men, during training in an overnight-fasted state (Stannard et al., 2010). Combined, these observations suggest that although females generally have a greater capacity than males to utilise fatty acids as a substrate during exercise, their ability to increase this through a dietary stimulus is reduced compared to men. This indicates that males possess greater metabolic flexibility than females.

The current study was designed to fill important gaps in the literature by providing a best-case scenario to test two hypotheses: 1) that chronic low CHO intake improves exercise performance in men and women and 2) that athletes become leaner (lose body fat) on a low CHO diet. The key points of the study were to allow for a sufficient adaptation period (>2 weeks) and exercise duration (>2 hr). The study design was a repeated measure, cross-over four-week dietary intervention (moderate and low CHO), with performance measured as a self-paced time trial (TT) to complete a fixed workload which was equivalent to cycling at 55% VO_{2max} for 5 hr.

6. 2. METHODS

6. 2. 1. Participants

Thirteen well-trained endurance cyclists and triathletes, who trained ten to fifteen hours per week and had a minimum of five years racing experience (eight males, VO_{2max}: 66.0 ± 9.5 ml/kg/min; five females, VO_{2max}: 50.6 ± 8.4 ml/kg/min) participated in this study. The participants were recruited from local cycling and triathlon clubs, as well as through personal contact with the researcher. The participants' characteristics are outlined in Table 7.1. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Massey University (14/09) in April 2014.

Table 6-1. Participant Overview.

Sex	Body Mass (Kg)	VO_{2max} (ml/min/kg)	Maximum heart rate (bpm)	Body fat (%)	Age (yrs)
Female	63.9 ± 10.5	50.6 ± 7.7	180 ±6.2	21.7 ± 5.5	35 ± 10
Male	79.9 ± 13.7	66.0 ± 9.2	188 ± 6.8	14.2 ± 3.1	33 ± 9.4

Data is presented as mean ± SD

6. 2. 2. Study Design

The study design is referred to in Chapter 4, which outlines the protocol used here.

6. 2. 3. Common Methodology

The methods used for the collection data below refer to the protocols outlined in Chapter 5 Common Methodology for body composition, VO_{2max} and submaximal exercise tests, standardised training, and dietary control.

6. 2. 4. Mood Questionnaire

A profile of mood state (POMS) test was used to assess the mood of each participant before and throughout each intervention. Each test gave a total mood disturbance score (TMD) which could be used for within and between subject comparisons. The test was completed weekly by the participants, so that their changes in their TMD within each intervention could be examined.

6. 2. 5. Nutritional Protocol for Exercise Test

In each intervention, all food consumed from the completion of the fasted, submaximal test to the commencement of the TT were standardised and strictly controlled (Prior day: 5g/kg/day CHO, breakfast: 3g/kg CHO). Specifically, ready-made meals were provided for lunch and dinner following the submaximal test, and for breakfast on the morning of the TT, as well as snacks; the exact foods provided are detained in Appendix D. The participants were required to eat all foods provided to them, and were told not to eat any other foods besides this. On arrival into the laboratory for the TT, participants were asked whether they had complied with these requirements; however, no further checks were made to ensure that these dietary requirements had been followed. The food provided was selected to give the participants a

CHO “load” to ensure a “like vs like” comparison for TT performance on each diet, in terms of immediate pre-exercise nutrition. It was assumed that this would mean participants started each TT with a similar glycogen status and thus conclusions drawn around performance could be related to the dietary intervention rather than pre-exercise glycogen status.

To ensure euglycemia was maintained during the TT, participants ingested 40 grams of CHO/hr during exercise via a commercially available sports drink (Gatorade, Frucor Beverages Ltd, New Zealand) for the first two hours. The quantity of 40g/hr CHO was selected to avoid gastro intestinal issues, which have been report for CHO intakes ≥60 g /hr. After two hours, solid food was introduced in the form of a small chocolate bar (Snickers, Mars Inc.) which accounted for 10g of the 40g CHO/hr. Snickers was selected for practicality and the unanimous agreement of enjoyment. Once solid food was introduced, the sports drink volume was decreased to maintain the glucose:H_2O concentration ratio. During the baseline TT, water was made available ad libitum and the amount drunk by each participant was recorded, and replicated for the remaining two TTs. In the last ten minutes of each hour during the TT, participants were warned that they only had a short period of time to consume the remaining sports drink and water that they had left for that hour. At the beginning of each hour, new bottles of sports drink and water were provided.

6. 2. 6. Self-Paced Total Workload Time Trial

Prior to commencement of the cycling TT, baseline respiratory gases, a venous blood sample (venepuncture) and HR (Polar RS800 HRM, Finland) measures were collected (approximately ten minutes before exercise started), following five minutes of rest in a seated position. The same measures were also taken ten minutes after the completion of the trial.

Following the pre-exercise resting measures, the participants mounted the electronically-braked cycle ergometer (Excalibur Sport, Lode BV, The Netherlands), which had previously been set up as closely as possible to the measurements of their own bike (this was replicated in each trial), and pedalled easily for ten minutes, to allow their legs to warm up. Subsequently, a linear factor was applied to the ergometer, which was designed so that a cadence of 90 rpm corresponded to a work rate which was predicted to elicit 55% of the participants' previously measured VO_{2max} (90^2 x 0.036 = 291 W). Thus, if the participant pedalled faster than 90 rpm, their power output and work rate would be greater than this, with the reverse occurring if they pedalled slower than 90rpm. This linear factor was chosen based on previous exercise tests from our laboratory, which have been conducted on athletes of similar fitness levels to the current study's participants. The total work requirement for the TT was based on an average power of 55% VO_{2max} for 5 hr expressed in kilojoules (1W = 3.6kJ); in other words, if a participant pedalled at 90 rpm for the total duration of the test, it would take them 5 hr to complete it, however, if they pedalled faster or slower than this, the duration of the TT would change accordingly. Participants were informed of their progress following the completion of each 5% segment of the TT; however, no other progress or performance feedback information was made available to the participants.

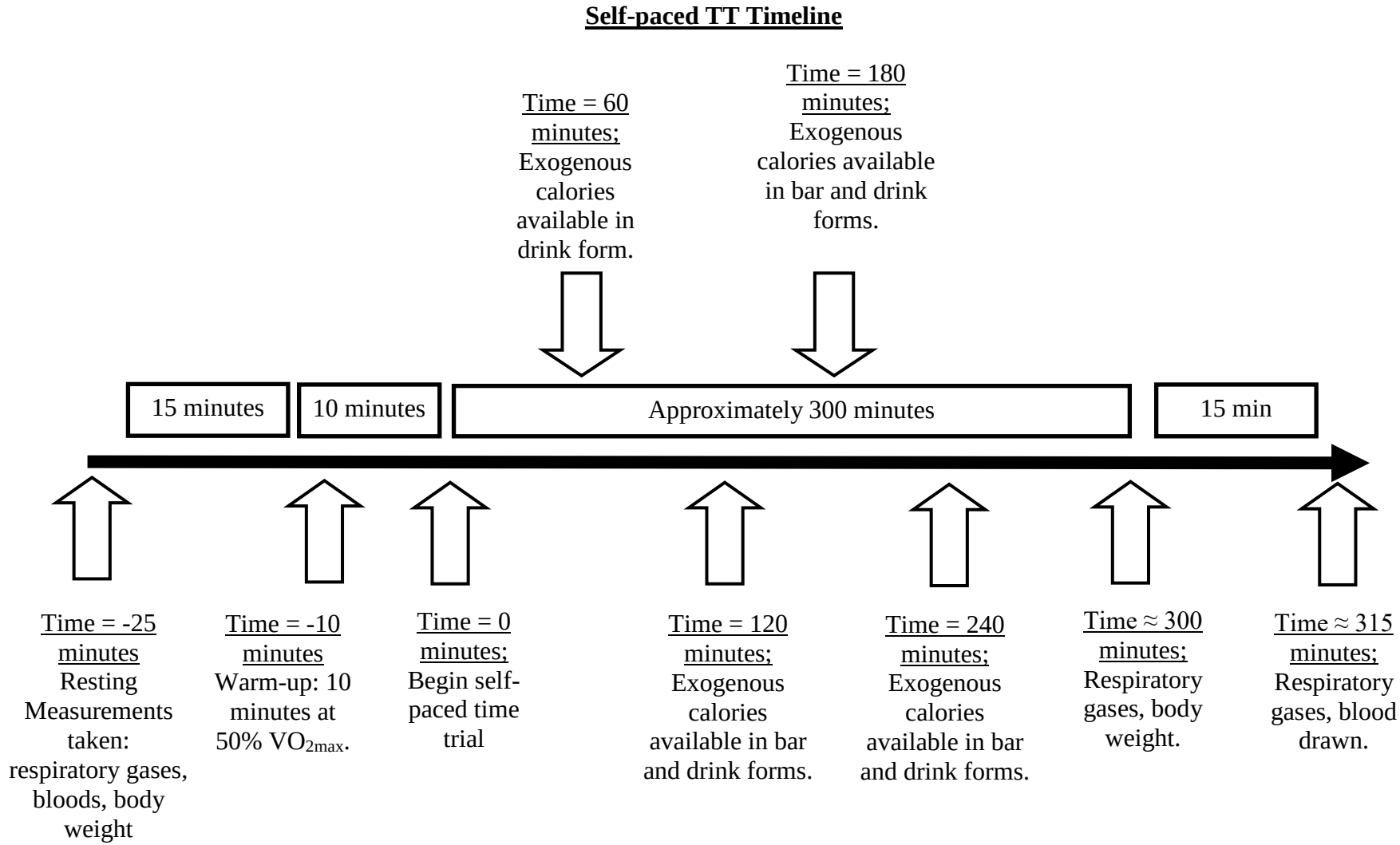


Figure 6-1. Timeline of events during the self-paced time trial.

6. 2. 7. Statistical Analysis

All data were analysed using SPSS Statistics for Windows, Version 23.0. (IBM Corp, NY, USA). A one-way repeated measure analysis of variance (ANOVA) identified the differences in week four performance, measured as time to complete a fixed workload and average W/kg. A two-way repeated measures ANOVA tested diet x time (baseline, week one–four) interactions for changes in body mass, BF% and resting RER. Sex was included as a between-subjects factor. Mauchly's test of sphericity was used to test the sphericity of the ANOVA output and unless otherwise specified, sphericity was assumed. Least squares difference post hoc tests were run to determine the locations of any significant ANOVA findings. To test the effect of order, the data was analysed, as trial one and trial two, with no reference of diet. A paired t-test was used to investigate the effect of order on the performance TT. A repeated measures two-way ANOVA tested the potential effect of order on changes in body mass and BF% within and between each intervention. Significance was set at $p \leq 0.05$. The effect sizes (ES) of significant interactions were calculated using Cohen's d (0.1 small; 0.5 medium; 0.8 large)

6. 3. RESULTS

6. 3. 1. Performance

6. 3. 1. 1. Time

There was no main effect of diet (p = 0.06) or sex on time to complete the time trial (p = 0.31) (Figure 6-2).

6. 3. 1. 2. Absolute Power Output

There were main effects of diet (p = 0.030) and sex (p = 0.030) on average power output produced during the time trial and a near significant interaction between the two (p = 0.050). Average power output was higher following the low CHO diet than at baseline and following the moderate CHO diet (mean difference = 17.38 W, 95% CI: 2.18 to 32.58: P = 0.03, ES = 0.35 & mean difference = 6.025 W, 95% CI: -0.15 to 12.20: p = 0.055, ES = 0.11).

6. 3. 1. 3. Relative Power Output

There was a main effect of diet (p = 0.023) on relative power output (W/Kg), but there were no main effect or interactions of sex (p = 0.087). The participants held significantly more watts relative to body mass on the low CHO diet compared to baseline (mean difference = 0.261 W/kg, 95% CI: 0.064 to 0.458: p = 0.01, ES = 0.46) and the moderate CHO diet (mean difference = 0.137 W/kg, 95% CI: 0.047 to 0.227: p = 0.07, ES = 0.21) (Figure 6-2).

The order effect tested for any difference in means between trial one and two for time to complete the TT. No significant difference was detected (mean difference = 0.042 h, 95% CI: -0.172 to 0.257: p = 0.68, ES = 0.083).

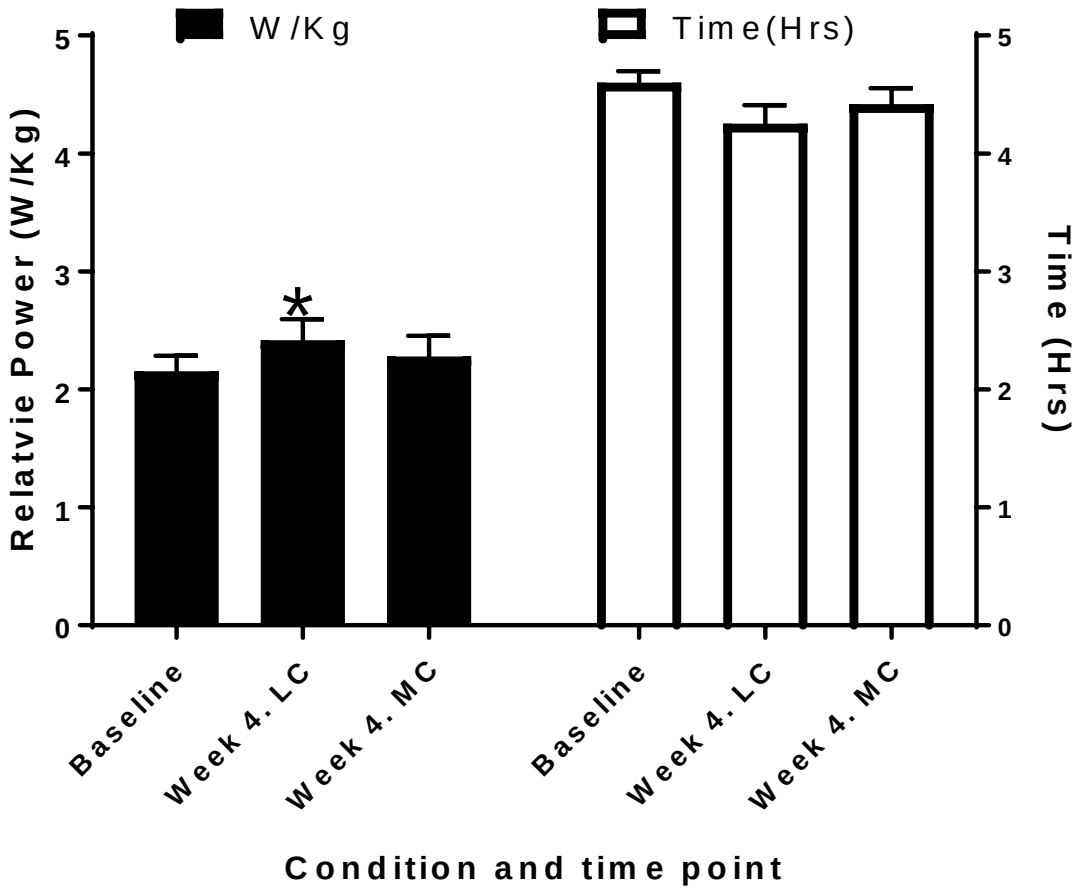


Figure 6-2. Overall performance for baseline and week four self-paced cycle time trials, measured as watts per kg (black) and time to completion (white). * denotes significant ($p \leq 0.05$) difference between diets.

6. 3. 2. Body composition

There was a significant main effect of diet on body mass (p = 0.01) but not sex (p = 0.09). Both time and the interaction of time and diet did not affect body mass (p = 0.14 and p =

0.11, respectively), nor was there any interaction between sex and the independent variables (diet, $p = 0.49$; and time, $p = 0.45$).

There were significant main effects of both independent variables on BF% (diet, $p = 0.05$, time $p = 0.04$). However, there was no interaction ($p = 0.16$) between the two. Sex significantly interacted with time ($p = 0.01$) and the diet-time interaction ($p = 0.03$) but not diet ($p = 0.99$). Post-hoc inspection showed that when compared to the start of the respective intervention, women lost BF% (mean difference = -0.82 %, 95% CI, -1.49, -0.15, $p = 0.02$, ES = 0.13) on the low CHO diet and trended toward gaining BF% (mean difference = 1.34 %, 95% CI, -0.25, 2.93, $p = 0.09$, ES = 0.21) on the moderate CHO diet, whereas men lost BF% on both low CHO (mean difference = -1.08 %, 95% CI, -1.60, -0.55, $p = 0.001$, ES = 0.18) and moderate CHO diets (mean difference = -1.64 %, 95% CI, -2.90, -0.38, $p = 0.015$, ES = 0.26) *(*Figure 6-3).

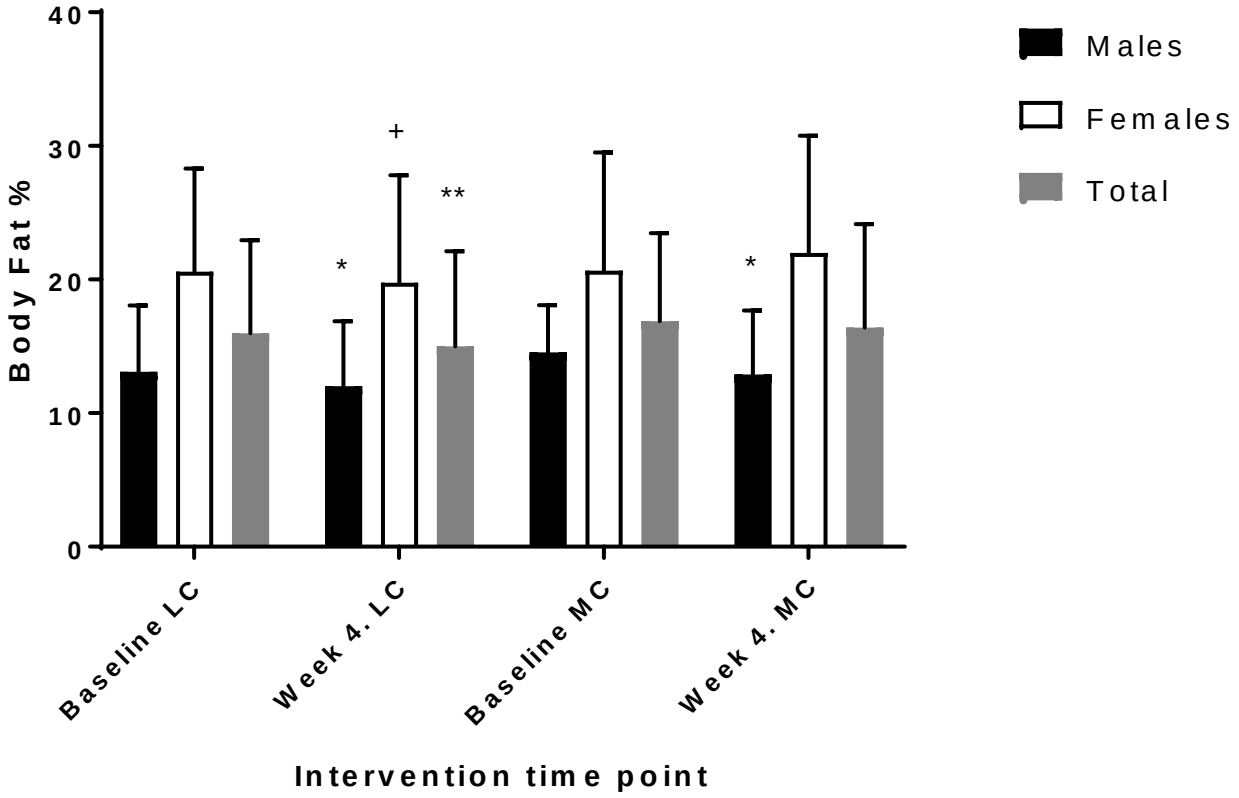


Figure 6-3. Total mean and sex specific percentage body fatness (BF%) measured before and after each four-week dietary intervention. * denotes significant ($p \leq 0.05$) difference between baseline and week four measures across both interventions in males. ** denotes significantly lower BF% in week four of the low CHO diet compared to all other time points. + denotes significant difference between baseline and week four of the low CHO diet in BF% for females.

6. 3. 3. Resting metabolism

The results of the two-way repeated measures ANOVA including sex as a dichotomous variable are displayed in Figure 6-4 and show main effects of diet (p = 0.001) and time (p = 0.001) as well as a diet-time interaction (p = 0.04) on fasted and CHO-loaded resting RERs. Sex had no effect on either of the independent variables or their interaction. The post hoc analysis showed that fasted RER was significantly lower during the low CHO diet than both

baseline (mean difference = - 0.108, 95% CI, -0.160, -0.056, p = 0.001, ES = 1.21) and the moderate CHO diet (mean difference = -0.072, 95% CI, -0.105, -0.038, p = 0.001, ES = 1.00). CHO loaded RER was higher than baseline and both fasted low CHO and moderate CHO diets (mean difference = 0.076, 95% CI, .017, 0.135, p = 0.02, ES = 0.61 and mean difference = 0.064, 95% CI, .019, .109, p = 0.01, ES = 0.68 respectively). There was a near significant difference between CHO-loaded low CHO and moderate CHO (p = 0.07).

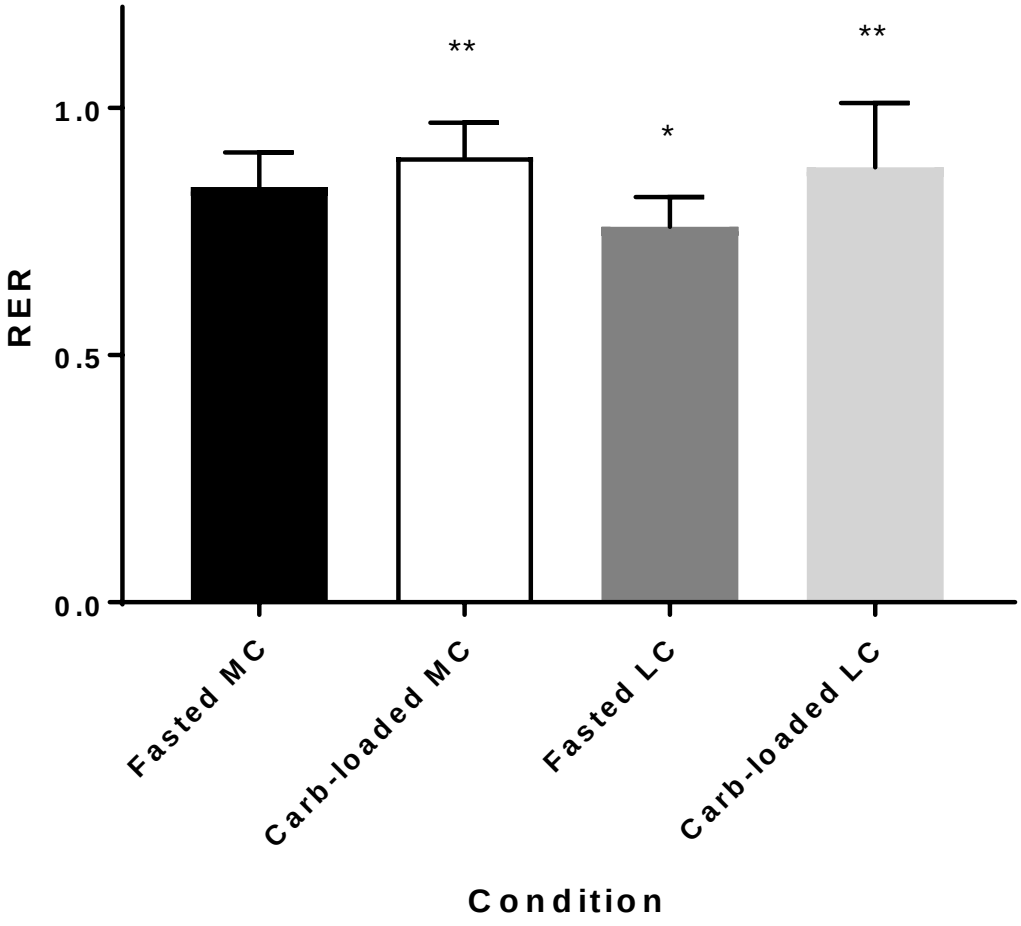


Figure 6-4. Resting RER values in fasted (prior to 2 hr fixed intensity ride) and CHO loaded (prior to TT) states in week four. * denotes significant ($p \leq 0.05$) difference between fasted low CHO and all other measures. ** denotes significant ($p \leq 0.05$) difference between CHO-loaded RER and fasting RER.

When testing for order, there were no significant effects of trial or time and no interaction between trial and time (p = 0.09) on body mass. Results were similar for BF%, with no effect of trial (p = 0.73), a significant effect of time (p =0.04), and no interaction between trial and time (p = 0.68).

6. 3. 4. Diet

Table 6-2. Average daily macronutrient and caloric intake.

		Female			Male			Total		
		Mean (kcal)	SD	%	Mean (kcal)	SD	%	Mean (kcal)	SD	%
Moderate CHO	CHO	1490	±320	66%	2013	±288	62%	1775	±396	62%
	Fat	432	±81	19%	790	±185	24%	627	±234	24%
	PRO	345	±83	15%	435	±124	14%	394	±112	14%
	Total	2267	±449		3237	±405		2796	±648	
Low CHO	CHO	360	±39	19%	566	±75	19%	472	±123	19%
	Fat	1211	±247	62%	1889	±486	62%	1581	±517	62%
	PRO	382	±144	19%	558	±119	19%	478	±154	19%
	Total	1952	±353		3012	±595		2530	±730	
Habitual	CHO	857	±405	43%	1266	±225	48%	1080	±369	46%
	Fat	766	±230	41%	1027	±521	37%	908	±419	38%
	PRO	301	±58	16%	410	±138	15%	360	±119	15%
	Total	1923	±297		2704	±427		2349	±541	

There were main effects of diet (p = 0.01) and sex (p = 0.002) on total caloric intake. Post hoc comparisons showed that participants consumed more calories on the moderate CHO diet compared to their habitual diet (mean difference = 498.28 kcal, CI 95%, -156.1, 493.58, p = 0.006, ES = 1.00) and the low CHO diet (mean difference = 269.54 kcal, CI 95%, -10.31, 549.38, p = 0.057, ES = 0.35) (mean difference = 438.28 kcal, 95% CI, 160.47, 716.09, p = 0.006, ES = 1.00) and as expected, women consumed less calories overall (mean difference = -937.06 kcal, CI 95%, -1429.36, -444.36, p = 0.002, ES = 2.41) (Table 6-2).

Similarly, there were main effects of diet and sex on caloric intakes from CHO (p = 0.001 and 0.006) and fat (p = 0.001 and p = 0.02). CHO caloric intakes were higher during the low CHO diet relative to the participants' habitual diets (mean difference = 598.92 kcal, CI 95%, 383.57, 814.27, p = 001, ES = 2.40) and lower on the moderate CHO diet (mean difference = -689.45 kcal, CI 95%, -939.47, -439.42, p = 0.001, ES = 2.04). Fat caloric intakes were lower on the moderate CHO diet (mean difference = -285.55 kcal, CI 95%, -539.69, -31.41, p = 0.032, ES = 0.84) and higher on the low CHO diet than the habitual diets (mean difference = 953.24 kcal, CI 95%, 365.29, 941.18, p = 0.001, ES = 1.49). There was a strong tendency for diet and sex to affect protein intake (p = 0.06).

6. 3. 5. Additional data

Diet had no significant effect on total mood disturbance (TMD) (p = 0.20), whereas time showed a nearly significant effect (p = 0.07). The interaction between diet and time was significant (p = 0.03) and there was a trend towards significance (p = 0.09) for the diet x sex interaction.

Post hoc comparisons showed no significant difference in TMD across the moderate CHO intervention. However, within the low CHO diet, a significant negative difference was found between baseline and week two (mean difference = -21.25, CI 95%, -40.34, -2.16, p = 0.033, ES = 0.76) and near significant positive differences between baseline and week three (mean difference = -13.50, CI 95%, -27.915, 0.92, p = 0.06, ES = 0.50) and week two compared to week four (mean difference = 19.81, CI 95%, -1.21, 40.84, p = 0.06, ES = 0.73).

6. 4. DISCUSSION

The primary purpose of this study was to investigate whether a four-week low CHO diet could improve endurance performance in men and women when compared to a moderate CHO diet. Our findings demonstrate that, when assessed in the context of absolute (W) and relative power outputs (W/kg), ultra-endurance cycling performance improved on a low CHO diet.

Whilst we do not present mechanistic data, our results very likely reflect contrasting changes in the oxidative capacity of the trained muscle on each diet. It can be seen from the fasted RER data (recorded the day prior as per Chapter 4) that a moderate CHO diet results in a higher reliance on CHO as an energy source at rest (Figure 6-4) while the converse occurs during a period of low CHO intake. It is likely that the low CHO intervention provided a strong adaptive stimulus to metabolically active tissue other than skeletal muscle, specifically the liver (Webster et al., 2016), which became more proficient at sparing CHO at rest (Nilsson & Hultman, 1973). Along these lines, it has previously been shown that fasted training or low

CHO training diets in either sex produce a greater capacity to accumulate and store CHO as glycogen in the muscle (Stannard et al., 2010) and liver (Nilsson & Hultman, 1973; Saitoh, Shimomura, & Suzuki, 1993), which has been reported to assist an increase in endurance exercise capacity (Lambert et al., 2001).

The fact that each sex responded similarly for performance but differently for body composition suggests that there are differing metabolic/mechanistic responses occurring between sexes. This could be related to the inherent metabolic inflexibility of women, which may result in a blunted adaptation to a chronic moderate CHO intake. It could be said that men are more metabolically flexible than women and more able to respond to large changes in macronutrient availability, whereas women, although able to handle low CHO intakes, are far less metabolically able to tolerate large loads of CHO outside of exercise, resulting in a trend toward fat gain. However, it is acknowledged that the overall small sample size may have impacted the gender comparison in the current study.

When CHO depletion is facilitating high rates of gluconeogenesis (Webster et al., 2016) and glycogen sparing (Sherman et al., 1981), it provides a “best case scenario” for both sexes to exploit their full lipid utilising capacities during exercise, enabling better ultra-endurance performances when compared to the traditional athlete dietary recommendations (Academy of Nutrition and Dietetics, 2016; Louise M. Burke, Hawley, Wong, & Jeukendrup, 2011). During rest and submaximal exercise, high rates of lipid oxidation can be advantageous for reducing body mass and, more specifically, BF loss (St-Onge & Jones, 2003). Accordingly, drawn from this was a secondary hypothesis that athletes would become leaner (lose BF) on the low CHO diet. Our data supported this hypothesis because women tended to gained BF on the moderate CHO diet (p =0.09) and lose body fat on the low CHO diet (p = 0.03), whereas

the men lost almost the same amount of BF on both diets (Figure 6-3). However, as with previous research (J. Volek et al., 2004), it was observed (albeit non-significantly) that women consumed less calories (approx. 300 kcal/day) on the low CHO diet, whereas men had near identical caloric intakes on both diets.

The POMs test results showed an initial reduction in TMD on the low CHO diet from baseline to week 2 (p = 0.03). This finding aligns with recent research which showed a significant decline in TMD over the first eight weeks of a very low CHO diet (Brinkworth, Buckley, Noakes, Clifton, & Wilson, 2009). Since baseline and week four TMD scores were not significantly different, mood could be acting as a proxy for the body adapting to a chronic change in macronutrient intake. This would align with previously reported negative impacts of low CHO diets on endurance performance when the dietary intervention period was greater than seven days (Ref table L. M. Burke and Hawley (2002)). Due to the nature of the diets, it was not possible to blind the athletes to either diet, so it is possible that prior perception of the diets may have affected the participants' behaviour. The authors also acknowledge the lack of control for the menstrual cycle, which was done due to time constraints and to allow for an easy inclusion of women in this study. In this regard, research is mixed and multiple references have shown little effect of the menstrual cycle on endurance performance (Dombovy et al., 1987; Nicklas et al., 1989).

Based on the results observed in the current research, it is suggested that further research in the area of low CHO and endurance performance should be focused on periodisation and sex differences, as there are periods in a training and competition cycle where high CHO oxidation rates are more important than high rates of fatty acid oxidation and vice versa. The approaches taken to account for the changing demands of a competition cycle may differ for

men and women. In a general sense, having high fatty acid oxidation levels during recovery and non-crucial periods of endurance events could be vital to preserving glycogen for high intensity bouts that can occur within the event itself or on subsequent days of competition. In addition, body composition is an important aspect of endurance sport where power–weight ratios can significantly influence performance and any means of improving body composition without sacrificing performance need to be considered. Another area for consideration is gastrointestinal (GI) distress, which is commonly associated with ultra-endurance events when exogenous CHO intake is high (Louise M. Burke et al., 2011). Being less reliant upon intake of CHO through improved rates of lipid oxidation may reduce the probability of overloading the GI tract and thereby improve fluid uptake and performance.

6. 4. 1. Conclusion

A low CHO diet resulted in improved ultra-endurance cycling performance in athletes of both sexes compared to the more traditional higher CHO dietary recommendations.

CHAPTER 7. PHYSIOLOGICAL RESPONSES OF MEN AND WOMEN TO A CHRONIC LOW CARBOHYDRATE TRAINING DIET.

7. 1. INTRODUCTION

CHO-based foods are derived from plants, and the availability of these is seasonal, particularly in temperate and colder climates. Periods of low CHO intake would have been experienced by many humans for millions of years prior to the development of agriculture and food storage techniques. Since the re-introduction of the needle biopsy technique in the 1960s for investigating skeletal muscle metabolism, much scientific effort has gone into describing the importance of CHO, and particularly muscle glycogen, as a substrate for contracting muscle. Evidence shows, without doubt, that optimal performance of skeletal muscle during strenuous activity is not possible in a muscle glycogen depleted state (Louise M. Burke et al., 2011). However, optimal performance aside, for the physically active (pre-agricultural) hunter-gatherer it would have been necessary to maintain physical adeptness when faced with a relative CHO depletion, to catch animals for food when plant-based food availability was reduced (Stannard & Johnson, 2004a). The necessity to maintain the capacity to exercise in a glycogen deplete state mirrors the environment of an endurance athlete (Johnson et al., 2003) or more specifically an ultra-endurance athlete nearing the end of an almost day-long bout of exercise.

If the physiological capacity to maintain performance in the face of glycogen depletion is present through our evolutionary heritage then an opportunity exists to utilise this capacity to gain a performance advantage. By mimicking the CHO depletion experienced by our ancestors during endurance training we may advance one's ability to cope with low muscle glycogen levels, either by reducing the reliance upon glycogen from the outset of exercise and/or maintaining relative work rate in the latter stages of endurance exercise when glycogen stores are low.

Low CHO diets have been experimented with since the early 20th century in an attempt to up-regulate the rate of lipid utilisation and more recently increase IMTG storage (Sherman & Leenders, 1995). Accretion of IMTG ensures a means of providing an intramuscular substrate store to best preserve physical work capacity in the face of challenged glycogen status (Stannard & Johnson, 2004b). However, one criticism of nearly all research performed on low CHO diets and endurance training has been the absence of sufficient time to adapt to the diet (J. S. Volek et al., 2015). As a result, the scientific literature describes more closely an acute response, rather than chronic adaptation to low CHO intake (Yeo et al., 2011). In addition, despite past research showing that the skeletal muscle of men and women adapt differently to training in the fasted state (Stannard et al., 2010) no research has been done comparing men and women adapting to a low CHO training diet.

In this regard a common and unsubstantiated claim is that "women are naturally better fat burners than men" (Gillen, 2014). This phrase remains entrenched regardless of the research being incongruous (Horton et al., 1998; Knechtle et al., 2004; Roepstorff et al., 2002; Zehnder et al., 2005). If women are shown to utilise more lipid than men at rest or during physical work it may be context-dependent and due to the degree of metabolic flexibility of each sex.

That is, women may rely on lipid utilisation because they are not metabolically flexible enough to adapt to oxidising CHO at great rates even if available. The "women use more fat" paradigm may have been shown to be the case because the majority of sex comparative research is done in a CHO abundant state (Horton et al., 1998; Knechtle et al., 2004; Powers, Riley, & Howley, 1980; Roepstorff et al., 2002; Zehnder et al., 2005). In the CHO loaded state, men are not seen to be as adept at utilising fat as women, not because they are not, but perhaps because their muscles are more metabolically flexible and thus able to burn CHO at a rate proportionate with availability. A chronic low CHO training diet, in this instance, may provide a best-case scenario for which men and women are able to maximise their natural fat burning capacity. Therefore, it is hypothesised that, when compared to a standard moderate CHO diet, a chronic low CHO training diet will allow for increases in fat oxidation and reductions in CHO oxidation that will be more pronounced in men than women during fasted exercise.

The primary aim of this study was to examine the physiological effects of chronic (>2 weeks) low CHO training diet on fasted endurance exercise as compared to a standard moderate CHO athlete diet. The secondary aim was to compare and contrast sex specific adaptations to each dietary intervention.

7. 2. METHODS

7. 2. 1. Participants

The study participants were thirteen well-trained endurance athletes with a minimum of five years racing experience (eight males, VO_{2max}: 66.0 ± 9.5 ml/kg/min; five females, VO_{2max}:

50.6 ± 8.4 ml/kg/min). The participants' characteristics are outlined in section 6. 2. 1. , Table 6-1. Participant Overview.

7. 2. 2. Fixed Intensity Ride

The participants presented to the laboratory in the morning after an overnight (water-only) fast. Baseline respiratory gases, a venous blood sample (via indwelling cannula) and HR (Polar RS800 HRM, Finland) measures were collected ten minutes before exercise, while at rest, in a seated position. The exercise bout began with a five-minute warm-up at a workload calculated to elicit 40% VO_{2max} before progressing immediately into the two hour fixed intensity ride at 60% VO_{2max}. Ventilatory gases and blood samples were collected for one minute at the following time points: ten minutes, forty minutes, eighty minutes and two hours (Figure 7-1). Water was made available to the participants *ad libitum* for the first ride and was recorded so that fluid intake during the subsequent rides would be the same. A more comprehensive methodology is outlined in Chapter 4.

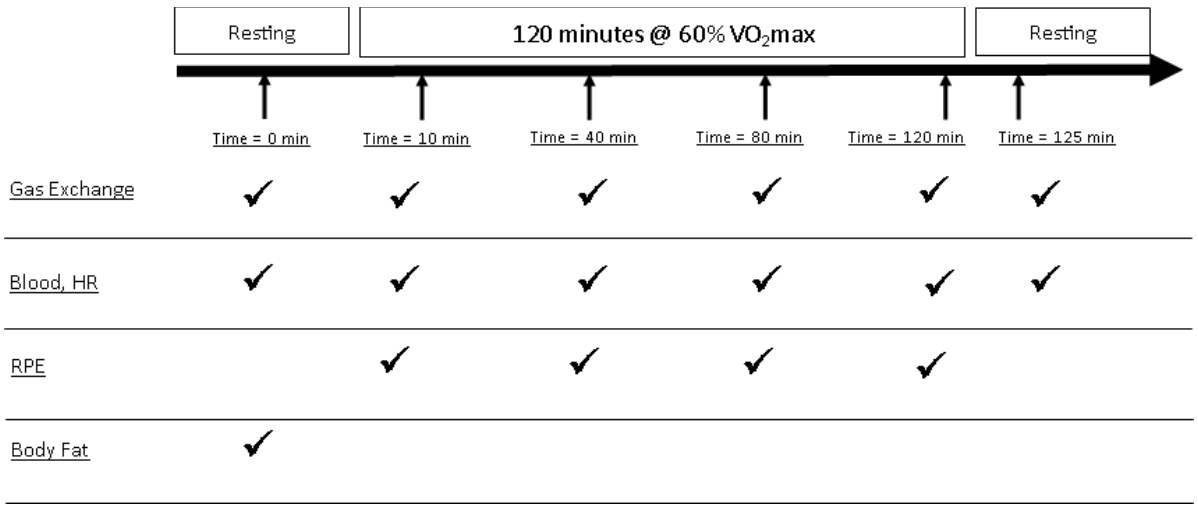


Figure 7-1. Timeline of experimental measurements.

7. 2. 3. Common Methodology

The methods used for the collection data referring to the protocols used for body composition, the VO_{2max} and submaximal tests, standardised training and dietary control are described in the Chapter 5 Common Methodology.

7. 2. 4. Statistical Analysis

All data were analysed using SPSS Statistics for Windows, Version 23.0. (IBM Corp, NY, USA). Three-way repeated measures ANOVAs tested for main effects of, and interactions between, diet (low CHO & moderate CHO) x trial (baseline, week two, week four) x time (10 minutes, 40 minutes, 80 minutes, 120 minutes) on substrate oxidation rates, RER, RPE, HR and VO_2, with sex included as a between-subjects factor. Mauchly's test of sphericity was used to test the sphericity of the ANOVA output and unless otherwise specified, sphericity was assumed. Least squares difference post hoc tests were employed to investigate the location of significant effects indicated in the ANOVAs. Pearson's correlation was used to test the relationship between substrate oxidation rates and RPE. To test the effect of order, the data was analysed as trial one and trial two, with no reference of diet. Repeated measures two-way ANOVAs tested the potential effect of order on changes in variables mentioned previously. Significance was set at $p \leq 0.05$. All data are presented as mean ± standard deviation (SD) or mean difference and 95% confidence interval (CI), as appropriate. The effect sizes (ES) of significant interactions were calculated using Cohen's d (0.1 small; 0.5 medium; 0.8 large)

7. 3. RESULTS

7. 3. 1. RER

During exercise, there was a significant main effect of time (p = 0.001) with RER decreasing with exercise duration (10–120 min) (mean difference = - 0.046, 95% CI, -0.034, -0.059, p = 0.001, ES = 1.579). There was also a significant main effect of diet (p = 0.002), RER being lower overall with the low CHO diet (mean difference = - 0.041, 95% CI, -0.073, -0.008, p = 0.0013, ES = 1.263) (Figure 7-2). No impact of sex was apparent, either as a main effect (p = 0.363) or through interactions with diet (p = 0.922) or time (p = 0.113). Significant interactions between time x diet (p = 0.045) and diet x trial (p = 0.034) occurred (Figure 7-2), indicating that proportional fuel selection (RER): a) changed during exercise duration to a different degree between diets; and b) that changes in fuel selection between trials (over the four-week intervention), occurred differently between trials.

The post hoc analysis indicated that RER decreased to a greater extent with exercise duration on the moderate CHO diet (mean difference = - 0.055, 95% CI, -0.075, -0.035, p = 0.0001, ES = 1.602) compared to the low CHO diet (mean difference = - 0.033, 95% CI, -0.044, -0.022, p = 0.0001, ES = 0.925). For the first and second trial, RER did not differ between diets during exercise (mean difference = - 0.009, 95% CI, -0.029, 0.010, p = 0.330, ES = 0.277 and mean difference = - 0.045, 95% CI, -0.105, 0.014, p = 0.122, ES = 0.734 respectively) but by the end of each intervention (week four), RER was significantly lower on the low CHO diet during exercise (mean difference = - 0.068, 95% CI, -0.105, -0.031, p = 0.002, ES = 1.352). (Figure 7-2).

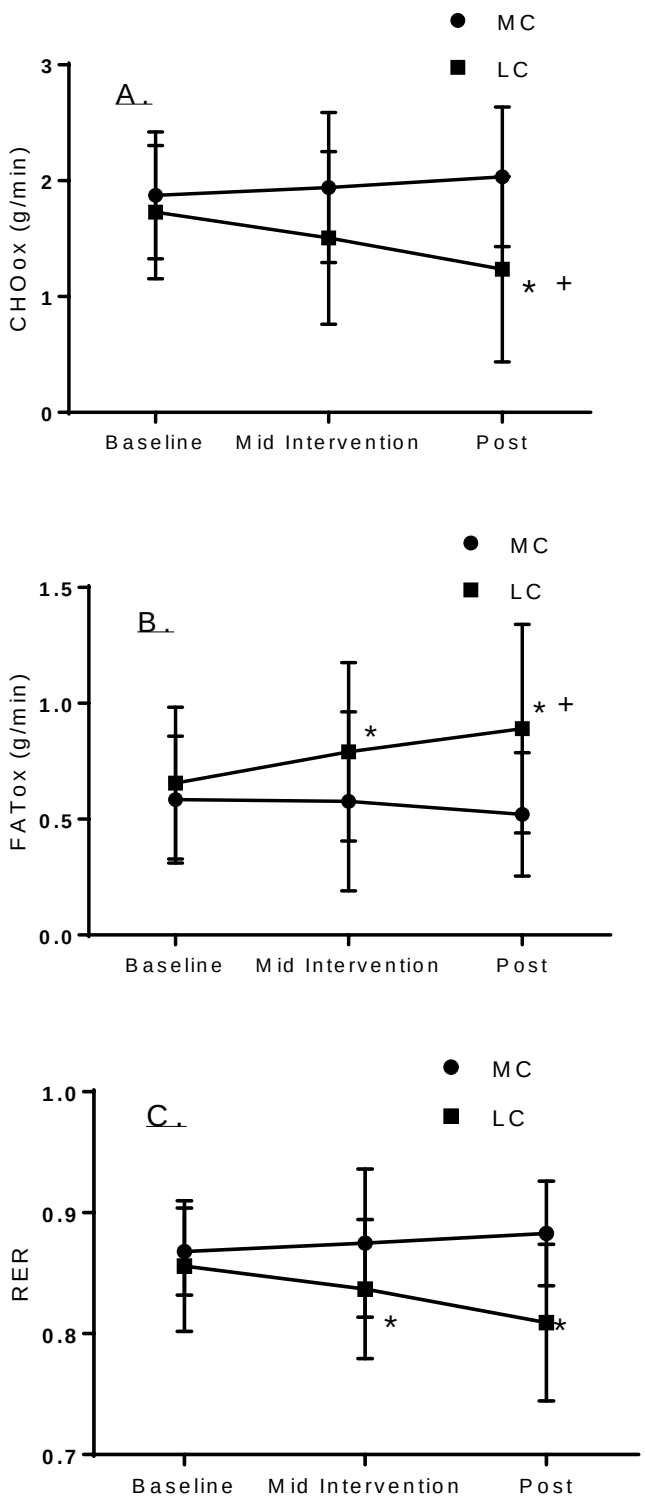


Figure 7-2. A. Average carbohydrate oxidation rate (g/min) during exercise. B. Average fat oxidation rate. C. Average respiratory exchange ratio (RER). * Denotes values from the same time point that are significantly different between interventions. + Denotes values that are significantly different from all time points in both interventions.

7. 3. 2. Fat Oxidation (FAT_{ox})

Aligning with the RER results, there was a main effect of (exercising) time on FAT_{ox} (p = 0.001), with increasing absolute rates of fat utilisation as the duration of exercise progressed (mean difference = 0.212 g/min, 95% CI, 0.143, 0.281, p = 0.001, ES = 0.764). There was also a main effect of diet (p = 0.03) as well as an interaction between diet and trial (p = 0.05) Figure 7-2B on FAT_{ox}. There was a main effect of sex on FAT_{ox}, with men having higher rates throughout exercise (mean difference = 0.409 g/min, 95% CI, 0.063, 0.754, p = 0.025, ES = 1.490); however, there were no significant sex-related interactions (Table 7-1).

Post hoc comparisons revealed that FAT_{ox} during exercise was significantly higher at the two week time-point compared to baseline (mean difference = 0.134 g/min, 95% CI, 0.032, 0.236, p = 0.015, ES = 0.374), and significantly higher post-intervention compared to both baseline and two weeks on the low CHO diet (mean difference = 0.234 g/min, 95% CI, 0.090, 0.378, p = 0.004, ES = 0.593 and mean difference = 0.10 g/min, 95% CI, -0.018, 0.182, p = 0.021, ES = 0.238 respectively) (Figure 7-2B).

7. 3. 3. Carbohydrate Oxidation (CHO_{ox})

For rates of CHO_{ox}, there were main effects of diet (p = 0.023) and time (p = 0.001) but no interactions between the two (Figure 7-2A). There was a main effect of sex on CHO_{ox} (p = 0.031), but no significant sex-related interactions (Table 7-1).

The increase in FAT_{ox} on the low CHO diet was coupled with significantly lower CHO_{ox} in week four compared to baseline and 2 weeks (mean difference = -0.491 g/min, 95% CI, -

0.971, -0.012, p = 0.046, ES = 0.704 and mean difference = -0.271 g/min, 95% CI, -0.493, -0.048, p = 0.021, ES = 0.635, respectively) (Figure 7-2A).

Table 7-1. Sex comparison for change in exercising substrate oxidation rates from baseline to week four.

	Moderate CHO				Low CHO			
	FATox (g/min)		CHOox (g/min)		FATox (g/min)		CHOox (g/min)	
Men	-0.08	±0.08	0.2	±0.2	.196*	±0.08	-0.4	±0.27
Women	-0.05	±0.1	0.12	±0.25	.272*	±0.1	-0.58	±0.34

*Denotes significantly higher value compared to moderate CHO.

7. 3. 4. Week four comparisons

Heart rate during exercise, submax fixed intensity ride, in week four was significantly affected by time (p = 0.001) but not diet (p = 0.616). Time and diet interacted significantly (p = 0.018), along with time and sex (p = 0.020).

As expected, HR increased from start (10 minutes) to finish (120 minutes) of exercise (mean difference = 11.67 bpm, 95% CI, 8.021, 15.317, p = 0.001, ES = 1.340), and this cardiovascular drift occurred more so with men than women (mean difference = 15.94 bpm, 95% CI, 11.413, 20.462, p = 0.001, ES = 1.882 and mean difference 7.40 bpm, 95% CI, 1.677, 13.123, p = 0.016, ES = 0.85, respectively).

There were main effects of diet (p = 0.002) and time (p = 0.011) on relative VO_2 during exercise in week four. Relative VO_2 increased from the beginning to the end of exercise in both diets (mean difference = 1.197 ml/kg/min, 95% CI, 0.277, 2.118, p = 0.015, ES = 0.172) and was significantly higher on the low CHO diet (mean difference = 3.282 ml/kg/min, 95%

CI, 1.503, 5.061, $p = 0.002$, ES = 0.461). The time x diet interaction was not significant ($p = 0.489$) and there were no interactions of diet or time with sex ($p = 0.958$ and 0.498, respectively).

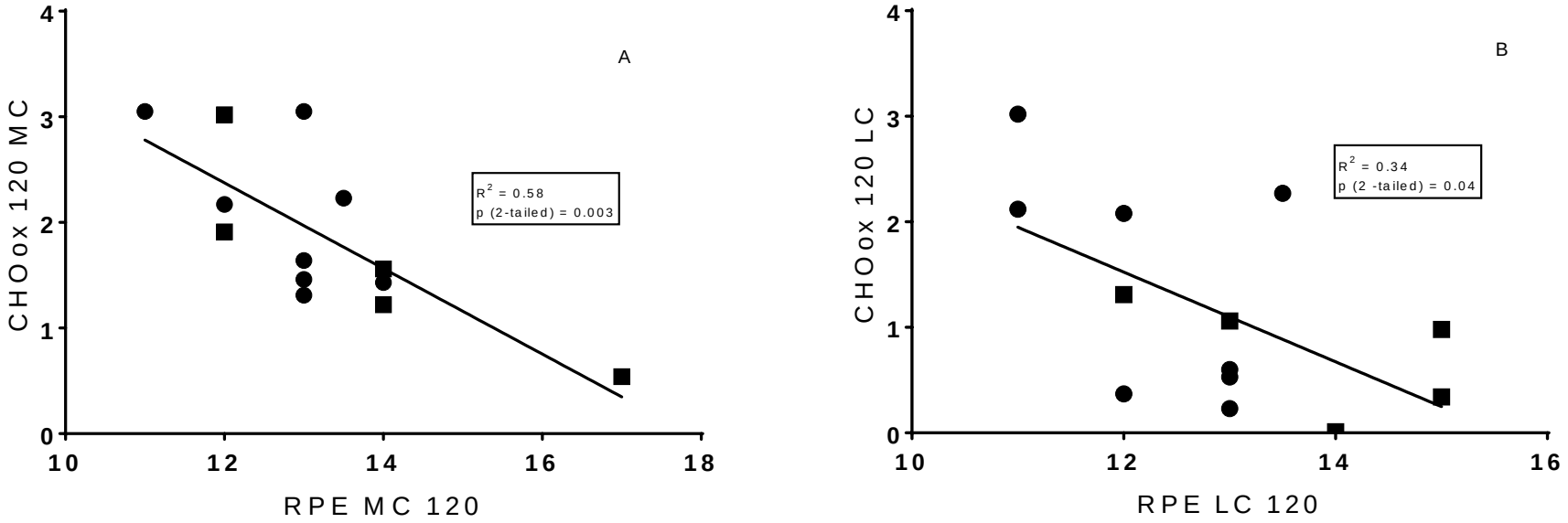


Figure 7-3. Pearson correlation of CHOox and RPE during exercise for each intervention at week four. ■ represents females, ● represents males.

Week four RPE was not significantly affected by diet (p = 0.426 but there was a main effect of time (p = 0.001). There was a significant interaction between diet x time x sex (p = 0.03). The post hoc analysis showed RPE increased from the start to the finish of the exercise (mean difference = 1.438, 95% CI, 0.742, 2.133, p = 0.001, ES = 1.063), with women having a higher RPE at the end of exercise finish (mean difference = 1.238, 95% CI, -0.229, 2.704, p = 0.09, ES = 0.727). Pearson's correlation analysis showed a significant negative correlation between RPE at 120–minutes and CHO_{ox} at the same time point in both diets (Figure 7-3), but no such correlation was found with FAT_{ox}.

7. 3. 5. Diet

Table 7-2. Total and sex specific dietary information for each intervention.

		Female			Male			Total		
		Kcal	SD	%	Kcal	SD	%	Kcal	SD	%
Moderate CHO	CHO	1490	±320	66%	2013	±288	62%	1775	±396	62%
	Fat	432	±81	19%	790	±185	24%	627	±234	24%
	PRO	345	±83	15%	435	±124	14%	394	±112	14%
	Total	2267	±449		3237	±405		2796	±648	
Low CHO	CHO	360	±39	19%	566	±75	19%	472	±123	19%
	Fat	1211	±247	62%	1889	±486	62%	1581	±517	62%
	PRO	382	±144	19%	558	±119	19%	478	±154	19%
	Total	1952	±353		3012	±595		2530	±730	
Habitual	CHO	857	±405	43%	1266	±225	48%	1080	±369	46%
	Fat	766	±230	41%	1027	±521	37%	908	±419	38%
	PRO	301	±58	16%	410	±138	15%	360	±119	15%
	Total	1923	±297		2704	±427		2349	±541	

There were main effects of diet (p = 0.01) and sex (p = 0.002) on total caloric intake. Post hoc comparisons showed that participants consumed more calories on the moderate CHO diet compared to their habitual diet (mean difference = 498.28 kcal, CI 95%, -156.1, 493.58, p = 0.006, ES = 1.00) and compared to the low CHO diet (mean difference = 269.54 kcal, CI 95%, -10.31, 549.38, p = 0.057, ES = 0.35), and, as expected, women consumed less calories overall (mean difference = -937.06 kcal, CI 95%, -1429.36, -444.36, p = 0.002, ES = 2.41) (Table 6-2).

Similarly, there was a main effect of diet and sex on the caloric intakes of CHO (p = 0.001 and 0.006) and fat (p = 0.001 and = 0.02). CHO caloric intakes were lower than habitual diets for low CHO (mean difference = 598.92 kcal, CI 95%, 383.57, 814.27, p = 001, ES = 2.40) and higher on moderate CHO (mean difference = -689.45 kcal, CI 95%, -939.47, -439.42, p = 0.001, ES = 2.04) respectively. Fat caloric intakes were lower on the moderate CHO diet (mean difference = -285.55 kcal, CI 95%, -539.69, -31.41, p = 0.032, ES = 0.84) and higher on the low CHO diet than the habitual diets (mean difference = 953.24 kcal, CI 95%, 365.29, 941.18, p = 0.001, ES = 1.49). There was a strong tendency for diet and sex to affect protein intake (p = 0.06)

7. 4. DISCUSSION

This study was undertaken to compare the physiological and sex specific responses to a low CHO training diet with a sufficient adaptation period (>2 weeks). We hypothesised that when compared to a standard moderate CHO athlete's diet, a chronic low CHO training diet would allow for increases in fat oxidation and reductions in CHO_{ox} that would be more pronounced in men than women during fasted exercise.

Our results showed no physiological differences in the response to chronic moderate and low CHO diets between men and women. That is, both sexes similarly altered their relative fat and CHO usage across the four-week intervention period, as measured during two hours of submaximal exercise (Figure 7-4). The most interesting findings were 1) despite some suggestion to the contrary (Mark A. Tarnopolsky, 2000), women did not, in absolute terms, oxidise more lipid than men and 2) the degree of adaptation to a low CHO diet, in relation to an increased reliance upon lipid oxidation, increased from baseline to week four in both sexes.

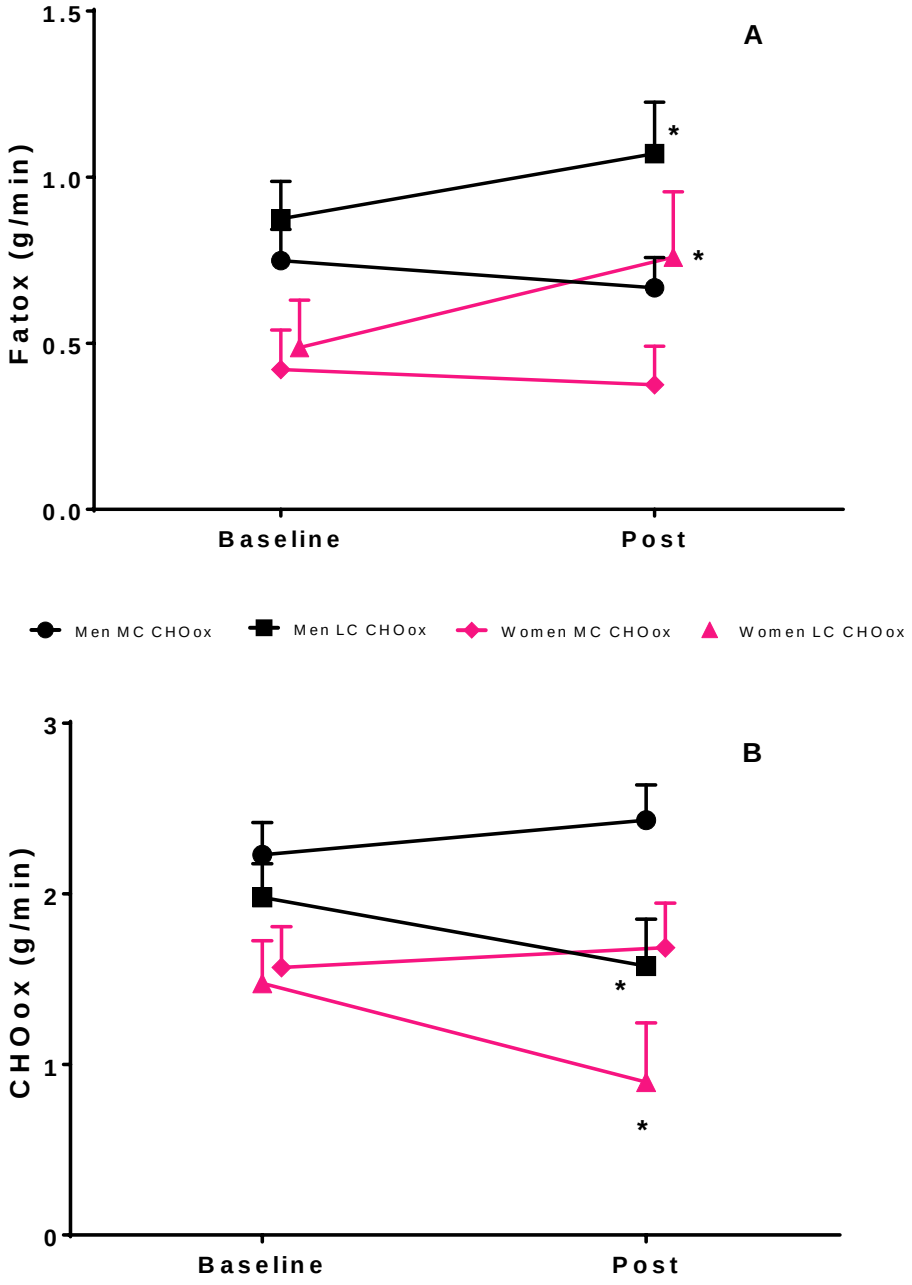


Figure 7-4. Sex specific carbohydrate (4A.) and fat (4B.) oxidation rates (g/min) for each trial taken as the average from four-time points during exercise at 60%VO_{2max}. *Denotes significantly different from moderate CHO.

The significant main effect of sex on FAT_{ox}, but not RER can be explained by the fact that RER represents proportional fuel utilisation, whereas FAT_{ox} represents the absolute rate of fat oxidation. Men have a larger lean body mass, on average, and work at higher absolute work rates (Figure 7-4), so when lipid oxidation is the same proportionately, fat oxidation will almost certainly be higher.

One of the key objectives of the study was to include an adequate adaptation period for the low CHO diet, as this has been a critique of previous low CHO research in the exercise science field (J. S. Volek et al., 2015). The chronic adaptation approach allowed for a significantly greater change in FAT_{ox} and CHO_{ox} during the latter part of the low CHO intervention, compared to baseline and week two (Figure 7-4). The continued increase in FAT_{ox} from week two to week four potentially represents an acute vs adaptive response, which was similarly seen in work by S. D. Phinney et al. (1983). Phinney *et al*. suggested the mechanism through which the subjects in his study adapted to be able to perform prolonged endurance exercise appeared to be simultaneous maximisation of glucose storage during periods of rest and limitation of its mobilisation during exercise. Without muscle biopsies, we cannot comment as to whether our results support this claim or not, but the results of others since have seemed to agree with this theory (Jeff S. Volek et al., 2016; Webster et al., 2016).

Our findings also align with the few previously published studies (S. D. Phinney et al., 1983; Stephen D Phinney et al., 1980; Venkatraman, Feng, & Pendergast, 2001; Jeff S. Volek et al., 2016) where adaptation occurred over four weeks and included exercise. Direct comparison, however, is not possible, because these studies used different levels of participant training status (Stephen D Phinney et al., 1980), sports (Venkatraman et al., 2001), and degrees of CHO restriction (S. D. Phinney et al., 1983; Jeff S. Volek et al., 2016). What sets this study

apart from those listed and almost the entire body of low CHO exercise research ((Yeo et al., 2011) is the sex comparison.

Although the change in macronutrient usage was similar in both sexes, women had lower absolute CHO_{ox} rates than that of men during both the moderate CHO and low CHO diets (Figure 7-4). In other words, men were utilising CHO at a greater rate on the low CHO diet than women were on the moderate CHO diet. Several studies have found greater fat metabolism in women than in men during exercise (S. L. Carter et al., 2001; Horton et al., 1998; Mark A. Tarnopolsky, 2000), whereas others have failed to show a sex difference (Roepstorff et al., 2002; Zehnder et al., 2005). Venables, Achten, and Jeukendrup (2005) reported higher maximal FAT_{ox} in females compared with males during an incremental running test, but such a finding was not replicated in the current study. In fact, the average FAT_{ox} for women shown here on the low CHO diet is almost double the previously reported maximums of FAT_{ox} for women (Stisen et al., 2006) and, to our understanding, represents the highest recorded FAT_{ox} rates in females during exercise.

The implication of a lower absolute CHO_{ox} in women indicates that recommendations for CHO intake before and during exercise should not be the same for men and women. The current American College of Sports Medicine (ACSM) guidelines recommend an intake of 60–90 g CHO/hr for ultra-endurance exercise with no reference to sex (Academy of Nutrition and Dietetics, 2016), yet based on the results presented here, women oxidise, on average, 60 g/hr less CHO than men. Gareth A. Wallis, Yeo, Blannin, and Jeukendrup (2007) have specifically stated that there is no added benefit from 1.0 g/min to 1.5 g/min CHO supplementation in women during two hours of cycling at 60% VO_{2max}; in fact, the higher ingestion rate actually increased the rate of glycogenolysis.

With this in mind, what we may actually be seeing is that women burn less CHO than men, rather than oxidising more fat. This theory is supported by Zehnder et al. (2005), who measured total fat and CHO oxidation in nine men and nine women during three hours of cycling at 50% VO_{2max}. Zehnder et al. (2005) showed that average FAT_{ox} was the same for men and women, whereas CHO_{ox} was significantly higher in males relative to females. Much of the female specific research on CHO oxidation and supplementation suggests endogenous that CHO_{ox} (CHO_{endo}) is far lower in females than males when exercise is completed without CHO supplementation (Mark A. Tarnopolsky, 2000; J. Tremblay, Peronnet, Massicotte, & Lavoie, 2010; Gareth A Wallis, Dawson, Achten, Webber, & Jeukendrup, 2006). Yet, when CHO is ingested during exercise, it has a far greater effect on women compared to men (J. Tremblay et al., 2010), This is a limitation of the present study as it was undertaken in the fasted state and potentially only represents the naturally lower CHO_{endo} of women compared to men.

In practice, only Applegate, O'Toole, and Hiller (1989); Downey and Hopkins (2001); Kimber, Ross, Mason, and Speedy (2002) have collected field-based data on nutritional intakes during endurance exercise in men and women. Applegate et al. (1989) and Downey and Hopkins (2001) either did not specify substrate intakes (CHO, fat, protein), or did not differentiate their data between sexes. The work of Kimber et al. (2002), on the other hand, found that average CHO intake was similar between sexes. Although CHO intake was similar, it was shown that in men— but not women—CHO intake during exercise bout was positively correlated with finishing time. Kimber and colleagues went on to conclude that increasing CHO ingestion for male—but not female—athletes may be a useful strategy for improving performance. This aligns with the current study's findings, which suggest that for women,

CHO intake may not play as an important role in endurance performance above a certain rate of ingestion (which is below the standard recommendation), due to their comparatively low lean body masses and maximal absolute rates of CHO oxidation.

In studies of exercise metabolism, the use of the RER as an estimate of the non-protein exercising muscle respiratory quotient (RQ) is not without risk. A sudden change in the acidity of the blood or a relative hyperventilation can cause the RER to be misleadingly high, and invalidate its use as a measure of substrate metabolism (Mansell & Macdonald, 1990a). However, the exercise intensity chosen for this study (60% VO_{2max}) is known to cause little or no net lactate production (David L. Costill, 1970) and the duration of the exercise task (120 minutes) was such that a sustained hyperventilation would have been an improbable event. Based on these observations, it seemed reasonable to use the gas exchange measures in the study to calculate an estimate of the non-protein RQ and relating rates of substrate utilisation.

Because the data presented in the current study was collected in the overnight fasted state means that our findings are not necessarily applicable to a situation where CHO is ingested immediately pre-exercise. It must also be acknowledged that we did not control for the menstrual cycle or oral contraceptives. However, as outlined in the “Sex” Section of the Literature Review the influence of the menstrual cycle on whole body substrate metabolism is equivocal at best. Nevertheless, there still remains the possibility that comparisons of female data within and between interventions were influenced by differences in acute sex-specific hormonal states.

In conclusion, these data show that both men and women are able to increase their rates of FAT_{ox} in a time dependent manner when the training diet is low in CHO. The time dependent

nature of the changes suggests that future research on a low CHO training diet needs to be conducted over four or more weeks, in order to guarantee that sufficient adaptation has taken place. Based on the large discrepancies in CHO_{ox} rates between sexes, there needs to be further work done to identify adequate and relevant CHO intake recommendations for women undertaking endurance exercise.

CHAPTER 8. METABOLOMIC ANALYSIS OF MODERATE AND LOW CARBOHYDRATE ENDURANCE TRAINING DIETS.

8. 1. INTRODUCTION

Following the seminal work by Levine, Bergstrom and others (Jonas Bergström et al., 1967; Christensen, 1931; Levine, Gordon, & Derick, 1924), which reported that dietary carbohydrate (CHO) can increase an individual's capacity to exercise over moderate durations, much research has focused on increasing the body's ability to store CHO in skeletal muscle, in order to improve performance (Jørn Wulff Helge, 2002). In contrast, much less effort has been applied to the alternative strategy of limiting CHO ingestion and storage to up-regulate rates of lipid oxidation within skeletal muscle, at rest and during exercise. Work that has been done is equivocal at best, but some studies have suggested that longer duration, lower intensity endurance exercise performance may benefit from adaptation to a low CHO intake (Yeo et al., 2011). To date, however, only a handful of low CHO studies have used exercise of three or more hours, with each citing equal or better performances, when compared to a high CHO diet (Carey et al., 2001; Lize Havemann et al., 2007; L. Havemann et al., 2006; Lambert et al., 2001; S. D. Phinney et al., 1983; Rowlands & Hopkins, 2002)

Metabolic flexibility describes the ability of a tissue to easily and quickly alter the flux through specific metabolic pathways, according to substrate supply (Storlien, Oakes, & Kelley, 2004). Having skeletal muscle metabolic flexibility is ideal for an endurance athlete,

because it enables both optimal lipid and/or CHO oxidation, depending on fuel availability and requirements. For example, a reliance on lipid oxidation for submaximal bouts of exercise can conserve muscle glycogen for intense periods of work—in particular, those which might occur in the latter stage of an event. Athletes who are metabolically inflexible, on the other hand, may struggle to shift between macronutrient fuel sources, resulting in a reduced capacity to buffer the loss of glycolytic-derived energy, thus compromising performance. However, to better understand metabolic flexibility, it is necessary to identify metabolic markers which relate to both endurance training and high vs low dietary CHO interventions; this may allow relationships between specific metabolic signatures and metabolic flexibility to be identified.

Metabolomics has formally been defined as the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification (Jeremy K Nicholson, Lindon, & Holmes, 1999). Results can be achieved by the combined use of a multiparametric analytical technique, most commonly mass spectrometry (MS) or nuclear magnetic resonance spectrometry (NMR), accompanied by a multivariate statistical analysis, such as principal component analysis (PCA) or projection on latent structure (PLS) analysis. Consequently, metabolomics is well adapted to simultaneously investigate most of the metabolic modifications that occur during endurance exercise and may be observed at the plasma level. It has been widely demonstrated in human and animals, such as rodents, that the metabolome is highly modified by physical exercise (Le Moyec et al., 2012; Pechlivanis et al., 2010).

The potential of metabolomics in pharmacology and in toxicology has been clearly recognised (J. K. Nicholson, Connelly, Lindon, & Holmes, 2002), and its role in human nutrition is an

emerging field of investigation (Lenz et al., 2004). One of the key aims of nutritional metabolomics is to identify the small molecules responsible for the effects of different diets and, in so doing, deepen the knowledge of human health and the interacting and regulatory roles of nutrition (Gibney et al., 2005; Rezzi, Ramadan, Fay, & Kochhar, 2007).

The identification of metabolomic profiles associated with diet and exercise related stresses will allow for a better understanding of metabolism at rest and during exercise in physically fit males on contrasting diets. During submaximal endurance exercise, muscular mitochondrial oxidative phosphorylation activity should be maintained at its maximal level for much of an endurance exercise bout. However, endurance exercise involves increased spatial muscle recruitment over time, due to cumulative fatigue of type 1 skeletal muscle fibres (Gollnick et al., 1972); this results in glycolysis providing a larger proportion of the muscle cells' energy needs. NMR metabolomics can investigate the distribution of small molecular weights and mobile molecules involved in the energetic metabolic pathways, such as the tricarboxylic acid (TCA) cycle or lipid beta-oxidation pathway.

High CHO diets are not only the recommended diet for endurance athletes, but also for the majority of Western society, as decided by the World Health Organisation (2010) along with many of the largest government health agencies (NZ Ministry of Health, 2015). These recommendations suggest that a diet low in CHO and high in fat may present potential health risks that are not outweighed by the potential performance benefits. Metabolomics should provide an overall snapshot of the metabolic changes occurring at rest and during exercise, within the circulation at least, across a low CHO dietary intervention.

This study was designed to investigate two diets, contrasting in CHO intake, to determine how the subsequent CHO status of the participants following each would affect their metabolome at rest and during exercise. It was hypothesised that a low CHO diet would increase concentrations of β-oxidative related metabolites, such as FFAs, ketone bodies, glycerol and triglycerides, at rest and during exercise. Specifically, it was proposed that the moderate CHO diet would induce higher concentrations of glycolytic metabolites (glucose, lactate, alanine and pyruvate) in the circulation.

8. 2. METHODS

8. 2. 1. Samples

Refer to section 6. 2. 1. for information regarding the participant's physiological characteristics. Samples were drawn from an indwelling cannula or venepuncture on the antecubital vein of the forearm (see section 4. 2. 1.). In total, 210 samples were analysed, pertaining to the following time points during the submaximal fixed-intensity ride for each participant: resting (pre), 10 minutes, 120 minutes, recovery (post), as well as pre- and post-exercise samples for the self-paced time trial. The protocol for the exercise tests is outlined in the previous chapter (section 7. 2. 2.).

8. 2. 2. Pre-Treatment

Plasma (heparin tubes) were taken from storage (-80ºC) and randomly assorted. Each sample was spun down for 5 minutes (max speed: 13,000 rpm) using a table top centrifuge (Gen-Probe Incorporated, San Diego California, U.S.A.). Precipitation was achieved by a mixing 300 uL sample + 600 uL MeOH (2:1 ratio) and vortexing for 30 s. Each sample was frozen at

-20°C for a minimum of two hr and a maximum of twelve hr. Subsequently, they were removed from the freezer and spun for 15 minutes at 4°C and 15,000 rpm. The supernatant formed was immediately transferred to a new tube and air-dried in the fume-hood overnight. Samples were then placed in a speed vacuum with heat applied, until all liquid was removed.

Dried samples were re-suspended in 100 uL 6 mM DFTMP–0.9 mM DSS–1x PO_4 buffer, and 500 uL D_2O-phosphate buffer (TSP-free) was added, to give a final concentration of 1 mM DFTMP and 0.15 mM DSS. Each sample was vortexed for 30 s and spun down at maximum speed (13,000 rpm) for 45 s, before being transferred (~580 uL) to an NMR tube via micropipette.

8. 2. 3. NMR Spectroscopy

8. 2. 3. 1. Spectral Acquisition

All samples were recorded using a Burker Avance 700 MHz NMR spectrometer (Bruker-Biospin, GmbH, Rheinstetten, Germany), operating at 700.13 Mhz and equipped with a three-channel inverse detection cryo-probe. The temperature for all measurements was 298 K, which was calibrated using the separation of the residual ^1H signals from a standard sample of d4-methanol. Water suppression was achieved during the recycled delay in all samples, via pre-saturation at the water offset frequency (4.71 ppm), using a field strength of 50 Hz. All methods used a recycle delay of 1.50 s at the end of each scan.

One-dimensional Nuclear Overhauser effect Spectroscopy (NOESY) spectra were recorded for all samples. The spectra were recorded using a Bruker ‘noesygppr1d’ pulse sequence, with spectral width (SW) of 8.33 kHz (11.90 ppm) and 58 k points, with an acquisition time of 3.5

s and averaged for 128 scans, with 4 dummy scans. A mixing time of 100 ms was used for all samples.

Two-dimensional heteronuclear Single Quantum Coherence (HSQC) spectra were recorded using the standard Bruker 'hsqcetgpsisp2.2' pulse program, with a SW of 8.39 kHz (11.98 ppm) in the F2 domain and 2048 points, using an acquisition time of 0.12 s, and SW of 2.92 kHz (166.05 ppm) and 512 points in the F1 domain, using an acquisition time of 0.01 s. Forty-four scans were used for all samples preceded, with 32 dummy scans.

Two-dimensional Total Correlation Spectroscopy (TCOSY) spectra were recorded using the standard Bruker 'mlevesgpph' pulse program, with a SW of 8.39 kHz (12.00 ppm) in the F1 domain and 400 points, using an acquisition time of 0.02 s. A mixing time of 60 ms was used. Forty-eight scans were used for all samples, preceded by 256 dummy scans.

8. 2. 3. 2. Spectral Processing

All 1D NOESY spectra were apodised using an exponential function with line broadening of 1 Hz, and zero, filled to 128 k points. All HSQC and TOCSY spectra were apodised using cosine bell in both dimensions, Fourier transformed, and zero filled to 16 k points in the F2 dimension and 256 points in the F1 dimension.

8. 2. 4. Statistical Analysis

8. 2. 4. 1. Pre-Processing

The following pre-processing steps were applied to all NMR spectral data in batch mode. The order in which they are presented is the order in which they were performed.

Prior to statistical analysis, all spectra were referenced, phased and baseline corrected using Topspin (version 2.5; Bruker-Biospin, GmbH, Rheinstetten, Germany). All spectra were referenced to the methyl peak from DSS (0.00 ppm). Spectra were imported into Chenomx (as a reference) and adjusted so that all spectra were in a consistent comparable state. On importing the data DSS, the reference was set to 0.15 mM and the pH reference was set to auto pick on the DMFTP peak from the buffer.

8. 2. 4. 2. Region Exclusions

Region exclusions were carried out before binning and normalisation. Spectra were binned within Chenomx, with an area of 0–10.0 ppm, width of 0.04 ppm, cuts of 4.7–5.1 ppm (H_2O), 3.33–3.36 ppm (methanol) and 0.4–0.8 ppm (DMFTP). All spectra were normalised by dividing the intensity of each point on each spectrum by the total sum of the intensities over the whole spectrum. Samples with poor spectral quality were excluded.

8. 2. 4. 3. Scaling

All data was mean-centred prior to model building. For PCA, PLS-DA and OPLS-DA, the data was Pareto scaled—every variable was divided by the square root of the standard

deviation for that particular variable, to help prevent large peaks from obscuring the systematic variation in the lower concentration metabolites.

Statistical analyses of the binned spectra were carried out with SIMCA statistical software. The primary variables were set as the column titled ‘NMR Tube #’. All other variables were secondary variables/classes, as follows: subject, diet, order, test, time point.

8. 2. 4. 4. Multivariate Statistical Analysis

For the multivariate statistical analysis, the quantification parameters generated by SIMCA, were organised in the form of a matrix, X (descriptor matrix), where the rows represented NMR spectra and each column (variable) represented one of the buckets in which each spectra was partitioned. The integral of the intensities of the spectral peaks in a given bucket represented the X-value. Initially, the data matrix X was processed by PCA, with the aim of exploring the dataset and identifying outliers to be excluded from the modelling. By applying PLS regression, it is possible to connect the information in two blocks of variables—X (descriptor matrix) and Y (response matrix)—to each other via a linear multivariate model. In this case, the response matrix, Y, is a matrix of dummy variables that describe the class membership of each observation in the descriptor matrix X.

PLS calculates latent variables (LV) as the linear combination of X, in such way that they will approximate X and Y and maximise the covariance between X and Y. The application of PLS as a classification method is indicated as a partial least squares discriminant analysis (PLS-DA) (Barker & Rayens, 2003). Furthermore, the model allows us to identify which descriptors explain most of the differences in the two groups by means of the variable influence in projection (VIP). The VIP is a weighted sum of squares of the PLS loading weights, taking into account the amount of explained Y-variation in each dimension. The rule “greater than one” is used for detecting the descriptors with the greatest importance in the projection (Eriksson, 2006)

8. 2. 4. 5. PLS-DA Model Validation

Supervised analysis techniques, PLS-DA and OPLS-DA, have the potential to over-fit data to produce visually significant results (group separation) that are unreliable (Figure 8-1). A permutation test gives an indication of whether the model can be considered statistically valid. Permutation tests were run using 20 models, with randomly assigned class labels, that were built from the data set. These 20 models were compared with the original model with correct labels.

Model over-fit is indicated if the original model's R2 and Q2 values differ by more than 0.2. This is because adding latent variables to the model will optimise its parameters, and R2 and Q2 will increase towards 1. However, at a certain point the two variables will diverge, with Q2 trending toward 0 as the model becomes over-fitted (Broadhurst & Kell, 2006). For Q2, all values must be below the original model's Q2 value, but for R2 it is only required that the majority of the values be below the original R2. Additional requirements have been proposed by (B. Worley & R. Powers, 2013), namely that Q2 must be below -0.2 and R2 must be below 0.3. For the analysis outlined, the value limits were set so that R2 could not exceed 0.4 and Q2 could not exceed 0.05 (Eriksson, 2006).

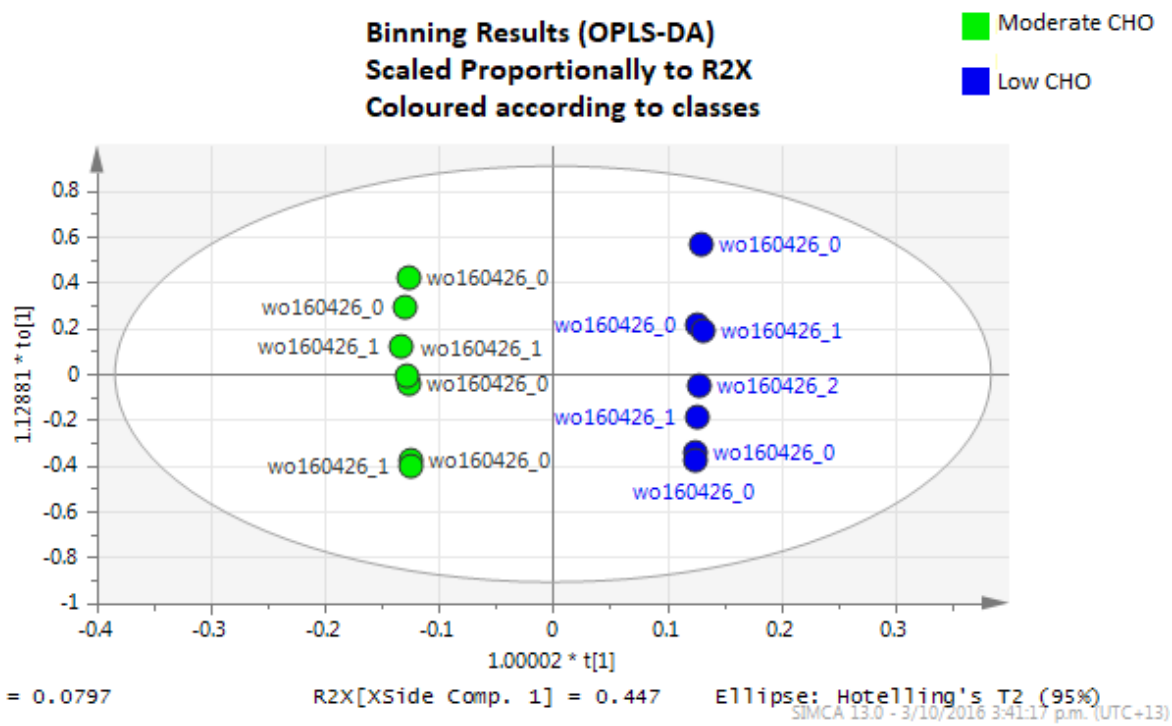


Figure 8-1. Example OPLSA-DA plot with pre-assignment of diet. Components are visibly separated.

8. 3. RESULTS

Both unsupervised (PCA) and supervised (PLSA-DA & OPLS-DA) models failed to statistically separate the sample groups in regards to the dietary intervention (i.e. moderate CHO and low CHO). However, both methods of supervised discriminant analysis separated groups based on time (i.e., pre- vs. post). Of these only a handful could be validated using the permutation technique outlined in the methods section.

Table 8-1. All validated supervised discriminant analysis models.

Test	Time point(s)	Model Type	R2X	Eigenvalue	R2Y	Q2	Validity
Low CHO, all tests	10–120 minutes	OPLS-DA	0.138	5.65	0.686	0.274	
		PLS-DA	0.246	10.1	0.413	0.244	
		Permutations	0.116			-0.779	Weak
Moderate CHO, all tests	10–120 minutes	PLS-DA	0.364	14.6	0.311	0.169	
		OPLS-DA	0.11	4.42	0.841	0.54	
		Permutations	0.0981			-0.078	Weak
Low CHO, all tests	Pre- vs. -Post	OPLS+PLS-DA	0.415	16.6	0.371	0.258	
		Permutations	0.0888			-0.0858	Valid
Moderate CHO, all tests	Pre- vs. Post	OPLS+PLS-DA	0.413	16.1	0.284	0.164	
		Permutations	0.0938			-0.0814	Valid

Unfortunately, only a small number of samples could be used in the statistical analysis as some metabolites were not detected in certain samples, thereby reducing the total number that were contributing to the group separation. In addition, some samples were lost during analysis or missed during the data collection period, due to unforeseen complications with blood drawing (i.e., clotting of the cannula line). This reduction to the sample pool considerably lowered the statistical power of the results.

8. 3. 1. Glucose

There were no main effects of diet (p = 0.944) or exercising time (p = 0.284) on glucose values obtained in samples from the post intervention exercise test. There was an interaction between diet and time at post intervention (p = 0.006). The post hoc analysis showed that this interaction was due to the glucose concentrations [Glu] starting lower on the moderate CHO diet and then increasing over time (mean difference = 0.919, CI 95%, -0.017, 0.205, p = 0.079, ES = 1.460), while the converse occurred during the low CHO diet (mean difference = -0.186, CI 95%, -0.328, -0.045, p = 0.022, ES = 1.569) (n = 5) (Figure 8-2). Overnight fasted glucose was similar between diets.

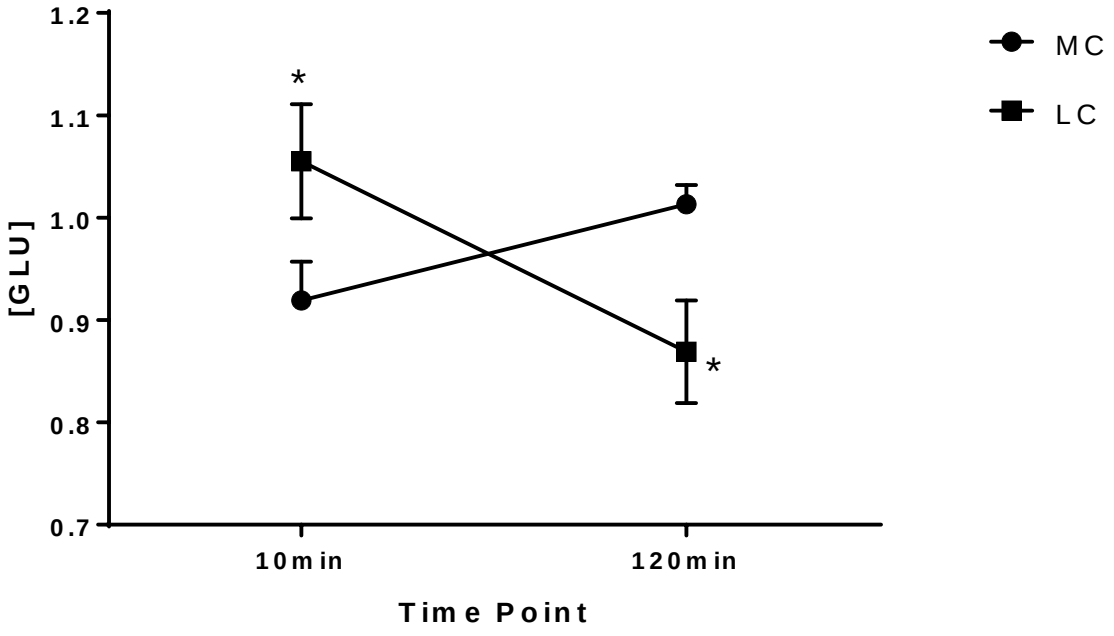


Figure 8-2. Glucose concentrations during exercise. * denotes a significant (p < 0.05) difference between diets.

8. 3. 2. Lactate

There were no main effects of diet ($p = 0.353$) or time ($p = 0.375$) on lactate concentrations during exercise. A paired t-test showed no difference between the means of the overnight fasted resting samples ($p = 0.327$, n = 5)

8. 3. 3. Valine

There were no main effects of diet ($p = 0.789$) or time ($p = 0.999$) on post intervention valine concentrations. A paired t-test did not show any difference in mean valine concentration, at rest, during week four of each intervention ($p = 0.544$).

8. 3. 4. 3-betahydroxybutyrate

There were no main effects of diet ($p = 0.583$) or time ($p = 0.566$) on 3-βHB, and a paired t-test showed no difference in means between pre ($p = 0.806$, n=4) and post ($p = 0.662$, n = 7) intervention exercise measures.

8. 4. DISCUSSION

This study was undertaken primarily to investigate how the CHO status evoked by two diets contrasting in CHO intake, would affect the circulating (blood plasma) human metabolome at rest and during exercise. The main finding was that there was no significant effect of the diet on the circulating metabolome at rest or during exercise. However, the metabolomic analysis

did resolve a pre- vs post-exercise effect on some variables. This indicates that the metabolic impact of exercise exceeds that of a four-week change in the CHO content of the diet.

The one significant metabolite-related finding identified was the contradicting response of blood glucose to exercise at the end of each dietary intervention. As expected, based on numerous prior studies on glucose metabolism during exercise (Ahlborg et al., 1974; Bonen, Malcolm, Kilgour, MacIntyre, & Belcastro, 1981; Louise M Burke et al., 2000; Christensen, 1931; A. R. Coggan & Coyle, 1987; Levine et al., 1924; Rowlands et al., 2009; A. Tremblay, Nadeau, & LeBlanc, 1983; Gareth A. Wallis et al., 2007), blood glucose declined during exercise on the high CHO diet. Interestingly, the converse occurred on the low CHO diet, indicating that glycaemic control was improved following adaptation to a low CHO diet. This maintenance of glycaemia seen on the low CHO diet during exercise may be related to an improved ability of the liver to produce glucose, to buffer skeletal muscle uptake. Alternatively, or perhaps concurrently, the skeletal muscle may have been less reliant on glucose during exercise, enabling plasma glucose concentrations to be more easily supported. Accordingly, it has been previously shown that the glucose–alanine and gluconeogenic pathways are up-regulated in low CHO diets (Webster et al., 2016).

Nutritional metabolomics measures subtle metabolic changes as the human biome responds to relatively low doses of bioactive food nutrients or supplements. The metabolic response is the product of many simultaneous regulations of biochemical pathways, which are difficult to target a priori as compared with conventional toxicological events (Rezzi et al., 2007). Different studies have attempted to describe these metabolic signatures with varying degrees of success (Bollard, Stanley, Lindon, Nicholson, & Holmes, 2005; Kochhar et al., 2006; Walsh, Brennan, Malthouse, Roche, & Gibney, 2006; Williams, Lenz, Lowden, Rantalainen,

& Wilson, 2005). In addition to the varied regulatory and physiologically factors, nutritional metabolomics relies on the separation of specific metabolic signatures related to the specific dietary intervention implemented, in this case CHO status.

The fitness state of the cohort tested in the current study confounds the discussed issues of nutritional metabolomics due, in part, to the participants' assumed metabolic flexibility. As shown in Figure 8-3 (Storlien et al., 2004) type I fibres (slow twitch), known to be the dominant fibre type of endurance athletes (Gollnick et al., 1972), are characterised by many positive adaptations to fuel utilisation, irrespective of the source (CHO or fat). Therefore, despite a large shift in the macronutrient intake of the participants, they can alter their metabolism to maintain homeostasis. As discussed above, homeostasis could not be maintained across a bout of endurance exercise and as such group separation was capable during exercise but unsuccessful between diets.

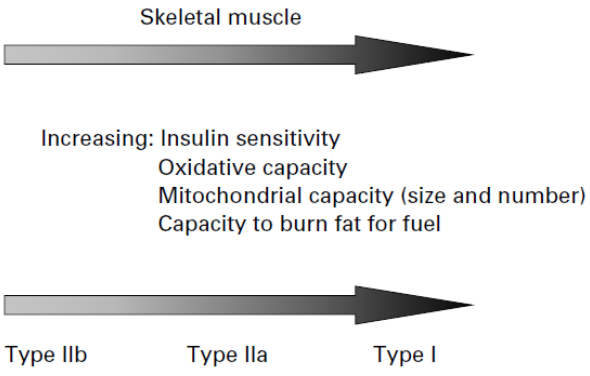


Figure 8-3. The range of skeletal muscle fibre types with some of their relevant associated metabolic characteristics. A predominance of type IIb fibre type is associated with obesity and insulin resistance, both conditions of metabolic inflexibility.

To our knowledge, this is the first study to investigate the impact of a chronic low CHO diet on the plasma metabolome at rest and following submaximal exercise. That the method did not identify changes accorded to the diet (alone), suggests that either the subjects were able to sufficiently adapt to maintain a constant metabolomic profile, or that the method was not precise enough to detect any changes. We speculate that indeed both situations probably occurred. That we were able to resolve changes due to exercise, but not at rest, certainly indicates, as mentioned above, the former, but also suggests that the "broad brush" metabolomic method may not be suitable for this type of work. Clearly more research needs to be applied to this area.

In conclusion, our data indicate that endurance trained individuals possess the metabolic flexibility to counter changes in dietary CHO availability and maintain a normal circulating metabolic profile. The complexity of the metabolome and the massive inter-subject metabolic variation found in humans, limits the effectiveness of high-throughput H^1 NMR metabolomics for analysing multiple physiological parameters within human biofluids in relation to diet, but not exercise.

CHAPTER 9. KETONE SUPPLEMENTATION: CASE STUDY

9. 1. INTRODUCTION

Ketosis occurs naturally in response to starvation, carbohydrate (CHO) restriction, or prolonged exercise, and is defined as a physiological state where blood ketones (acetoacetate (AcAc) and β-hydroxybutyrate (β-HB)) are consistently above 0.2 mM (Robinson & Williamson, 1980). Ketones can be utilised by the brain and other organs when the glucose supply is limited (George F. Cahill, 2006). In theory, ketosis provides a suitable metabolic model for endurance exercise, especially ultra-endurance events, because ketones are able to partly off-set CHO needs, especially those of the central nervous system. However, when exercise performance is of primary concern no level of ketosis can buffer the need for CHO. At low intensities (<60% VO_{2max}) the recruitment of type Π (or fast glycolytic) muscle fibres is still required due to muscle recruitment patterns associated with cumulative fatigue (Grimby & Hannerz, 1977). Therefore, a proposed ideal physiologic state is one in which glycogen storage is high and high rates of fatty acid oxidation can still be achieved. Ketone supplementation (KS) can theoretically provide this scenario by elevating circulating [β-HB] to concentrations normally only experienced during starvation or severe CHO restriction, but in a state in which glycogen stores are still plentiful. The scientific theory behind KS is, in short, that the Gibbs free energy (G) of KS states are more negative than that of glucose supplementation; the more negative the ΔG, the more "free" energy that exists in the system. On a per carbon basis, β-HB generates more adenosine triphosphate (ATP) than glucose or pyruvate.

When exogenous CHO is ingested at rest, insulin is released. This activates the pyruvate dehydrogenase (PDH) complex within muscle mitochondria, enabling decarboxylation of pyruvate to acetyl–CoA (Kashiwaya, King, & Veech, 1997). However, β-HB is thought to "mimic" the effect of additional insulin, without the complex cascade of metabolic processes brought on by additional insulin (e.g., decreased lipolysis, activation of glucose transporters, accumulation of glycogen, stimulation of PDH inside the mitochondria and increased conversion of pyruvate decarboxylation). Therefore, supplementing with exogenous ketones offers more potential energy with less oxygen consumption. Further, ketones easily cross cell membranes so are able to enter the cell and mitochondria, and participate in the tricarboxylic acid (TCA) cycle directly. In doing so they are able to bypass the rate-limiting PDH complex (Sato et al., 1995).

Supplementation with KS is novel, and as such only a small amount of published research exists on the topic (Clarke et al., 2012; Desrochers et al., 1995; Hashim & VanItallie, 2014; Kies, Tobin, Fox, & Mehlman, 1973; Sherwin et al., 1975; Veech, 2004). Notably only three published studies reference an application of KS to exercise (P. J. Cox & Clarke, 2014; Pete J Cox et al., 2016; Rodger, 2015).

The aim of this case study was therefore to firstly test the effects of ingesting a KS on endurance performance in one individual, and secondly to identify whether this effect is altered by different metabolic states. The experimental design and findings of this case study will provide direction for future research as presented in the Review of Literature "Ketone Supplementation".

It is hypothesised that ingesting a KS could artificially elevate blood [β-HB] in the presence of CHO supplementation, allowing for the simultaneous benefits from both ketosis and CHO supplementation, as outlined above. In addition, it is thought that a cellular setting that is adapted to a low CHO environment (i.e., nutritional ketosis) may respond better to utilising ketones than that of CHO abundance (high CHO). It is expected that these two factors will work together to result in improved endurance performance.

9. 2. METHODS

9. 2. 1. The athlete

The athlete (the researcher) was a highly trained male triathlete completing a minimum of 15 hr of aerobic training per week (age: 26 years, body mass: 77.1 kg, BMI: 22, height: 1.87 m, body fat: 4%). The athlete ate a habitually low CHO diet (2–4 g/kg/day).

9. 2. 2. Common Methodology

The methods used for the collection of data referring to the protocols of VO_{2max} and submax tests are outlined in the "Common Methodology".

9. 2. 3. Ketone Supplement

The ketone being supplemented was an alcohol ketone precursor—1, 3 butanediol ($C_4H_{10}O_2$, molecular weight = 90.12 g/mol, density = 1.005 g/mL at 25^{o}C, caloric content = 4.69 kcal/g, Sigma Aldrich, New Zealand). The ketone precursor, 1, 3 butanediol, is metabolised in the liver by alcohol dehydrogenase, to β-HB (Kies et al., 1973). Based on the research of Clark et

al (2012) a dose of 260 mg/kg was chosen to avoid any adverse effects, while still raising blood ketones to above 0.1 mM. In this athlete, this corresponded to a dose of 20.2 g (95.11 kcal). The KS was ingested three times (60.6 g total) for each performance session as follows: at rest (with 250 mL H_2O), during the first hour of fixed intensity riding (600 mL sports drink (SpD); 40 g CHO, Powerade, Coca-Cola Oceania Ltd, New Zealand) and during the second hour of fixed intensity riding. For the control trials, the same concentration of SpD was made available over the same time points.

9. 2. 4. Diet

The secondary aim of the study was to test the interaction between the athlete's metabolic state and the KS. To achieve this, the athlete's macronutrient intake was manipulated in the form of either a ketogenic diet or a moderate CHO diet, undertaken for 14 days. During the ketogenic diet, the athlete was limited to 1.5 g CHO/kg/day. This is higher than common CHO intakes for ketogenic diets (<50 g/day) (Paoli et al., 2012; S. D. Phinney et al., 1983; Westman et al., 2007), but for this athlete who was completing a minimum of 90 minutes of endurance training per day, it was deemed a sufficient restriction to deplete liver glycogen and maintain regular resting blood [β-HB] below 0.2 mM (Hashim & VanItallie, 2014; Robinson & Williamson, 1980; R. Smith, Fuller, Wedge, Williamson, & Alberti). β-HB was measured daily during the ketogenic diet by the participant using a handheld ketone meter (Precision Xtra, Freestyle, Abbott Diabetes Care Inc.). A concentration of 0.2 mM was used instead of the more well referenced 0.5 mM because the intent was to induce an upregulation of kotengic pathways which could be achieved with at a level of 0.2 mM. Diets were run in a consecutive fashion with no washout period, due to time restrictions.

During the moderate CHO diet, 50% of total energy came from CHO (approximately 5 g CHO/kg/day), which was deemed to be representative of the common endurance athlete's diet (van Erp-Baart et al., 1989). The composition of daily food intake was monitored using "MyFitnessPal®" online and mobile software (MyFitnessPal® Inc., San Francisco). In both diets, protein intake was fixed at between 15 to 20% of total calories. There were no caloric restrictions to allow the diets to be as habitual as possible. Because the researcher was the one undertaking the study the knowledge of the macronutrient composition of foods was extremely comprehensive and therefore compliance was extremely high.

The meal before the performance test was standardised as a low CHO smoothie and a coffee (caffeine = 160 mg) with cream (approx. 30 mL). Standardising the pre-exercise diet allowed the comparison of the dietary and supplement conditions not to be influenced by the level of CHO loading. The low CHO smoothie consisted of 100 mL cream, 100 mL coconut cream, 150 mL full cream milk, 50 g butter, 100g blueberries (1052 kcal; CHO = 24 g, Fat = 104 g, Pro = 9 g).

9. 2. 5. Performance Session

To remove any bias, the study design was a double-blinded randomised cross-over. A researcher who was not involved in the project prepared the drink (KS vs. placebo) before each trial; both drinks had similar tastes, odours and appearances. The order of drinks was random, and was determined by the outside researcher. The performance session was carried out a total of four times as follows: Day 14: post-ketogenic diet with SpD (K + SpD), day 16: post-ketogenic diet with KS (K + KS), day 30: post-moderate CHO diet and SpD (CHO + SpD), day 32: post-moderate CHO diet and KS (CHO + KS).

The performance session consisted of a two hr fixed intensity ride at 60% VO_{2max} (power: 213 Watts), immediately followed by a time to exhaustion (TTE) ride at 90% VO_{2max} (power: 340 Watts). The protocol for VO_{2max} and calculations of percentage work rates are explained in detail in the 'Common Methodology: VO_{2max} and submaximal exercise test'.

9. 2. 6. Physiological Measurements.

The athlete was weighed on arrival to the performance laboratory on a set of calibrated weighing scales (Jadevar, Taiwan). The athlete was then seated for five minutes before ingesting either 250 mL H_2O or 250 mL H_2O + KS. Immediately after ingesting the KS, breath-by-breath respiratory gas analysis (K4 b^2, COSMED s. r. l., Rome, Italy) was carried out for 10 minutes. At the same time (t = 0 min), HR (Garmin, Schaffhausen, Switzerland) and blood metabolite measures were taken using handheld meters and finger prick samples as follows: glucose ([Glu]) (ACCU-CHEK® Advantage, Roche Diagnostics), lactate ([Lac]) (Lactate Pro 2, Arkray Inc, Japan) and β-HB (Precision Xtra, Freestyle, Abbott Diabetes Care Inc.). Measures were repeated during the submaximal ride (t = 10, 60, 120 minutes) and 5 minutes into the TTE (t = 125 minutes) (Figure 9-1).

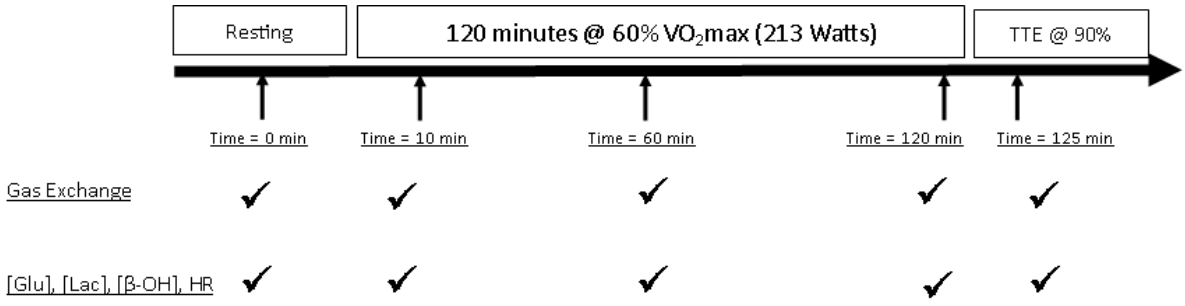


Figure 9-1. Exercise protocol with sampling time points and physiological measures. Lactate [Lac], glucose [Glu], beta-hydroxybutyrate [β-HB], heart rate (HR).

9. 3. RESULTS

Performance during the TTE was improved with the KS (K + KS and CHO + KS) compared to the SpD (K + SpD, CHO + SpD). When the KS was taken in conjunction with a moderate CHO diet, TTE performance was the longest (Figure 9-2).

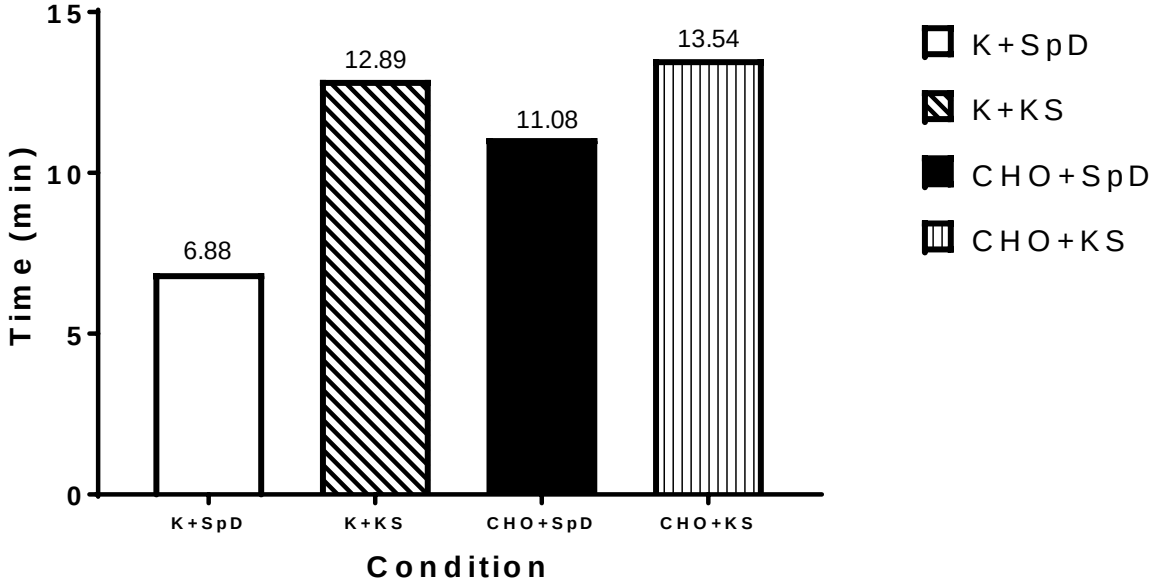


Figure 9-2. Time to exhaustion (TTE) at a fixed intensity (90% VO_{2max}, 340W) under four different dietary and supplemental conditions; ketogenic diet with SpD (K + SpD), ketogenic diet with KS (K+KS), moderate CHO diet (>5 g CHO/kg/day) and SpD (CHO + SpD), moderate CHO diet and KS (CHO + KS).

The KS had a greater effect on [β-HB] than the diet intervention. Taken as the mean value of the 2 KS conditions, [β-HB] during exercise peaked at 0.8 mM with the KS in both treatments. Ketosis without supplementation still reached 0.3 mM with SpD (Figure 9-3a). The [Glu] trend in response to exercise was similar in all conditions, with the exception of K + KS (Figure 9-3b).

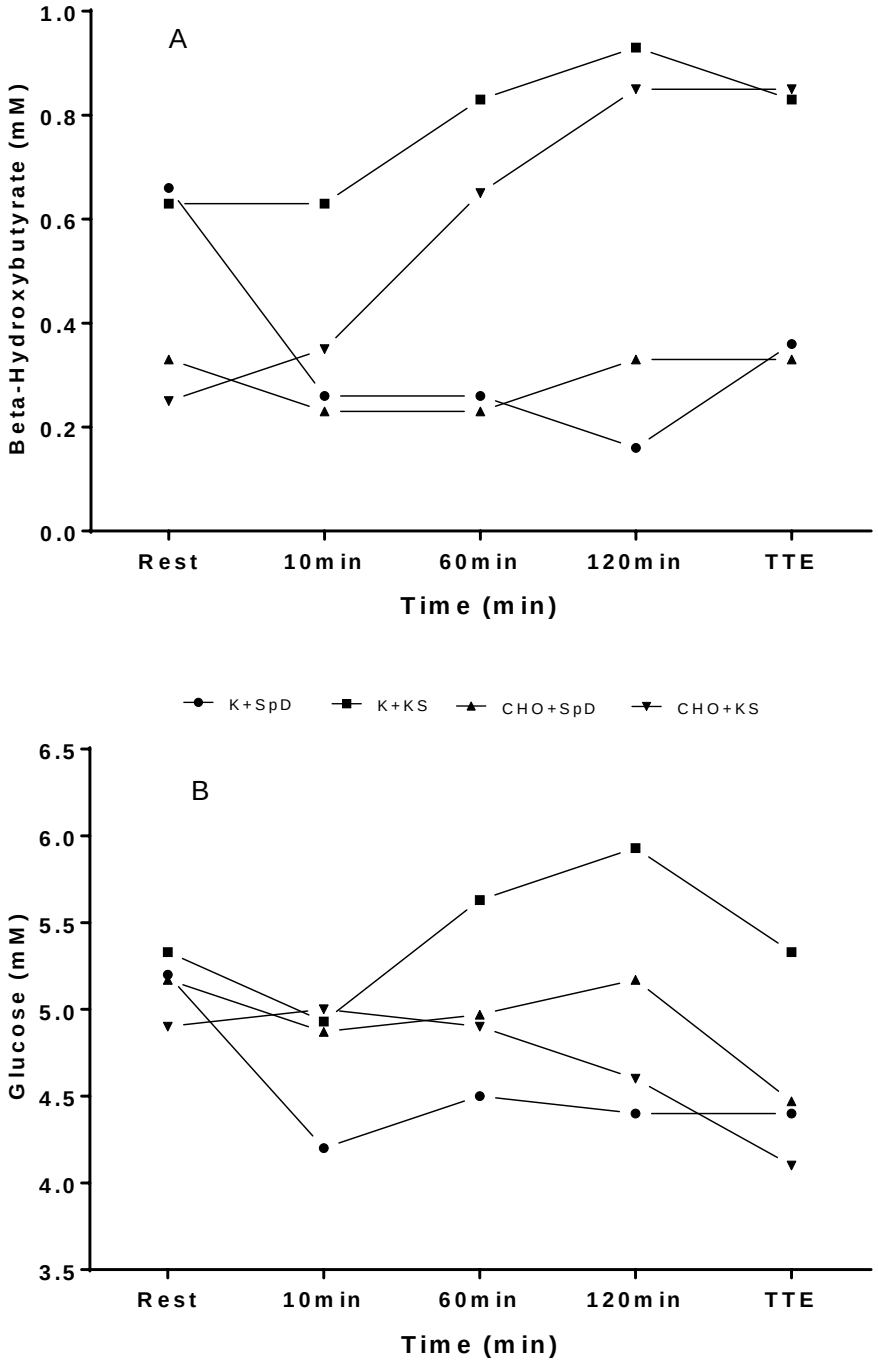


Figure 9-3. β-Hydroxybutyrate [β-HB] (3a) and blood glucose [Glu] (3b) at rest, during fixed intensity cycling and TTE. Ketogenic diet and SpD (K + SpD), ketogenic diet and KS (K + KS), moderate CHO and SpD (CHO + SpD), moderate CHO diet and KS (CHO + KS).

As expected, RER values at rest and during exercise were lower during the ketogenic diet compared to the moderate CHO diet. However, RER values did not differ greatly across all conditions during the TTE (Figure 9-4). During the K condition, resting VO_2 was higher compared to all other conditions and continued to rise during the submaximal ride, but did not reach the same peak during the TT as the other conditions. Lactate concentrations showed no differences across conditions.

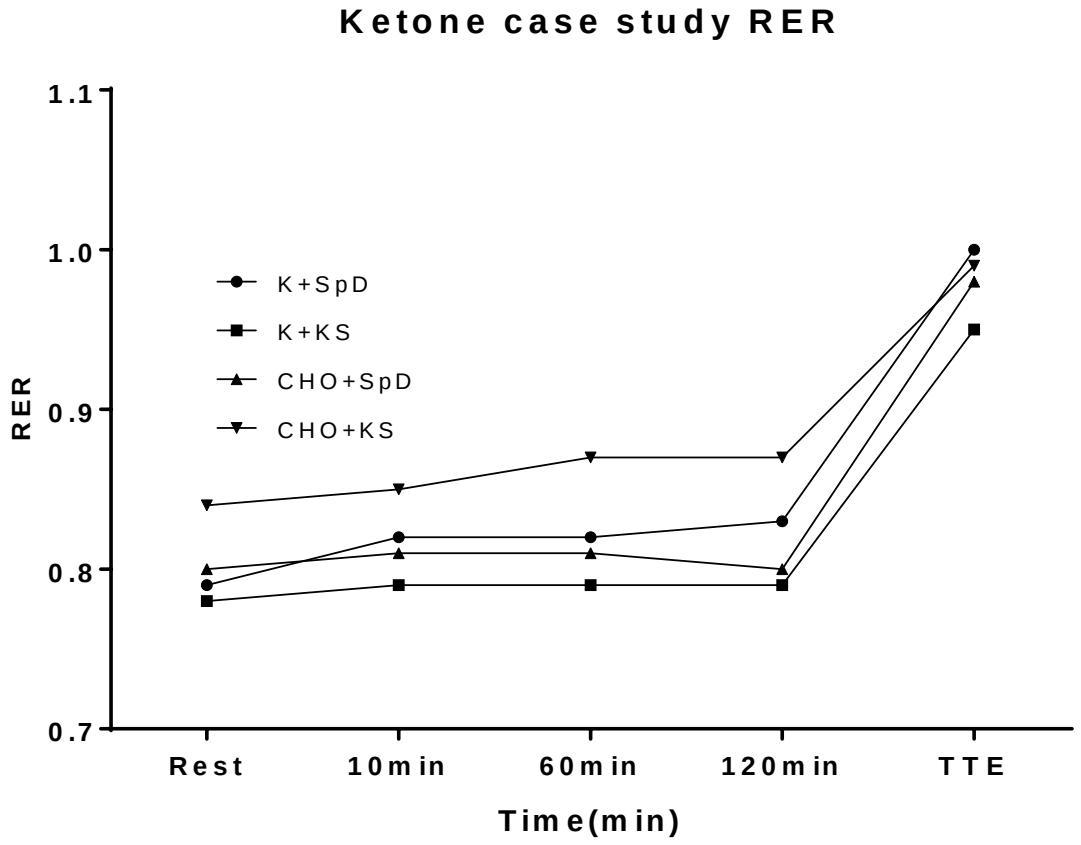


Figure 9-4. Respiratory exchange ratio (RER) at rest, during fixed intensity cycling and TTE. Ketogenic diet and SpD (K + SpD), ketogenic diet and KS (K + KS), moderate CHO and SpD (CHO + SpD), moderate CHO diet and KS (CHO + KS).

The athlete's adherence to both diets was high and all macronutrient goals were met. Even without caloric control, the average daily caloric intake only differed by 72 kcal between the two diets (Table 9-1).

Table 9-1. Average daily macronutrient and caloric intake during each two-week dietary intervention.

Diet	CHO (g)	Fat (g)	Protein (g)	Calories (kcal)
Ketogenic	89.46	248.15	123.15	3131.00
Moderate CHO	371.18	118.91	114.73	3058.64
Difference	281.72	-129.24	-8.42	-72.32

9. 4. DISCUSSION

A circulating [β-HB] of >0.50 mM was associated with improved TTE performances and high rates of CHO oxidation. Ingesting a KS while consuming a moderate CHO diet had an additive effect, producing the best overall performance. An RER >0.9 is well known to represent predominantly non-β-oxidative energy sources (Frayn, 1983) suggesting that an up-regulation of fatty acid oxidation cannot explain the performance effect of KS, as has been suggested in the past (Cameron-Smith et al., 2003). The possible reasoning for an increase in CHO oxidation, despite a 14-day ketogenic diet, is likely to relate to the metabolic effects of exogenous ketone bodies and the difference between the metabolic conditions of nutritional ketosis. That is, endogenous ketone body production is dependent upon circulating free fatty acids (FFA) and depleted hepatic and muscle CHO reserves (P. J. Cox & Clarke, 2014), whereas the KS can induce the same [β-HB] response, independent of this requisite metabolic state.

When considering the mechanism of action responsible for improved performance, the cerebral metabolic system can quickly be ruled out, despite its ability to utilise ketone bodies as an alternative fuel to glucose (Gasior, Rogawski, & Hartman, 2006; Yudkoff et al., 2001). The threshold concentration (Michaelis constant (Km)) at which ketone bodies enter the cerebral system, via the monocarboxylate transporter (MCT), is 5 mM—almost five times the peak concentration reported here (0.90 mM). The Km for neuronal and mitochondrial MCTs, however, is only 0.5 mM, indicating that the point of action is likely to be outside the brain (Veech, 2004).

In the context of past research, only one study (at the time of writing) has attempted to research the effects of a KS on exercise performance directly (Rodger, S. *et al.* 2015). Additionally a review also reported the potential application of KS to exercise (P. J. Cox & Clarke, 2014). In the study of Rodger, S *et al*., (2015) oral administration of a sodium β-HB salt (Na^+ β-HB) was used to raise ketone levels three-fold (0.2 to 0.6 mM/L). The acute increase in β-HB levels had a non-significant impact on performance with the ketone salt being associated with slightly higher power outputs in a four-minute cycling TT (2.3 ± 4.8%). The review from P. J. Cox and Clarke (2014) did not report published data, but did present well-supported theories on the application of a KS to improve exercise performance by elevating circulating ketone levels without the requisite depletion of muscle glycogen. The results presented here align with both Rodger, S. *et al*. (2015) and P. J. Cox and Clarke (2014), showing that a KS can increase ketone levels, even in the presence of a normal CHO status and has the potential to improve endurance performance. The results from the current study also support the claims by P. J. Cox and Clarke (2014) that no prior adaptation to ketosis is required when using exogenous KS.

Although the athlete was extremely experienced in similar testing protocols and test situations, the lack of a familiarisation protocol and washout periods could have impacted the results. The continuation from the ketogenic diet to the moderate CHO diet could have had a follow-on effect from the ketogenic adaptation and potential super-compensation of glycogen stores (Yeo et al., 2011). The justification for the decision to not incorporate a washout period was that the athlete had a habitual low CHO diet and would have entered the second arm of the study in a similar metabolic state. In addition, being a case study, the performance tests and dietary interventions could not be balanced, which could have impacted results due to a training adaptation effect. Also, worth noting, is the energy content of the KS (95 kcal per serve), which meant the energy intake during the ride was not even between trials. However, a calculation of the total energy expenditure (EE) of each TTE shows that EE far exceeded that ingested from the supplement.

In conclusion, the potential for a KS to improve exercise performance is supported by the results of this preliminary case study. However, the method of best application and mechanism of action require further research. Additionally, the improved performance demonstrated here may be the result of mechanisms other than KS offering an alternative fuel for energy production. Based off these findings it is recommended that a statistically well-powered follow-up study be carried out, as outlined in “Ketone Supplementation and Exercise Performance”.

CHAPTER 10. KETONE SUPPLEMENTATION AND EXERCISE PERFORMANCE

10. 1. INTRODUCTION

Endurance exercise performance is affected by many co-founders, which, when combined in the right way, can act to delay the onset of fatigue. For example, an appropriate nutritional strategy for an event lasting over one hour is to maximise muscle glycogen stores ('glycogen load') through elevating carbohydrate (CHO) consumption prior to an event. This can help support the high rates of energy and CHO utilisation associated with competition, thereby delaying the deterioration in muscle power production, which occurs when glycogen levels drop (Louise M. Burke & Kiens, 2006; Kies et al., 1973; Laeger, Metges, & Kuhla, 2010; Laffel, 1999; Westman et al., 2007). Based on the weight of experimental evidence, this strategy appears to work in lesser trained men and during cycling exercise, though the effects on elite athletes, females, and during weight-bearing exercise are less clear (Andrews, Sedlock, Flynn, Navalta, & Ji, 2003; Louise M. Burke et al., 2000; M. A. Tarnopolsky et al., 2001). Nevertheless, and with regard to the latter, CHO loading is generally recommended for endurance sports and alternative strategies to the same end (i.e., improved performance) have subsequently received less attention.

An alternative ergogenic nutritional strategy to that of glycogen loading, has been based on encouraging adaptation to a low CHO diet, in order to increase maximal rates of fatty acid oxidation during competition and, consequently, spare muscle and liver glycogen (S. D. Phinney et al., 1983). Along these lines, there has recently been considerable interest in the

role that supplemental ketones might play in exercise metabolism (Pete J Cox et al., 2016; Evans, Cogan, & Egan, 2016; Hashim & VanItallie, 2014; Kesl et al., 2016; Pinckaers, Churchward-Venne, Bailey, & van Loon, 2016; Rodger, 2015). Ketone bodies are lipid-derived organic compounds that can serve as a circulating energy source for tissues during starvation/fasting or prolonged exercise (F. Fery & Balasse, 1983). The ketogenic state produced during starvation is not a function of total energy restriction per se, but is due specifically due to low CHO intake and the subsequent decrease in the insulin:glucagon ratio (McGarry & Foster, 1980). This means that low CHO diets can produce a state of ketosis without caloric restriction (Johnson et al., 2006; Kelley, Goodpaster, Wing, & Simoneau, 1999).

Acetyl–CoA, derived from fatty acids, is converted to ketone bodies via hepatic mitochondria (up to ~150 g/day) (Pete J Cox et al., 2016; Lestan, Walden, Schmaltz, Spychala, & Fox, 1994). The term "ketone bodies" refers to the compounds acetoacetate (AcAc), β-HB, and acetone. However, only AcAc and acetone are actual "ketones"; β-HB is a ketone body but technically not a ketone. While the majority of acetone is secreted through urine and lost via expiration, AcAc and β-HB are transported in the bloodstream to extrahepatic tissues, such as the brain, heart, and skeletal muscle (Laeger et al., 2010; Laffel, 1999). Ketone bodies can cross the plasma and mitochondrial membranes by monocarboxylate transporters (MCT) and are converted back to acetyl–CoA and used as an alternative source of energy by the tricarboxylic acid (TCA) cycle (Pete J Cox et al., 2016; F. Fery & Balasse, 1983; Webster et al., 2016).

In many instances, a low CHO ketogenic diet has been known to impair exercise performance due to reduced intra- and extra-muscular CHO availability (Louise M. Burke

& Kiens, 2006). Ketone body supplements (KS), on the other hand, have the potential to rapidly increase ketone body availability, without the need to first adapt to a ketogenic diet through a low CHO intake (Clarke et al., 2012). Outlined in the previous section (Ketone Supplementation: Case Study), is a case study demonstrating the potential applications of KS to endurance performance, but as yet, little is known about their effects on substrate metabolism during endurance-type exercise.

In theory, ketosis provides an ideal metabolic setting for endurance exercise, especially events lasting over four hours, because of the reduced reliance on muscle glycogen and blood glucose at the muscle. However, a point of concern with nutritional ketosis and exercise performance is the need for CHO even at higher intensities (e.g., >50% VO_{2max}). At these intensities, a proportion of type Π (or fast glycolytic) skeletal muscle fibres will still be recruited, particularly as type I fibres become fatigued over time; type Π rely heavily on CHO to support contraction, while type I fibres have the flexibility to utilise alternative fuel. Recently, the ingestion of ketone body supplements has emerged as a strategy to induce hyperketonaemia (van Erp-Baart et al., 1989), with considerable media speculation about ketone body supplements being used by professional cyclists (Abraham, 2015). Until now, humans have been unable to exogenously alter the ketone body concentrations in the blood (excluding infusion). However, by digesting a KS, in this case 1, 3-butanediol (here forth referred to as KS), in beverage form, it is possible to raise blood ketones to a level the body has only previously encountered during starvation, or during a period of severe CHO restriction. The KS, 1, 3-butanediol, is metabolised in the liver by alcohol dehydrogenase to form β-HB (Kies et al., 1973).

The primary aim of this study is thus to test the effects of a KS on endurance performance. The secondary aim is to test the interaction between the KS and different metabolic environments.

10. 2. METHODS

10. 2. 1. Participants

Participants were six well-trained male endurance athletes, who were completing at least 15 hrs/wk of aerobic training (age: 29 ± 9 yrs, body mass: 74.1 ± 7.7 kg, VO_{2max}: 64.1 ± 5.8 ml/kg/min) and had a minimum of five years of racing experience. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Massey University (15/74) March 2016.

10. 2. 2. Study Design

Before the start of the study proper, a VO_{2max} and submaximal test were undertaken to calculate the performance test workloads, as outlined in Chapter 5.
The study design was a randomised cross-over and therefore participants were randomly separated in to two groups; A and B (Figure 10-1). Group A was assigned a ketogenic and Group B a moderate CHO diet for the first intervention period of fourteen days, after which, a washout period was enforced (two weeks minimum) before the alternative dietary intervention was initiated (cross-over). Randomisation of participants was done using a random number generator.

Throughout each fourteen day dietary intervention, participants completed three different supplemented performance sessions (see "*Supplements*" below) at baseline, day eleven and day fourteen (Figure 10-1).

10. 2. 3. Performance Test

The performance protocol comprised a progressive 40-minute fixed intensity ride: 10 minutes at 40% VO_{2max} power, 10 minutes at 50% VO_{2max} power and 20 minutes at 75% VO_{2max} power, immediately followed by a self-paced time trial (TT). The TT was in the format of time to complete a fixed workload (progress blinded) equivalent to 20 minutes at 75% VO_{2max} (workload expressed in kilojoules, 1W = 3.6kJ).

The performance protocol was designed to give an indication of which intensities the KS could provide the most benefit: low intensity (40–50% VO_{2max}), anaerobic/ventilatory threshold (~75% VO_{2max}) or anaerobic capacity (>90% VO_{2max}).

The performance protocol was carried out three times each intervention and a total of six times across the entire study as follows:
baseline (BASE-CHO, BASE-K), placebo + moderate CHO (PLB+CHO), KS + moderate CHO (KS+CHO), placebo + ketogenic diet (PLB+K), KS + ketogenic diet (KS+K).

10. 2. 4. Physiological Measures

The physiological measures collected during the performance tests were VO_2, blood GLU, blood Lac and blood β-HB.

Participants were weighed on arrival to the performance laboratory on a set of calibrated weighing scales (Jadevar, Taiwan). The participants then consumed one of the supplements and sat passively for 60 minutes to allow peak concentration of β-HB to be reached. At 60

minutes post ingestion (t = 0 min), resting respiratory gases were collected for five minutes while heart rate (Polar RS800 HRM, Finland) and blood metabolite measures were taken, using handheld meters and finger prick samples: glucose [Glu] (Precision Xtra, Freestyle, Abbott Diabetes Care Inc.), lactate [Lac] (Lactate Pro 2, Arkray Inc, Japan) and β-Hydroxybutyrate [β-HB] (Precision Xtra, Freestyle, Abbott Diabetes Care Inc.). During the performance test, all physiological measures were collected at 20 minutes and 40 minutes. However, during the TT, only blood GLU, blood Lac and blood β-HB were collected to avoid the VO_2 apparatus interfering with the participants' performances.

10. 2. 5. Supplements

The KS was an alcohol ketone precursor, 1, 3 butanediol ($C_4H_{10}O_2$), molecular weight = 90.12 g/mol, density = 1.005 g/mL at 25^oC, caloric content = 4.69 kcal/g (Sigma Aldrich, New Zealand). Based on the data presented in the previous section ("Ketone Supplementation: Case Study") a dose of 500 mg/kg was chosen to avoid any adverse effects, while still raising blood ketones to above 0.1 mM. The KS was mixed into 250 mL of H_2O and 50 g of powdered sports drink (SpD) (40 g CHO, Powerade, Coca-Cola Oceania Ltd, New Zealand). The placebo (PLB) was of equivalent nutritional value and made up as: 150 mL of tonic water, 100 mL of H_2O, 50 g of powdered SpD and 75 mL of white vinegar (to replicate taste). PLB and KS supplements were administered in a double-blinded fashion. The baseline condition (BASE+CHO & BASE+K) was 250 mL H_2O and 50 g powdered SpD. Within each performance session, the assigned supplement was ingested three times: at rest 60 minutes before exercise (rest), immediately before the onset of exercise (pre) and during the final five minutes of the 75% VO_{2max} fixed intensity ride (t = 35 minutes).

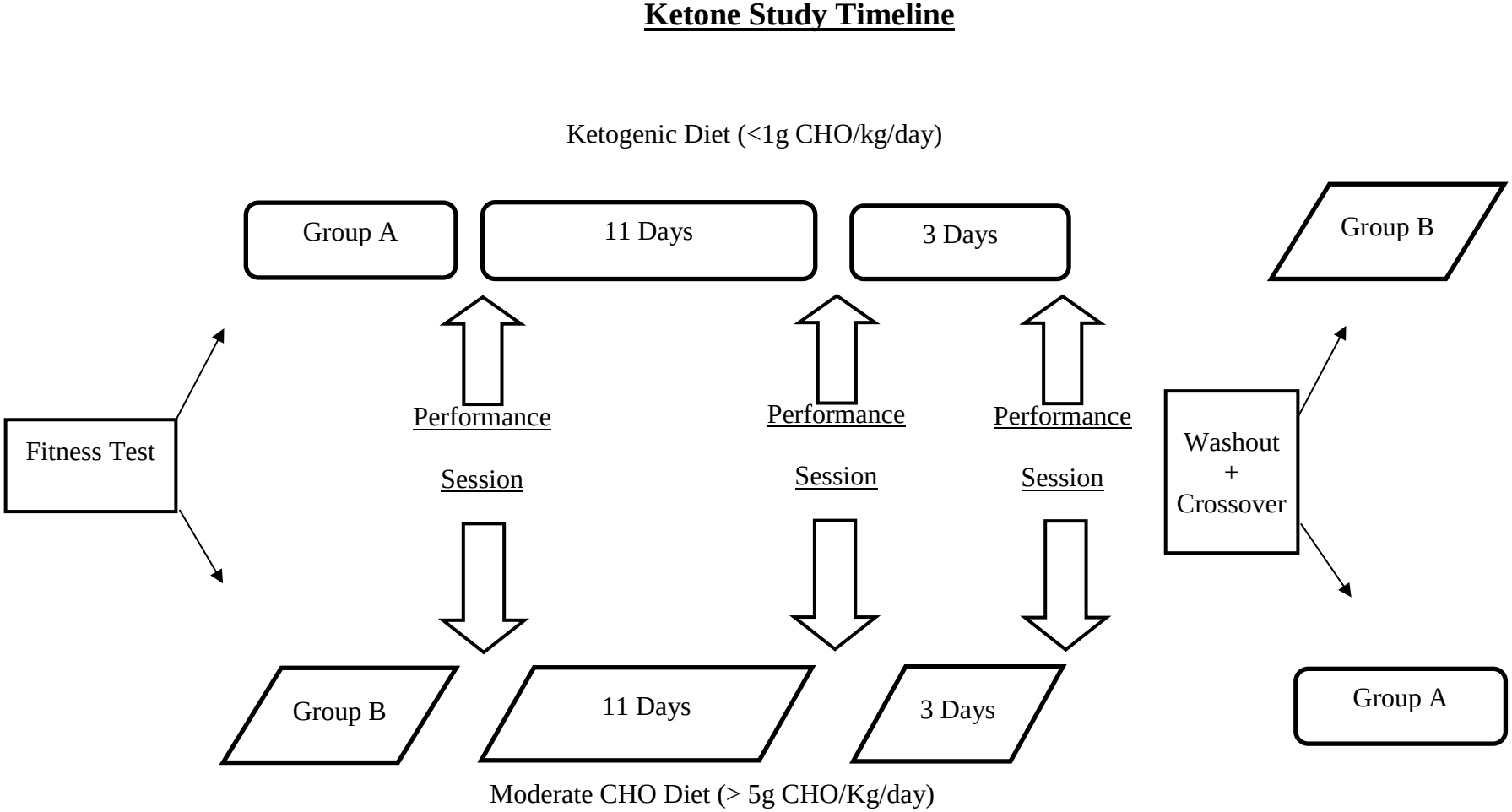


Figure 10-1. Study outline of the fourteen-day protocol. Each participant completed day one to day fourteen twice: high CHO and ketogenic.

10. 2. 6. Diet

The secondary aim of the study was to test the interaction between the participants' metabolic states and the KS. To achieve this, participants' macronutrient intakes were manipulated in the form of a ketogenic diet or a moderate CHO diet. During the ketogenic diet, the participants were limited to 1 g/kg/day of CHO. This is higher than common CHO intakes for ketogenic diets (<50 g/day) (Paoli et al., 2012; S. D. Phinney et al., 1983; Westman et al., 2007), but for this cohort, who were completing a minimum of 60 minutes of endurance training per day, it was deemed sufficient to elicit an appropriate level of glycogen depletion. The level of restriction was supported by regular resting blood β-HB values of over 0.2 mM (Hashim & VanItallie, 2014; Robinson & Williamson, 1980; R. Smith et al.). A concentration of 0.2 mM was used instead of the more well referenced 0.5 mM because the intent was to induce an upregulation of kotengic pathways which could be achieved with at a level of 0.2 mM. Outside of the performance tests, β-HB was only measured during the ketogenic trial in the lab after 5 days on the diet.

During the moderate CHO diet, 50% of total energy came from CHO (5 g CHO/kg/day) which is representative of recommendations for the common endurance athlete's diet (van Erp-Baart et al., 1989). The composition of daily food intake was monitored using "MyFitnessPal®" online and mobile software (MyFitnessPal® Inc., San Francisco). Both diets had protein intake fixed at between 15–20% of the total caloric intake. Participants were educated on using this software prior to the start of each dietary intervention. To allow the diets to be as habitual as possible, there were no caloric or other restrictions. A minimum washout period of two weeks separated the two dietary interventions. Extended methodology, referring to dietary control, can be found in the "Common Methodology" section.

A CHO load was used for both diets—before the performance session to remove the possibility of glycogen content affecting performance. The CHO load was standardised from dinner the night before (3 g/kg CHO, Fat = 21 g, Pro = 24 g) and breakfast the morning of the performance session (1 g/kg CHO, Fat = 16 g, Pro = 21 g). The standardised breakfast was consumed a minimum of 60 minutes and maximum of 120 minutes before the first measures took place. Specific information referring the standardised meals can be found in the Appendices.

10. 2. 7. Statistical Analysis

All data was analysed using SPSS Statistics for Windows, Version 23.0. (IBM Corp, NY, USA). Three-way repeated measures ANOVA tested the diet x supplement x time (Rest, pre, 15 minutes, 35 minutes, TT) interactions for changes in RER, [β-HB], [Lac], [Glu], HR and VO_2. A two-way repeated measures ANOVA tested the diet x time interaction for changes in body mass. Mauchly's test of sphericity was used to test the sphericity of the ANOVA output and unless otherwise specified, sphericity was assumed. On the occasion that sphericity could not be assumed, the Greenhouse–Geisser (GG) adjustment was used. Least squares difference post hoc tests were run, to inspect the comparisons of the mean differences. To test the effect of order, the data was analysed as trial one and trial two, with no reference of diet. Repeated measures two-way ANOVA tested the potential effect of order on changes in variables mentioned previously. Significance was set at $p \leq 0.05$. All data are presented as mean ± standard deviation (SD) or mean difference and 95% confidence interval (CI), as appropriate.

10. 3. RESULTS

10. 3. 1. Performance

There were no main effects of diet or supplement, and no interaction of the two, on TT performance in terms of time (minutes), power output (Watts) or relative power output (W/kg).

There were no main effects of diet or time on body mass, although the interaction between diet and time trended toward significance ($p = 0.080$) (Figure 10-2). Body masses during the two diets were as follows: moderate CHO (baseline = 74.2 ± 9.1 kg, post = 75.1 ± 9.8 kg), low CHO (baseline = 74.9 ± 8.6 kg, post = 74.2 ± 9.9 kg).

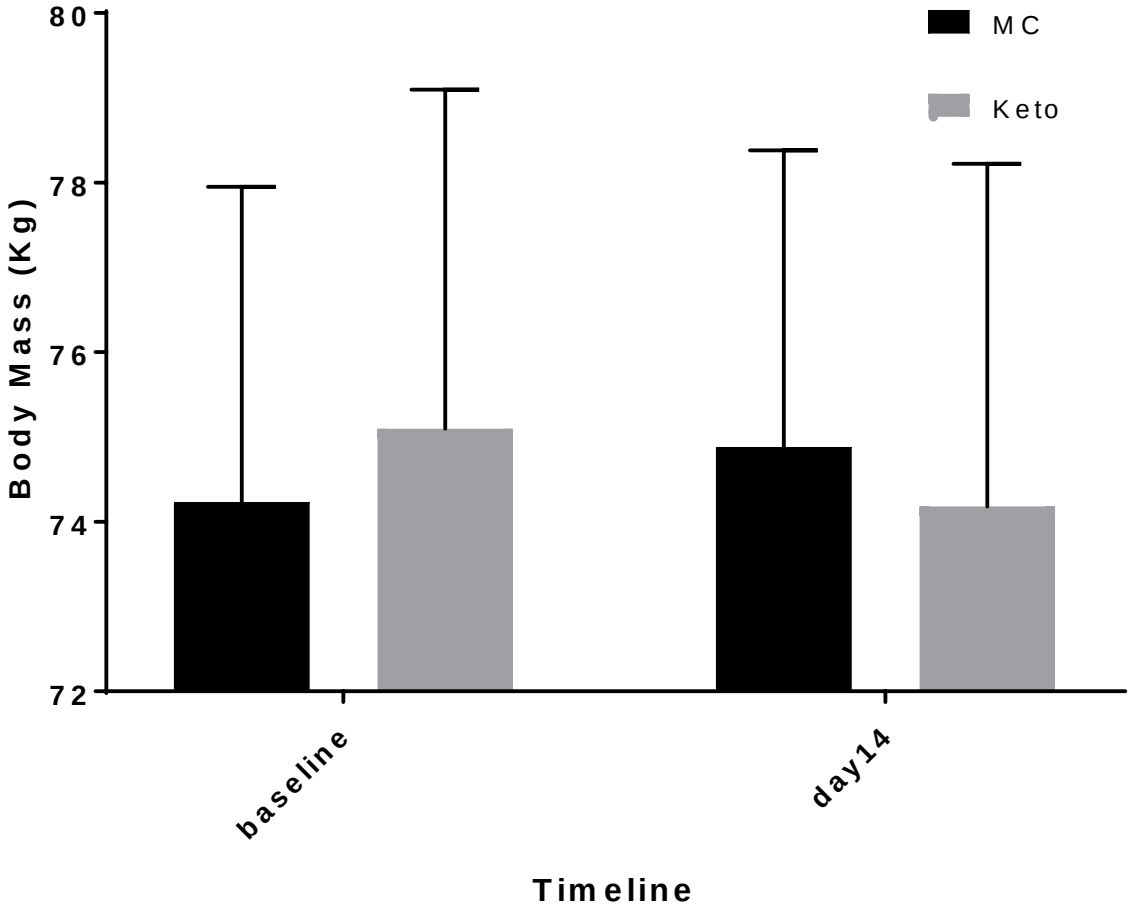


Figure 10-2. Body mass at baseline and day fourteen of each dietary intervention.

10. 3. 2. Beta-hydroxybutyrate

There was a main effect of supplementation on [β-HB] (p = 0.001) with the mean [β-HB] being higher than in the placebo condition (mean difference = 0.400 mM, CI 95%, 0.305, 0.495, p = 0.001, ES = 5.60) and at baseline (mean difference = 0.394 mM, CI 95%, 0.300, 0.488, p = 0.001, ES = 5.58) (Figure 10-3B). There was a main effect of time on [β-HB] (p = 0.001). There was no main effect of diet (p = 0.367). There was a significant interaction between supplementation and time (p = 0.003). The post hoc analysis showed. that 60

minutes after ingestion of the KS, [β-HB] was higher than prior to ingestion (mean difference = 0.575 mM, CI 95%, 0.262, 0.888, p = 0.005, ES = 3.229)) and during exercise (75%, mean difference = 0.308 mM, 95% CI, 0.087, 0.530, p = 0.016, ES = 2.466 and TT, mean difference = 0.342 mM, 95% CI, 0.084, 0.600, p = 0.019, ES = 2.058). [β-HB] during exercise did not differ between non-KS conditions (p = 0.415) (Figure 10-3B).

There was no interaction between diet and time (p = 0.095), diet and supplementation (p = 0.462) or diet x time x supplementation (p = 0.352).

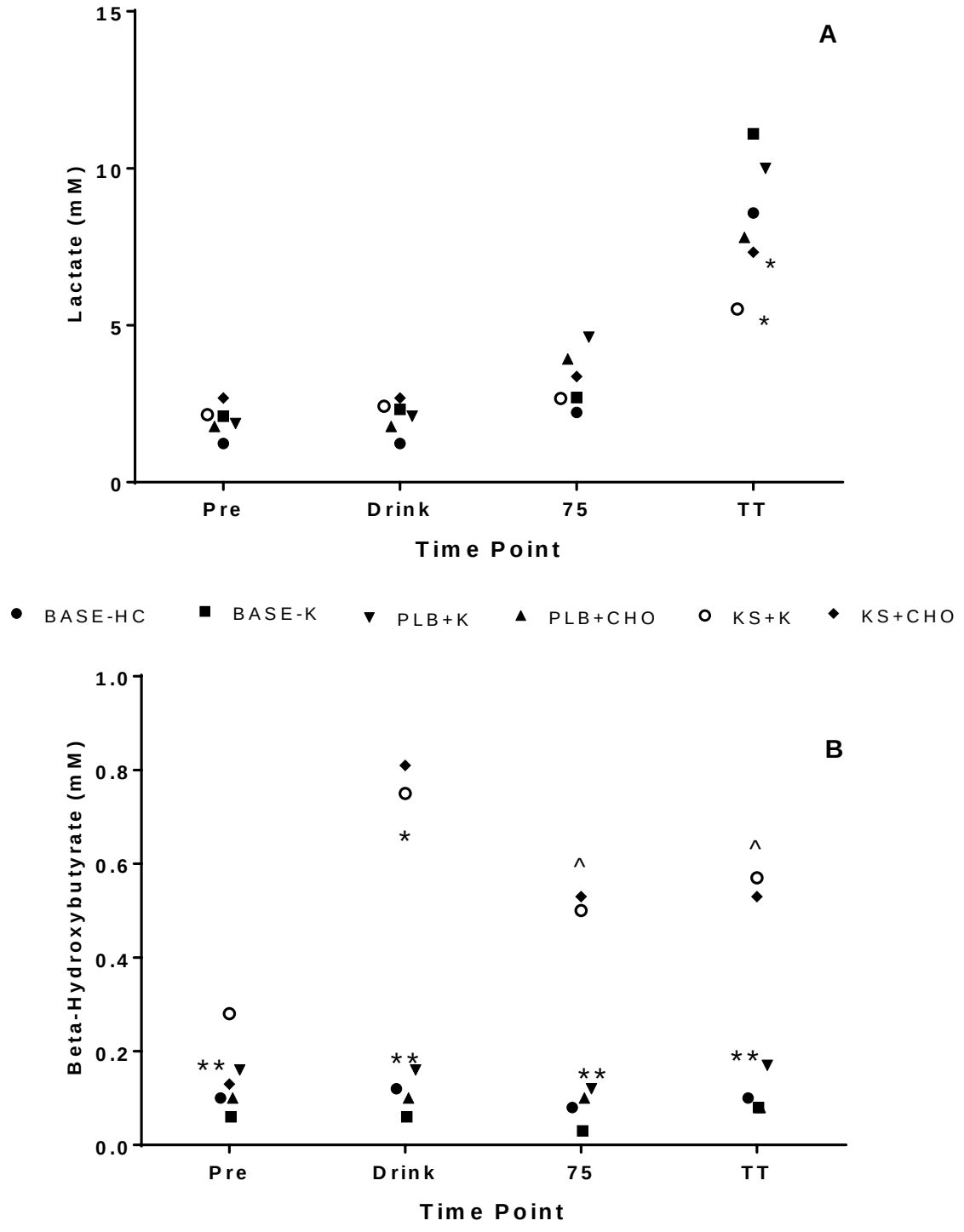


Figure 10-3. Lactate (A) and β-HB (B) concentrations taken at each sample point during all conditions. Graph A: * denotes a significantly lower [Lac] from all other conditions during the TT. Graph B: * denotes a significantly higher [β-HB] from all other time points and conditions. ** denotes a significantly lower [β-HB] from KS. Significance is set at ($p < 0.05$).

10. 3. 3. Lactate

There was a main effect of time on [Lac] (p = 0.001, Figure 10-3A). Supplementation also had a main effect on [Lac] (p = 0.01). Diet did not significantly affect [Lac] (p > 0.05). There was a significant interaction between supplementation and time (p = 0.749). The post hoc analysis showed that KS resulted in significantly lower lactate concentrations during the TT than with the PLB and at baseline (mean difference = -2.725 mM, 95% CI, -4.844, -0.606, p = 0.021, ES = 0.981 and mean difference = -3.867 mM, 95% CI, -6.046, -1.687, p = 0.006, ES = 1.625 respectively). PLB and baseline conditions did not differ from one another (p = 0.361) (Figure 10-3A). There were no other significant interactions.

10. 3. 4. Additional data

With the exception of an expected main effect of time on VO_2 (p = 0.034) there were no main effects of diet, supplementation or time on the remaining physiological measures (VO_2, RER and glucose) nor were there any interactions between them (Table 10-1). No adverse effects were noted by any participants.

Table 10-1. Additional physiological measures (mean +- SD)

		Moderate CHO						Ketogenic diet					
		VO_2		RER		GLU		VO2		RER		GLU	
Rest	BASE	0.37	± 0.07	0.98	± 0.11	5.70	± 0.34	0.37	± 0.09	0.93	± 0.12	5.28	± 0.59
	PLB	0.39	± 0.06	0.97	± 0.05	5.53	± 0.47	0.39	± 0.05	0.92	± 0.09	5.83	± 0.55
	KS	0.41	± 0.07	0.92	± 0.08	5.05	± 0.75	0.39	± 0.04	0.84	± 0.08	5.33	± 0.77
75%	BASE	3.32	± 0.60	0.97	± 0.07	4.62	± 0.86	2.92	± 1.58	1.59	± 1.59	4.52	± 0.50
	PLB	3.36	± 0.61	0.97	± 0.06	4.43	± 0.43	3.48	± 0.53	0.92	± 0.06	4.45	± 0.49
	KS	3.26	± 0.55	0.97	± 0.07	4.57	± 0.59	3.39	± 0.50	0.90	± 0.05	4.18	± 0.77
TT	BASE	4.17	± 0.58	1.03	± 0.04	4.22	± 0.45	4.22	± 0.79	0.98	± 0.07	4.58	± 0.72
	PLB	3.94	± 0.39	1.00	± 0.05	3.80	± 2.01	4.20	± 0.51	1.03	± 0.16	4.85	± 0.69
	KS	4.16	± 0.60	1.00	± 0.06	4.87	± 1.44	4.17	± 0.60	0.96	± 0.08	4.58	± 0.79

10. 4. DISCUSSION

Ketone supplementation did not result in the hypothesised improved performance, despite significantly increasing [β-HB] on both diets. On the other hand, an interesting and unintended finding of this study, was the indifferent TT performances between dietary conditions. For many decades, low CHO intake, in conjunction with endurance exercise, has been seen as ill-advised, due its potentially detrimental effects on performance (Louise M. Burke & Kiens, 2006). Yet, the results presented here show that, with the allowance of a CHO-load prior to exercise, low CHO intake to the extent of nutritional ketosis is not detrimental to endurance exercise performance. In fact, there was a trend toward weight loss following the ketogenic diet (Figure 10-2) which would be beneficial to sports where the power-to-weight ratio is an important determinant for performance, assuming preservation of skeletal muscle (not measured here).

The lack of a significant performance benefit could be explained by the [β-HB] achieved with the KS used in the current study. [β-HB] did not exceed a maximum level of 1.1 mM, irrespective of metabolic state. In the previous two studies, where KS has been applied to endurance exercise, the [β-HB] reached were far above the average resting concentrations (0.78 mM) achieved here (Figure 10-3B) (F. Fery & Balasse, 1983). It is possible that circulating [β-HB] needs to reach a minimum of around 2 mM in order to stimulate the effect of starvation (Pete J Cox et al., 2016; Hashim & VanItallie, 2014). Below 2 mM there is little metabolic stimulus to utilise ketones at a rate that would provide adequate additional acetyl–CoA to the TCA cycle, as described in Review of Literature - Ketosis: Ketone supplementation.

The key difference in the two referenced studies and the one presented here is the method of delivery for the exogenous ketones. Cox et al., (2016) used an oral *(R)*-3-hydroxybutyl *(R)*-3-hydroxybutyrate ketone monoester (KE) while Françoise Fery and Balasse (1988) used a sodium acoaceatate (AcAc) infusion. Both methods allowed for considerably higher concentrations of β-HB to be obtained. Ingestion of KE resulted in [β-HB] of ~3 mM after 10 minutes and rising to ~6 mM 30 minutes after ingestion. Françoise Fery and Balasse (1988) infused sodium AcAc after an overnight fast to achieve ketone body concentrations of ~6 mM (β-HB ~3.5 mM, AcAc ~2.5 mM). In the study by Pete J Cox et al. (2016), [β-HB] was reduced by ~2 and 3 mM during exercise lasting 45 minutes, at 40% and 75%Wmax, respectively. Expired air analysis, adjusted for the oxidation of ketone bodies, explains the reduction in [β-HB]; β-HB oxidation contributed 18% and 16% of oxygen consumption to energy provision at the respective intensities. Françoise Fery and Balasse (1988) reported a similar [β-HB] decline throughout exercise, from 3.5 mM to ~2 mM, at the end of a 2 hr walk at 55% VO_{2max}. Results here only show an 0.2 mM reduction in [B-HB], which would relate to around a 2% energy contribution. This minimal contribution to energy supports the previously discussed claim for a minimum 2 mM [β-HB] limit.

Françoise Fery and Balasse (1988) reported that the exercise induced decline in exogenous [β-HB] coincided with a progressive rise in the exercise induced uptake of β-HB. In contrast, this effect was not present when similar [β-HB] were achieved via fasting. Although the inhibition of ketone oxidation by hyperketonaemia was present during exogenous ketosis, there was no feedback inhibition, as seen in fasting ketosis. This occurred when the initial rise in exercise induced ketone oxidation caused a reduction in [β-HB] which, in turn, further increased cellular uptake of [β-HB]. Additionally, the threshold concentration at which hyperketonaemia inhibited [β-HB] uptake was higher in exogenous ketosis than in fasting

ketosis. The nutritional ketosis induced here did not appear to influence the exercise induced disposal (0.70 mM–0.50mM), although, as previously mentioned, the [β-HB] were not at a level representative of hyperketonaemia (>6 mM) (Kanikarla-Marie & Jain, 2016).
The comparatively lower [Lac] seen during the TT with the KS has been reported similarly by both Françoise Fery and Balasse (1988) and Pete J Cox et al. (2016). Pete J Cox et al. (2016) associated the drop in lactate production with inhibition of glycolytic metabolism, sparing of muscle glycogen, reduced deamination of branched-chain amino acids, and increased reliance on intramuscular triglyceride (IMTG) during exercise with ingestion of the KE. If this is the case, it would support the idea, outlined in section 2. 8. 5. ("Review of Literature - Ketosis: Ketone supplementation"), that [β-HB] is able to enter directly into the TCA cycle as acetyl–CoA and would reduce need for glycolysis.

Due to time constraints, a two-day washout period was employed after the initial CHO-loaded performance session (day eleven–Figure 10-1) however this may not have been enough time to cause a metabolic shift back to ketosis. The end results, however, indicate this was of little impact to the final outcome. The strength of the KS was not adequate to produce exercising [β-HB] >1.0 mM and, as discussed, this could have influenced results significantly when compared to previous studies. Potential adverse effects of KS should be considered, as Clarke et al. (2012) reported several side effects of KE ingestion in humans, such as flatulence, nausea, diarrhoea and dizziness, at doses ranging from 420 to 1071 mg/kg BM.

In conclusion, the use of 1, 3 butanediol as an exogenous KS does not impact performance during an endurance cycling time trial (workload equivalent to 20 minutes at 75% VO_{2max} power). Further research into KS and similar substances and exercise performance should ensure a minimum of 2 mM [β-HB] is obtained to guarantee an adequate metabolic stimulus.

The CHO status of the individual can be largely ignored as supplementation appears to be equally effective irrespective of the metabolic environment. Nevertheless, ketogenic diets should not be dismissed and show potential as an effective weight loss strategy without compromising endurance performance.

CHAPTER 11. SUMMARY OF FINDINGS

This thesis proposed to:

1. Expand on the term 'metabolic flexibility' and used it to describe the ideal metabolic state for endurance athletes, by testing the responses of highly trained endurance athletes exposed to periods of contrasting macronutrient availability (low CHO & moderate CHO).
2. Compare the metabolic flexibility of endurance trained men and women.
3. Test the ability of a novel supplement to induce favourable metabolic changes, mimicking the hypothesised benefits of metabolic flexibility.

As has been discussed in detail throughout this thesis, metabolic flexibility refers to the ability of cells or tissues to alter specific metabolic pathways to account for chronic and acute changes to substrate availability. From the data presented within the preceding chapters, it is apparent that endurance trained males possess an exceptional degree of metabolic flexibility. Endurance trained women, on the other hand, appear to be less metabolically flexible than their male counterparts, despite similar levels of relative physical fitness.

These differences can be attributed to sex specific differences in fibre type distribution, body composition, muscle recruitment patterns, hormonal status, enzyme concentrations and biochemical pathway regulation. On a more holistic level, the results from studies one and two indicate that the inability of women to oxidise CHO (CHO_{ox}) at the same maximal rate as men, limits their metabolic flexibility. An upshot of a naturally lower CHO_{ox} capacity is a comparatively more favourable response to a low CHO diet. It has been stated that when running distances of >50km, women can produce performances close to that of equally

trained men (Bam, Noakes, Juritz, & Dennis, 1997; Zingg et al., 2014). From the data gathered here, it is proposed that this occurs because with longer distances, less CHO_{ox} contributes to the total muscular energy usage; consequently, performance over prolonged durations is more closely associated with maximal FAT_{ox}, which favours women over men. Due to men possessing greater metabolic flexibility, their capacity to gain from CHO_{ox} is reduced in this situation. As exercise duration increases (>3 hours) the contribution of FAT_{ox} to energy production becomes more predominant and the contribution of CHO_{ox} is reduced. Therefore, both men and women do not benefit as much from CHO_{ox}, endogenous or exogenous, when exercising over prolonged durations. Thus, as the duration (distance) continues to increase the naturally lower dependence on CHO_{ox} (not higher FAT_{ox}) allows for a similar performance between sexes, due to the inability of men to buffer the loss of energy derived from their greater CHO_{ox} capacity.

Metabolic flexibility can help to explain the lack of significant alterations in the physiological and performance capacities of men across all studies presented in this thesis. The nature of the cohort—highly trained endurance athletes—meant that the years of training each participant had undergone had induced favourable adaptions resulting in the male athletes, who possessed high degrees of metabolic flexibility. That is, when presented with the metabolic challenges described within this thesis (i.e., low CHO intake, ketone supplementation) the male participants were able to alter their regulation of the relevant metabolic pathways to ensure the continuation of whole-body and cellular ATP production, ensuring the continuation of exercise at a given workload.

Ketone supplementation, in theory, has the potential to quickly induce the favourable metabolic alterations associated with metabolic flexibility. However, within this thesis, its

application to already well-trained males seems equivocal. The ability of this highly trained cohort to regulate ATP yielding pathways, specifically within working muscle, meant that when blood ketone concentrations were increased, relevant biochemical shifts took place that resulted in ketone body oxidation. The associated lowering of lactate production during high intensity exercise may represent potential benefits of exogenous ketones for repeated high intensity bouts, for example, in intermittent type events, like cycle racing, which require quick recovery between bouts of high level anaerobic work. However, referring back to the principle of exercise-associated metabolic flexibility, a potential performance enhancing increase or decrease of metabolic substrates may be masked by a corresponding performance reducing increase or decrease of substrates. As such, further studies must look holistically to ensure an isolation bias is avoided.

CHAPTER 12. IMPLICATIONS

1. A low CHO diet should be considered an appropriate nutritional tool for both male and female endurance athletes. Although the incorporation of a low CHO diet does not appear to cause reductions in endurance performance, its application should be trialed during non-crucial periods of competition (i.e., off-season).

2. Women appear to be less metabolically flexible than men. That is, women are not better at burning fat but rather, less able to burn CHO. As a result, we suggest that the appropriate level of CHO intake for endurance training is lower than the current recommendations.

3. It is recommended that, for women more so than men, a chronic adaption to low CHO intake is considered >14 days.

4. Both men and women improved body composition on a low CHO diet. This finding should be taken into account when trialing dietary methods in an attempt to improve body composition of endurance athletes.

5. Ketone supplementation has sound theory but as yet lacks enough conclusive evidence to allow for a complete recommendation for the application to endurance sport for performance enhancement.

6. If ketone supplementation is to be researched in the future, the concentration threshold of 2 mM [β-HB] should be applied, to ensure an adequate metabolic stimulus and or

contribution to energy provision. The use of ketone esters, such as (R, S)-1, 3-butanediol-acetoacetate monoesters, may be able to achieve these results.

7. The broad-brush approach of H^1 NMR metabolomics does not provide the best means for analysing complex human biofluids, especially in the absence of specific high dose interventions, like that of a single xenobiotic in toxicological studies.

CHAPTER 13. REFERENCES

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CHAPTER 14. APPENDICES

14. 1. TABLE OF APPENDICES

14. 2. APPENDIX A

Table 14-1. Individual macronutrient intakes for habitual diet.

		Habitual						
		Grams		S.D	Calories		S.D	Percentage
AB	CHO	243.57	±	79.59	974.29	±	318.34	48%
	Fat	81.86	±	22.97	736.71	±	206.72	37%
	Pro	75.71	±	30.56	302.86	±	122.22	15%
	Total				2013.86		621.37	
CB	CHO	375.55	±	72.84	1502.18	±	291.34	55%
	Fat	88.64	±	32.28	797.73	±	290.48	29%
	Pro	114.09	±	27.66	456.36	±	110.65	17%
	Total				2756.27		590.39	
DF	CHO	186.75	±	50.61	747.00	±	202.44	48%
	Fat	49.50	±	18.11	445.50	±	162.98	28%
	Pro	93.33	±	25.49	373.33	±	101.97	24%
	Total				1565.83		346.30	
EC	CHO	220.55	±	135.92	882.18	±	543.69	42%
	Fat	90.00	±	18.68	810.00	±	168.11	39%
	Pro	96.36	±	23.15	385.45	±	92.61	19%
	Total				2077.64		533.55	
HH	CHO	229.25	±	39.39	917.00	±	157.54	26%
	Fat	216.92	±	30.77	1952.25	±	276.92	56%
	Pro	148.25	±	21.35	593.00	±	85.40	17%
	Total				3462.25		270.81	
JB	CHO	194.25	±	46.47	777.00	±	185.90	51%
	Fat	44.50	±	22.07	400.50	±	198.64	26%
	Pro	87.75	±	35.45	351.00	±	141.82	23%
	Total				1528.50		448.35	
KS	CHO	349.11	±	88.95	1396.44	±	355.79	61%
	Fat	69.56	±	21.25	626.00	±	191.21	28%
	Pro	63.00	±	18.12	252.00	±	72.47	11%
	Total				2274.44		498.46	
LM	CHO	375.20	±	116.33	1500.80	±	465.34	58%
	Fat	69.00	±	13.84	621.00	±	124.58	24%
	Pro	120.00	±	22.76	480.00	±	91.04	18%
	Total				2601.80		454.70	
MM	CHO	326.17	±	124.95	1304.67	±	499.80	56%
	Fat	78.00	±	16.16	702.00	±	145.40	30%
	Pro	75.83	±	14.48	303.33	±	57.94	13%
	Total				2310.00		472.80	
MB	CHO	68.63	±	12.40	274.50	±	49.60	16%
	Fat	125.38	±	22.16	1128.38	±	199.42	65%
	Pro	80.13	±	16.53	320.50	±	66.13	19%
	Total				1723.38		256.49	
MBU	CHO	189.50	±	58.50	758.00	±	234.00	50%
	Fat	58.50	±	19.50	526.50	±	175.50	34%
	Pro	60.50	±	19.50	242.00	±	78.00	16%
	Total				1526.50		487.50	
VH	CHO	282.43	±	70.63	1129.71	±	282.53	49%
	Fat	83.29	±	23.44	749.57	±	211.00	33%
	Pro	106.14	±	19.49	424.57	±	77.96	18%
	Total				2303.86		482.21	
WO	CHO	311.00	±	124.52	1244.00	±	498.08	45%
	Fat	149.13	±	40.64	1342.13	±	365.73	48%
	Pro	50.75	±	19.85	203.00	±	79.39	7%
	Total				2789.13		433.91	

Table 14-2. Individual macronutrient intakes for moderate CHO diet.

		Moderate Carb Grams		S.D	Calories		S.D	Percentage
AB	CHO	376.21	±	80.02	1504.83	±	320.07	71%
	Fat	42.83	±	11.87	385.50	±	106.80	18%
	Pro	60.83	±	22.41	243.33	±	89.64	11%
	Total				2133.67		410.51	
CB	CHO	538.63	±	42.07	2154.53	±	168.28	63%
	Fat	74.13	±	20.81	667.20	±	187.25	20%
	Pro	144.33	±	27.42	577.33	±	109.68	17%
	Total				3399.07		334.43	
DF	CHO	428.53	±	69.96	1714.11	±	279.84	65%
	Fat	48.05	±	24.20	432.47	±	217.80	16%
	Pro	125.89	±	15.72	503.58	±	62.90	19%
	Total				2650.16		419.99	
EC	CHO	312.27	±	54.79	1249.08	±	219.14	63%
	Fat	43.69	±	23.25	393.23	±	209.21	20%
	Pro	82.23	±	16.36	328.92	±	65.43	17%
	Total				1971.23		310.46	
HH	CHO	524.81	±	64.17	2099.23	±	256.69	62%
	Fat	91.31	±	25.77	821.77	±	231.96	24%
	Pro	121.19	±	28.92	484.77	±	115.66	14%
	Total				3405.77		489.04	
JB	CHO	323.89	±	86.21	1295.58	±	344.83	59%
	Fat	49.58	±	15.83	446.21	±	142.46	20%
	Pro	113.26	±	20.98	453.05	±	83.92	21%
	Total				2194.84		409.08	
KS	CHO	507.31	±	74.35	2029.23	±	297.39	67%
	Fat	62.77	±	15.80	564.92	±	142.16	19%
	Pro	108.35	±	31.34	433.38	±	125.34	14%
	Total				3027.54		393.93	
LM	CHO	411.32	±	73.72	1645.27	±	294.90	62%
	Fat	67.59	±	23.93	608.32	±	215.38	23%
	Pro	97.86	±	28.58	391.45	±	114.31	15%
	Total				2645.05		507.78	
MM	CHO	444.89	±	85.65	1779.56	±	342.60	61%
	Fat	77.04	±	28.96	693.33	±	260.62	24%
	Pro	109.56	±	30.19	438.22	±	120.75	15%
	Total				2911.11		603.03	
MB	CHO	351.25	±	30.58	1405.00	±	122.31	62%
	Fat	50.14	±	12.12	451.29	±	109.09	20%
	Pro	106.54	±	21.07	426.14	±	84.30	19%
	Total				2273.19		206.64	
MBU	CHO	323.88	±	57.82	1295.52	±	231.30	66%
	Fat	41.72	±	9.70	375.48	±	87.28	19%
	Pro	75.08	±	17.04	300.32	±	68.16	15%
	Total				1971.32		316.19	
VH	CHO	485.93	±	87.76	1943.71	±	351.05	59%
	Fat	91.64	±	20.36	824.79	±	183.21	25%
	Pro	124.96	±	36.82	499.86	±	147.29	15%
	Total				3268.36		520.26	
WO	CHO	561.28	±	200.77	2245.11	±	803.08	66%
	Fat	109.78	±	35.12	988.00	±	316.08	29%
	Pro	47.72	±	17.75	190.89	±	71.00	6%
	Total				3424.00		1039.79	

Table 14-3. Individual macronutrient intakes for low CHO diet.

		Low CHO						
		Grams		S.D	Calories		S.D	Percentage
AB	CHO	100.58	±	35.71	402.31	±	142.83	27%
	Fat	102.08	±	34.38	918.69	±	309.38	61%
	Pro	43.38	±	20.01	173.54	±	80.02	12%
	Total				1494.54		352.82	
CB	CHO	166.42	±	46.11	665.67	±	184.44	17%
	Fat	279.92	±	64.10	2519.25	±	576.91	64%
	Pro	182.92	±	46.19	731.67	±	184.78	19%
	Total				3916.58		758.88	
DF	CHO	169.92	±	45.13	679.69	±	180.50	17%
	Fat	272.65	±	59.33	2453.88	±	533.95	63%
	Pro	187.92	±	50.24	751.69	±	200.98	19%
	Total				3885.27		666.31	
EC	CHO	76.15	±	16.73	304.62	±	66.91	17%
	Fat	127.88	±	24.15	1150.96	±	217.33	63%
	Pro	95.08	±	21.56	380.31	±	86.23	21%
	Total				1835.88		298.48	
HH	CHO	157.19	±	33.22	628.74	±	132.86	18%
	Fat	245.33	±	53.09	2208.00	±	477.85	65%
	Pro	146.56	±	30.77	586.22	±	123.10	17%
	Total				3422.96		576.07	
JB	CHO	219.00	±	35.98	876.00	±	143.92	23%
	Fat	252.12	±	43.72	2269.04	±	393.51	59%
	Pro	177.73	±	63.60	710.92	±	254.42	18%
	Total				3855.96		307.82	
KS	CHO	97.07	±	20.38	388.30	±	81.53	16%
	Fat	159.11	±	31.16	1432.00	±	280.40	60%
	Pro	144.11	±	23.02	576.44	±	92.06	24%
	Total				2396.74		353.60	
LM	CHO	138.38	±	31.48	553.54	±	125.93	23%
	Fat	138.27	±	35.06	1244.42	±	315.50	51%
	Pro	159.23	±	30.24	636.92	±	120.94	26%
	Total				2434.88		413.36	
MM	CHO	119.42	±	56.93	477.69	±	227.70	20%
	Fat	160.50	±	33.00	1444.50	±	297.02	59%
	Pro	129.85	±	29.59	519.38	±	118.34	21%
	Total				2441.58		393.54	
MB	CHO	84.40	±	15.18	337.60	±	60.74	15%
	Fat	166.44	±	23.79	1497.96	±	214.07	82%
	Pro	91.68	±	16.22	366.72	±	64.86	20%
	Total				2202.28		237.42	
MBU	CHO	91.23	±	21.76	364.92	±	87.02	20%
	Fat	117.23	±	42.29	1055.08	±	380.60	58%
	Pro	102.96	±	37.10	411.85	±	148.40	22%
	Total				1831.85		492.99	
VH	CHO	143.44	±	63.64	573.78	±	254.55	19%
	Fat	219.26	±	49.49	1973.33	±	445.40	65%
	Pro	123.26	±	30.00	493.04	±	120.02	16%
	Total				3040.15		454.87	
WO	CHO	128.70	±	25.26	514.81	±	101.05	18%
	Fat	221.67	±	67.81	1995.00	±	610.33	69%
	Pro	99.52	±	34.33	398.07	±	137.32	14%
	Total				2907.89		748.66	

Studies 1–3 Intervention Timeline

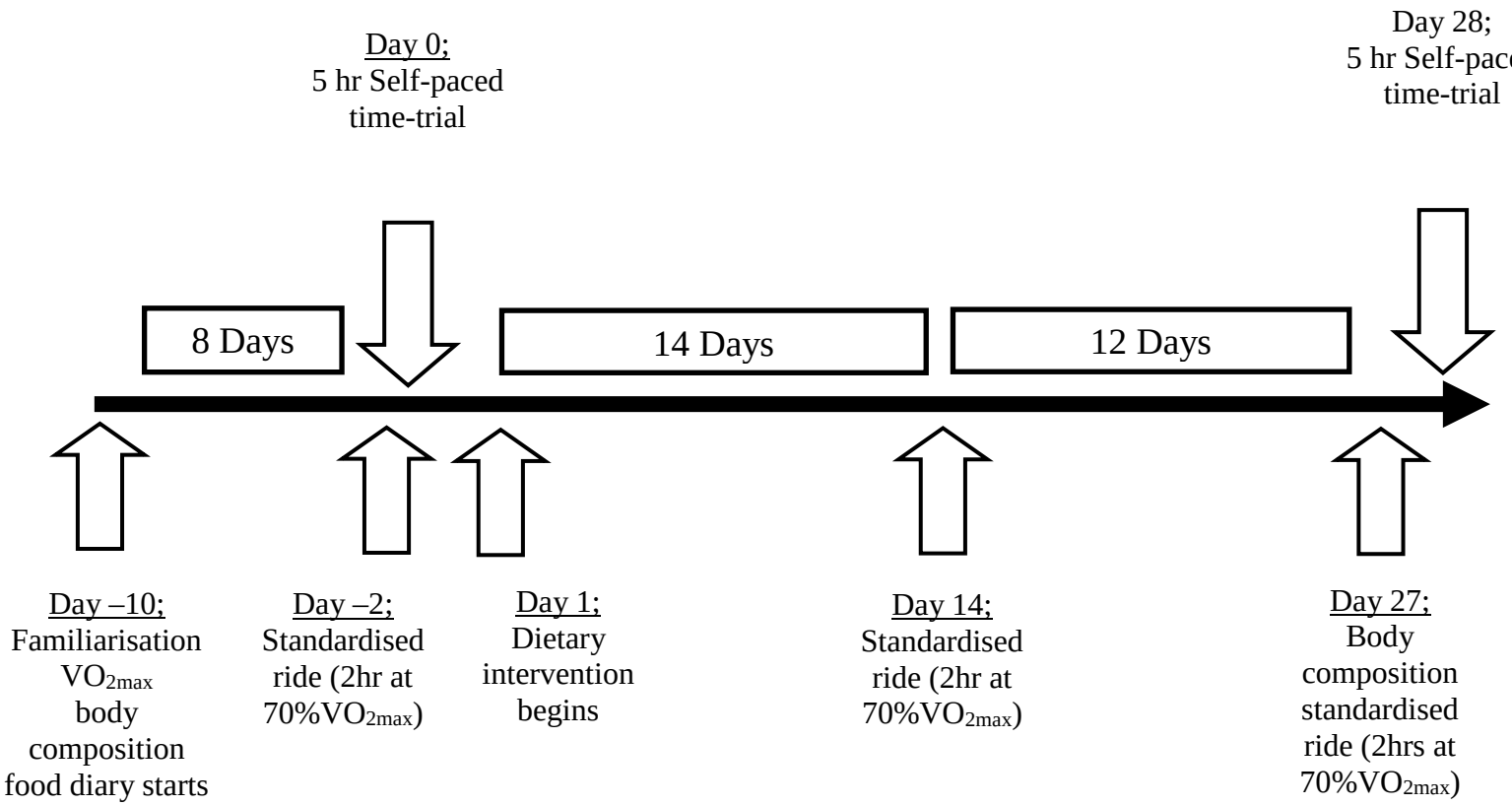

Table 14-4 Overall timeline for each dietary intervention.

Table 14-5. Individual participant overview Study One - Three,

Participant	Peak Power	Body Mass	VO_{2max}(L/min)	VO_{2max}(mL/min/Kg)	Heart Rate	Body Fat %	Age	Height
EC	318.00	53.00	3.41	64.38	181.00	20.30	25	160.00
KS	285.00	59.30	3.17	53.54	185.00	20.20	50	172.00
AB	297.00	86.05	3.32	38.60	175.00	32.80	33	175.00
MBU	268.00	58.35	2.81	48.11	189.00	21.00	22	167.00
MB	287.00	62.90	3.05	48.54	170.00	14.20	46	169.00
JB	310.00	72.60	3.71	51.08	187.00	14.20	39	180.00
DF	409.00	88.20	5.10	57.83	171.00	15.30	40	185.00
CB	478.00	109.00	6.25	57.32	191.00	14.30	38	194.00
MM	345.00	61.20	4.09	66.87	189.00	16.10	29	167.00
WOC	467.00	82.00	6.37	77.73	186.00	9.30	25	187.00
VH	338.00	75.76	5.56	73.43	188.00	20.70	52	180.00
LM	428.00	64.66	5.11	78.96	198.00	12.90	21	174.00
HH	571.00	90.80	6.61	72.85	192.00	10.60	23	193.00
Average	369.31	74.14	4.51	52.42	184.77	21.70	35	168.60
SD	86.25	15.12	1.28	11.97	7.73	5.76	10.01	9.95

Table 14-6. Body composition pre- and post each four-week dietary intervention.

	Body Mass				Skeletal Muscle Mass				Body Fat %			
	Low CHO		Moderate CHO		Low CHO		Moderate CHO		Low CHO		Moderate CHO	
ID	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
JB	72.6	71.8	75.6	75.9	35.6	36.0	37.7	38.2	14.2	12.8	13.8	13.2
DF	88.3	86.6	90.8	91.7	42.7	42.4	42.4	43.5	15.3	15.0	18.7	17.8
CB	103.4	98.7	106.9	101.1	52.5	51.2	53.1	50.8	12.0	10.0	14.3	12.9
MM	61.6	62.6	61.9	60.4	29.4	30.3	29.4	29.2	16.0	15.1	16.1	15.0
WOC	78.2	75.8	79.3	78.9	43.4	42.4	41.3	42.1	3.7	3.0	9.3	7.0
VH	74.9	74.3	75.5	76.3	33.5	34	34.3	34.3	20.7	19.1	19.5	20.4
LM	65.1	64.8	65.4	65.3	32.2	32.4	32.0	34.1	12.9	12.2	14.0	8.7
HH	91.8	90.5	90.6	91.1	47.8	47.7	46.7	48.4	10.0	9.0	10.6	8.2
EC	55.0	54.6	55.0	54.9	24.8	24.7	24.8	24.4	18.8	18.6	18.4	19.6
KS	59.8	57.7	59.3	58.4	29	27.6	28.5	27.3	14.2	14.0	14.2	16.6
AB	85.2	84.4	90.5	92.3	32.1	31.9	32.8	32.7	32.8	32.4	35.1	37.0
MBU	59.4	57.5	59.7	60.2	25.3	24.8	25.5	26.0	22.9	21.8	22.4	21.7
MB	61.2	62.2	66.2	65.7	29.4	30.8	32.4	31.5	14.2	12.0	13.2	15.1
Mean ± SD	73.58 ± 14.12	72.42± 13.57	75.13 ± 15.17	74.788 ± 14.75	35.21 ± 8.39	35.09 ± 8.16	35.45 ± 8.10	35.58 ± 8.15	15.98 ± 6.70	15.00 ± 6.83	16.89 ± 6.31	16.40 ± 7.45

Body Mass (BM), Skeletal Muscle Mass (SMM), Body Fat Percentage (BF%).

Table 14-7. Profile of mood state (POMS) scores for men throughout the moderate and low CHO interventions.

		Moderate Carb					Low Carb				
ID		**Base**	**Wk 1**	**Wk 2**	**Wk 3**	**Wk 4**	**Base**	**Wk 1**	**Wk 2**	**Wk 3**	**Wk 4**
JB	Tension	6.00	4.00	4.00	6.00	4.00	7.00	6.00	11.00	2.00	3.00
	Depression	1.00	1.00	1.00	1.00	4.00	7.00	7.00	24.00	1.00	1.00
	Anger	1.00	0.00	3.00	1.00	6.00	11.00	14.00	21.00	8.00	6.00
	Fatigue	4.00	5.00	4.00	7.00	4.00	3.00	8.00	6.00	6.00	6.00
	Confusion	5.00	4.00	3.00	5.00	5.00	7.00	6.00	13.00	5.00	3.00
	Vigour	10.00	7.00	13.00	9.00	13.00	14.00	15.00	16.00	18.00	13.00
	TMD	7.00	7.00	2.00	11.00	10.00	21.00	26.00	59.00	4.00	6.00
DF	Tension	5.00	4.00	5.00	4.00	3.00	1.00	1.00	2.00	6.00	6.00
	Depression	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	3.00	3.00
	Anger	1.00	1.00	0.00	0.00	1.00	0.00	0.00	2.00	4.00	6.00
	Fatigue	6.00	6.00	6.00	6.00	5.00	0.00	0.00	5.00	6.00	6.00
	Confusion	2.00	3.00	0.00	1.00	2.00	1.00	1.00	4.00	3.00	1.00
	Vigour	21.00	17.00	21.00	24.00	13.00	22.00	23.00	21.00	20.00	23.00
	TMD	-6.00	-3.00	-9.00	-13.00	-1.00	-20.00	-21.00	-8.00	2.00	-1.00
CB	Tension	3.00	3.00	3.00	2.00	4.00	1.00	2.00	1.00	1.00	1.00
	Depression	4.00	2.00	0.00	2.00	6.00	0.00	4.00	0.00	1.00	1.00
	Anger	7.00	5.00	3.00	8.00	6.00	3.00	10.00	3.00	2.00	6.00
	Fatigue	7.00	7.00	7.00	14.00	12.00	2.00	10.00	4.00	3.00	8.00
	Confusion	4.00	6.00	4.00	6.00	8.00	3.00	8.00	5.00	4.00	5.00
	Vigour	17.00	18.00	17.00	12.00	11.00	21.00	14.00	16.00	18.00	14.00
	TMD	8.00	5.00	0.00	20.00	25.00	-12.00	20.00	-3.00	-7.00	7.00
MM	Tension	7.00	4.00	11.00	7.00	9.00	2.00	2.00	2.00	2.00	2.00
	Depression	0.00	0.00	2.00	2.00	7.00	0.00	0.00	0.00	0.00	0.00
	Anger	1.00	1.00	2.00	2.00	3.00	0.00	0.00	0.00	0.00	0.00
	Fatigue	6.00	3.00	7.00	6.00	11.00	0.00	0.00	3.00	0.00	0.00
	Confusion	7.00	5.00	9.00	5.00	7.00	2.00	1.00	2.00	2.00	2.00
	Vigour	14.00	15.00	10.00	8.00	6.00	16.00	14.00	16.00	16.00	16.00
	TMD	7.00	-2.00	21.00	14.00	31.00	-12.00	-11.00	-9.00	-12.00	-12.00
WO	Tension	5.00	4.00	5.00	7.00	4.00	9.00	1.00	3.00	3.00	3.00
	Depression	2.00	0.00	3.00	3.00	2.00	2.00	2.00	0.00	1.00	1.00
	Anger	3.00	3.00	5.00	5.00	5.00	5.00	0.00	4.00	6.00	3.00
	Fatigue	5.00	6.00	8.00	9.00	12.00	12.00	2.00	5.00	6.00	3.00
	Confusion	4.00	2.00	5.00	9.00	9.00	10.00	2.00	3.00	1.00	1.00
	Vigour	18.00	17.00	19.00	15.00	16.00	14.00	23.00	18.00	17.00	22.00
	TMD	1.00	-2.00	7.00	18.00	16.00	24.00	-16.00	-3.00	0.00	-11.00
VH	Tension	1.00	1.00	1.00	1.00	3.00	4.00	3.00	6.00	1.00	6.00
	Depression	0.00	0.00	0.00	0.00	0.00	0.00	3.00	9.00	2.00	0.00
	Anger	0.00	0.00	0.00	0.00	0.00	3.00	7.00	10.00	1.00	0.00
	Fatigue	3.00	4.00	0.00	2.00	4.00	4.00	6.00	20.00	7.00	6.00
	Confusion	1.00	1.00	1.00	1.00	1.00	2.00	5.00	10.00	2.00	2.00

	Vigour	24.00	24.00	24.00	26.00	24.00	21.00	22.00	8.00	22.00	22.00
	TMD	-19.00	-18.00	-22.00	-22.00	-16.00	-8.00	2.00	47.00	-9.00	-8.00
LM	Tension	11.00	12.00	13.00	9.00	12.00	8.00	14.00	11.00	14.00	12.00
	Depression	11.00	7.00	9.00	7.00	7.00	8.00	7.00	4.00	11.00	8.00
	Anger	9.00	6.00	8.00	7.00	9.00	11.00	6.00	5.00	11.00	8.00
	Fatigue	10.00	8.00	10.00	8.00	7.00	13.00	6.00	9.00	12.00	9.00
	Confusion	10.00	6.00	11.00	7.00	10.00	8.00	6.00	5.00	11.00	9.00
	Vigour	17.00	16.00	14.00	19.00	19.00	16.00	21.00	20.00	15.00	15.00
	TMD	34.00	23.00	37.00	19.00	26.00	32.00	18.00	14.00	44.00	31.00
HH	Tension	6.00	8.00	4.00	3.00	1.00	3.00	0.00	1.00	5.00	1.00
	Depression	0.00	4.00	0.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00
	Anger	3.00	3.00	1.00	3.00	3.00	2.00	3.00	2.00	4.00	2.00
	Fatigue	4.00	6.00	0.00	7.00	7.00	2.00	6.00	7.00	4.00	3.00
	Confusion	7.00	7.00	4.00	6.00	5.00	3.00	5.00	5.00	5.00	4.00
	Vigour	17.00	19.00	18.00	18.00	19.00	22.00	24.00	24.00	24.00	21.00
	TMD	3.00	9.00	-9.00	2.00	-2.00	-11.00	-10.00	-9.00	-6.00	-11.00

Table 14-8. Profile of mood state (POMS) scores for women throughout the moderate CHO and low CHO interventions.

ID		Moderate Carb					Low Carb				
		Base	**Wk 1**	**Wk 2**	**Wk 3**	**Wk 4**	**Base**	**Wk 1**	**Wk 2**	**Wk 3**	**Wk 4**
EC	Tension	4.00	4.00	2.00	2.00	1.00	4.00	6.00	8.00	9.00	3.00
	Depression	0.00	0.00	0.00	0.00	0.00	0.00	4.00	14.00	17.00	0.00
	Anger	1.00	6.00	2.00	1.00	2.00	1.00	9.00	17.00	11.00	1.00
	Fatigue	2.00	5.00	2.00	2.00	0.00	3.00	3.00	24.00	28.00	4.00
	Confusion	4.00	5.00	2.00	4.00	2.00	3.00	7.00	14.00	13.00	4.00
	Vigour	12.00	13.00	15.00	5.00	21.00	6.00	4.00	1.00	0.00	18.00
	TMD	-1.00	7.00	-7.00	4.00	-16.00	5.00	25.00	76.00	78.00	-6.00
KS	Tension	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	3.00	1.00
	Depression	0.00	0.00	0.00	0.00	0.00	0.00	4.00	4.00	0.00	0.00
	Anger	1.00	2.00	0.00	0.00	0.00	0.00	11.00	4.00	0.00	1.00
	Fatigue	1.00	4.00	0.00	2.00	2.00	0.00	1.00	12.00	3.00	5.00
	Confusion	2.00	0.00	1.00	0.00	0.00	0.00	1.00	5.00	0.00	1.00
	Vigour	22.00	19.00	24.00	23.00	23.00	24.00	21.00	14.00	20.00	19.00
	TMD	-17.00	-12.00	-22.00	-20.00	-20.00	-23.00	-3.00	12.00	-14.00	-11.00
MBU	Tension	3.00	2.00	3.00	3.00	2.00	2.00	4.00	4.00	3.00	4.00
	Depression	11.00	12.00	12.00	11.00	9.00	11.00	15.00	13.00	12.00	13.00
	Anger	8.00	11.00	9.00	11.00	10.00	13.00	16.00	13.00	15.00	11.00
	Fatigue	2.00	2.00	2.00	1.00	1.00	3.00	1.00	1.00	2.00	2.00
	Confusion	10.00	5.00	8.00	9.00	9.00	11.00	11.00	6.00	13.00	8.00
	Vigour	0.00	0.00	0.00	0.00	0.00	2.00	2.00	2.00	0.00	1.00
	TMD	34.00	32.00	34.00	35.00	31.00	38.00	45.00	35.00	45.00	37.00
AB	Tension	3.00	5.00	6.00	3.00	4.00	2.00	7.00	9.00	13.00	4.00
	Depression	14.00	14.00	15.00	12.00	18.00	10.00	20.00	15.00	21.00	15.00
	Anger	15.00	11.00	18.00	16.00	14.00	10.00	17.00	22.00	17.00	16.00
	Fatigue	1.00	5.00	4.00	5.00	2.00	3.00	3.00	3.00	8.00	5.00
	Confusion	11.00	12.00	13.00	10.00	13.00	10.00	14.00	16.00	10.00	14.00
	Vigour	0.00	2.00	9.00	3.00	1.00	1.00	3.00	1.00	17.00	2.00
	TMD	44.00	45.00	47.00	43.00	50.00	34.00	58.00	64.00	52.00	52.00
MB	Tension	6.00	10.00	9.00	16.00	7.00	2.00		2.00	0.00	1.00
	Depression	16.00	20.00	15.00	25.00	16.00	11.00		12.00	9.00	12.00
	Anger	17.00	19.00	15.00	24.00	14.00	11.00		13.00	11.00	14.00
	Fatigue	6.00	9.00	8.00	10.00	6.00	3.00		4.00	1.00	4.00
	Confusion	10.00	7.00	9.00	6.00	9.00	13.00		11.00	10.00	11.00
	Vigour	8.00	12.00	6.00	12.00	6.00	0.00		2.00	0.00	0.00
	TMD	47.00	53.00	50.00	69.00	46.00	40.00		40.00	31.00	42.00

14. 3. APPENDIX B

Table 14-9. Absolute VO_2 (L/min) during the low CHO intervention during two hour fixed intensity ride.

			Low CHO					
			Pre	T10	T40	T80	T120	Post
Baseline	Men	JB	0.25	2.32	2.31	2.44	2.58	0.58
		DF	0.30	3.04	2.94	3.02	3.09	0.49
		CB	0.54	3.83	3.44	3.59	3.61	0.49
		MM	0.30	2.52	2.45	2.53	2.66	0.37
		WOC	0.44	3.90	4.19	4.24	4.32	0.61
		VH	0.42	2.90	2.87	2.97	3.12	0.41
		LM	0.40	3.11	3.08	3.09	2.99	0.40
		HH	0.39	4.09	4.30	4.32	4.36	0.57
	Women	EC	0.28	2.04	1.99	1.90	1.77	0.26
		KS	0.30	2.22	2.31	2.57	2.46	0.37
		MB	0.35	1.95	2.17	2.05	2.08	0.37
		MBU	0.21	1.46	1.67	1.71	1.71	0.28
		AB	0.24	1.86	1.86	2.04	2.00	0.29
Mid	Men	JB	0.32	2.57	2.52	2.73	2.78	0.34
		DF	0.40	3.56	3.62	3.81	3.88	0.63
		CB	0.53	4.08	3.68	3.94	3.97	0.65
		MM	0.33	2.78	2.77	2.87	2.83	0.42
		WOC	0.42	4.15	4.21	4.25	4.48	0.55
		VH	0.39	2.86	2.97	3.06	3.03	0.45
		LM	0.35	3.11	3.11	3.10	3.04	0.36
		HH	0.39	3.91	4.02	3.93	4.03	0.47
	Women	EC	0.29	2.07	2.15	2.16	2.21	0.30
		KS	0.28	2.13	2.18	2.04	2.21	0.31
		MB	0.29	2.01	2.11	2.09	2.11	0.40
		MBU	0.27	1.52	1.64	1.57	1.57	0.30
		AB	0.31	2.13	2.28	2.25	2.34	0.34
Post	Men	JB	0.40	2.60	2.57	2.78	2.59	0.44
		DF	0.43	3.25	3.58	3.36	3.57	0.54
		CB	0.49	4.26	4.05	4.01	4.17	0.58
		MM	0.32	2.91	2.79	2.93	2.91	0.41
		WOC	0.41	3.62	3.77	4.03	4.00	0.49
		VH	0.34	2.47	2.62	2.60	2.58	0.38
		LM	0.39	3.04	3.09	3.03	3.16	0.37
		HH	0.40	4.13	4.27	4.32	4.31	0.53
	Women	EC	0.33	2.23	2.28	2.35	2.31	0.31
		KS	0.31	2.37	2.41	2.57	2.42	0.40
		MB	0.31	2.18	2.22	2.21	2.21	0.37
		MBU	0.26	1.52	1.47	1.49	1.54	0.26
		AB	0.27	2.10	2.16	2.10	2.07	0.30

Table 14-10. Absolute VO_2 (L/min) throughout the moderate CHO intervention during the two hour fixed intensity ride.

			Moderate CHO					
			Pre	T10	T40	T80	T120	Post
Baseline	Men	JB	0.32	2.50	2.40	2.72	2.78	0.44
		DF	0.39	3.16	3.32	3.37	3.69	0.55
		CB	0.45	3.79	3.75	3.63	3.70	0.42
		MM	0.33	2.59	2.71	2.49	2.54	0.37
		WOC	0.44	3.75	3.97	3.88	4.00	0.55
		VH	0.34	2.31	2.39	2.46	2.45	0.34
		LM	0.50	3.08	2.99	2.93	2.61	0.40
		HH	0.46	3.89	4.11	3.91	4.09	0.55
	Women	EC	0.28	2.03	2.13	2.00	2.14	0.30
		KS	0.31	2.18	2.15	2.20	2.18	0.33
		MB	0.37	1.94	1.94	1.88	1.89	0.40
		MBU	0.28	1.56	1.61	1.64	1.68	0.28
		AB	0.31	2.12	2.13	2.21	2.17	0.37
Mid	Men	JB	0.31	2.85	2.88	3.05	3.45	0.47
		DF	0.45	3.89	4.13	3.90	3.90	0.59
		CB	0.53	3.98	3.73	3.70	3.88	0.64
		MM	0.30	2.47	2.46	2.52	2.53	0.32
		WOC	0.51	3.84	3.90	3.64	3.78	0.55
		VH	0.29	2.06	2.05	2.22	2.21	0.37
		LM	0.31	2.90	2.91	2.85	2.81	0.35
		HH	0.39	4.04	4.04	4.10	4.19	0.49
	Women	EC	0.24	1.97	2.01	2.08	1.93	0.25
		KS	0.24	2.02	2.09	2.17	2.07	0.28
		MB	0.36	1.93	2.01	1.88	2.19	0.39
		MBU	0.29	1.59	1.48	1.53	1.59	0.27
		AB	0.35	1.94	1.96	1.98		0.28
Post	Men	JB	0.33	2.60	2.62	2.82	2.85	0.45
		DF	0.43	3.32	3.46	3.47	3.67	0.52
		CB	0.42	3.60	3.34	3.39	3.47	0.56
		MM	0.28	2.22	2.26	2.27	2.34	0.40
		WOC	0.44	3.84	3.97	4.08	4.16	0.50
		VH	0.32	2.33	2.24	2.31	2.39	0.36
		LM	0.47	3.06	2.97	3.05	3.02	0.36
		HH	0.46	3.95	4.08	4.20	4.12	0.53
	Women	EC	0.27	2.19	2.13	2.15	2.10	0.27
		KS	0.29	2.19	2.12	2.19	2.32	0.35
		MB	0.33	1.94	2.10	1.90	1.94	0.40
		MBU	0.30	1.50	1.62	1.54	1.61	0.29
		AB	0.31	1.89	1.92	2.07	2.05	0.32

Table 14-11. Absolute CO_2 (L/min) throughout the low CHO intervention during the two hour fixed intensity ride.

			Low CHO					
			Pre	T10	T40	T80	T120	Post
Baseline	Men	JB	.21	2.26	2.14	2.21	2.46	.51
		DF	.24	2.68	2.49	2.49	2.51	.44
		CB	.51	3.11	2.71	2.80	2.81	.35
		MM	.22	1.96	1.89	1.91	2.24	.25
		WOC	.33	3.27	3.42	3.43	3.51	.41
		VH	.37	2.53	2.49	2.56	2.71	.28
		LM	.44	2.72	2.77	2.75	2.65	.36
		HH	.31	3.59	3.37	3.54	3.50	.43
	Women	EC	.20	1.68	1.60	1.54	1.41	.18
		KS	.26	2.08	2.12	2.24	2.17	.37
		MB	.34	1.84	2.00	1.88	1.83	.42
		MBU	.20	1.34	1.53	1.52	1.49	.22
		AB	.17	1.52	1.51	1.65	1.64	.19
Mid	Men	JB	.23	2.36	2.22	2.42	2.40	.26
		DF	.29	3.28	3.19	3.39	3.41	.48
		CB	.37	3.17	2.66	2.90	2.84	.43
		MM	.23	2.14	2.10	2.16	2.11	.27
		WOC	.30	3.20	3.21	3.28	3.50	.34
		VH	.33	2.27	2.32	2.48	2.31	.34
		LM	.26	2.68	2.69	2.52	2.56	.28
		HH	.28	3.08	3.16	3.20	3.06	.36
	Women	EC	.19	1.68	1.76	1.72	1.69	.20
		KS	.21	1.85	1.80	1.70	1.84	.23
		MB	.33	1.94	1.87	1.93	1.87	.43
		MBU	.23	1.38	1.55	1.40	1.38	.24
		AB	.23	1.89	2.02	2.01	1.91	.22
Post	Men	JB	.31	2.48	2.27	2.49	2.33	.32
		DF	.31	2.99	3.25	3.05	3.18	.37
		CB	.34	3.36	3.02	2.99	3.08	.37
		MM	.24	2.29	2.12	2.19	2.13	.28
		WOC	.30	2.71	2.77	2.96	2.82	.34
		VH	.29	2.04	2.06	2.11	2.11	.30
		LM	.33	2.58	2.60	2.56	2.70	.31
		HH	.30	3.35	3.30	3.45	3.50	.38
	Women	EC	.22	1.65	1.69	1.71	1.68	.19
		KS	.24	2.06	1.91	1.89	1.82	.24
		MB	.23	1.73	1.71	1.81	1.78	.27
		MBU	.23	1.35	1.28	1.28	1.32	.21
		AB	.20	1.83	1.81	1.76	1.67	.21

Table 14-12. Absolute CO_2 (L/min) throughout the moderate CHO intervention during the two hour fixed intensity ride.

			Moderate CHO					
			Pre	T10	T40	T80	T120	Post
Baseline	Men	JB	.26	2.41	2.22	2.51	2.50	.33
		DF	.29	2.82	2.90	2.83	3.08	.41
		CB	.33	3.07	2.94	2.82	2.85	.38
		MM	.29	2.25	2.28	2.04	1.99	.30
		WOC	.34	3.15	3.28	3.21	3.42	.41
		VH	.32	2.06	2.08	2.18	2.17	.27
		LM	.41	2.82	2.72	2.67	2.12	.32
		HH	.43	3.44	3.60	3.53	3.65	.49
	Women	EC	.23	1.84	1.86	1.70	1.80	.21
		KS	.28	1.96	1.91	1.98	1.92	.27
		MB	.44	1.70	1.63	1.59	1.64	.40
		MBU	.24	1.44	1.42	1.45	1.43	.24
		AB	.24	1.94	1.85	1.89	1.78	.28
Mid	Men	JB	.26	2.52	2.48	2.56	2.48	.33
		DF	.34	3.32	3.48	2.69	2.67	.39
		CB	.48	3.56	3.17	3.15	3.28	.63
		MM	.25	2.25	2.18	2.19	2.22	.26
		WOC	.42	3.30	3.24	2.98	3.08	.44
		VH	.30	2.03	1.97	2.11	2.09	.30
		LM	.29	2.76	2.70	2.77	2.62	.32
		HH	.34	3.55	3.53	3.64	3.60	.38
	Women	EC	.17	1.69	1.65	1.70	1.58	.17
		KS	.21	1.93	1.98	1.94	1.90	.22
		MB	.36	1.72	1.68	1.56	1.74	.29
		MBU	.24	1.42	1.29	1.33	1.33	.20
		AB	.29	2.02	1.87	1.82		.22
Post	Men	JB	.27	2.36	2.27	2.49	2.50	.34
		DF	.35	2.93	2.97	2.95	3.06	.37
		CB	.33	3.12	2.77	2.70	2.76	.47
		MM	.24	1.94	1.97	1.88	2.01	.32
		WOC	.35	3.42	3.62	3.59	3.60	.38
		VH	.29	2.05	2.00	2.07	2.10	.29
		LM	.47	3.03	2.92	2.93	2.80	.31
		HH	.36	3.35	3.41	3.54	3.58	.39
	Women	EC	.22	1.96	1.84	1.81	1.77	.20
		KS	.22	1.92	1.84	1.89	1.96	.27
		MB	.29	1.98	2.05	1.70	1.63	.33
		MBU	.27	1.44	1.52	1.41	1.48	.24
		AB	.26	1.70	1.63	1.75	1.78	.22

Table 14-13. CHO oxidation rates (g/min) throughout the low CHO intervention during the two hour fixed intensity ride.

			Low CHO					
			Pre	T10	T40	T80	T120	Post
Baseline	Men	JB	.15	2.82	2.34	2.25	2.89	.45
		DF	.11	2.43	1.88	1.63	1.47	.43
		CB	.59	1.85	1.29	1.21	1.20	0.00
		MM	.02	.81	.74	.57	1.68	0.00
		WOC	.07	2.36	2.11	2.00	2.07	0.00
		VH	.37	2.21	2.13	2.09	2.31	0.00
		LM	.69	2.40	2.71	2.58	2.45	.33
		HH	.13	3.20	1.53	2.23	1.91	.12
	Women	EC	0.00	1.11	.88	.88	.73	0.00
		KS	.22	2.30	2.25	1.94	1.97	.50
		MB	.44	2.10	2.12	1.96	1.65	.75
		MBU	.24	1.41	1.60	1.45	1.32	.12
		AB	.04	.95	.91	.96	1.05	0.00
Mid	Men	JB	.03	2.48	2.02	2.22	2.02	.09
		DF	.02	3.46	2.91	3.19	3.06	.18
		CB	0.00	1.34	.30	.53	.16	0.00
		MM	0.00	.81	.68	.63	.52	0.00
		WOC	0.00	1.22	1.07	1.31	1.52	0.00
		VH	.27	1.15	.99	1.47	.78	.12
		LM	.07	2.19	2.28	1.52	1.87	.12
		HH	.05	1.47	1.46	1.93	.99	.15
	Women	EC	0.00	.98	1.10	.88	.63	0.00
		KS	.08	1.56	1.22	1.18	1.29	.04
		MB	.56	2.39	1.74	2.06	1.75	.65
		MBU	.19	1.40	1.76	1.34	1.26	.15
		AB	.04	1.74	1.88	1.95	1.21	0.00
Post	Men	JB	.14	2.94	2.09	2.41	2.27	.04
		DF	.05	3.16	3.32	3.07	3.02	0.00
		CB	0.00	1.63	.73	.72	.60	0.00
		MM	.05	1.05	.69	.55	.37	0.00
		WOC	.03	.72	.52	.52	.01	0.00
		VH	.26	1.34	.97	1.25	1.31	.12
		LM	.27	2.02	1.92	1.93	2.12	.19
		HH	.05	2.00	1.34	1.83	2.08	.05
	Women	EC	0.00	.32	.36	.22	.23	0.00
		KS	.09	1.77	.96	.37	.53	0.00
		MB	.08	.84	.65	1.13	.98	.04
		MBU	.19	1.30	1.10	1.04	1.06	.12
		AB	.13	1.60	1.27	1.27	.94	0.00

Table 14-14. CHO oxidation rates (g/min) throughout the moderate CHO intervention during the two hour fixed intensity ride.

			Moderate CHO					
			Pre	T10	T40	T80	T120	Post
Baseline	Men	JB	.14	2.92	2.38	2.70	2.45	.05
		DF	.10	2.69	2.53	2.07	2.18	.12
		CB	.05	1.79	1.32	1.18	1.08	.39
		MM	.28	1.92	1.68	1.28	.89	.15
		WOC	.11	2.30	2.17	2.15	2.72	.13
		VH	.37	1.97	1.77	2.01	2.01	.16
		LM	.25	2.96	2.80	2.74	1.25	.16
		HH	.48	3.18	3.21	3.54	3.48	.47
	Women	EC	.15	1.87	1.62	1.33	1.34	.00
		KS	.26	1.93	1.79	1.94	1.70	.17
		MB	.84	1.54	1.20	1.23	1.38	.55
		MBU	.21	1.57	1.31	1.33	1.09	.16
		AB	.20	2.03	1.55	1.48	1.14	.08
Mid	Men	JB	.18	2.32	2.07	1.86	.22	0.00
		DF	.11	2.62	2.55	0.00	0.00	0.00
		CB	.45	3.42	2.44	2.46	2.47	.78
		MM	.20	2.31	2.02	1.88	2.01	.16
		WOC	.28	2.71	2.23	1.87	1.86	.22
		VH	.45	2.64	2.39	2.47	2.41	.19
		LM	.34	3.24	2.93	3.46	2.90	.31
		HH	.27	3.18	3.08	3.38	2.94	.17
	Women	EC	.04	1.37	1.05	1.06	.99	0.00
		KS	.19	2.29	2.32	1.86	2.02	.13
		MB	.49	1.62	1.20	1.08	.87	.07
		MBU	.15	1.35	1.15	1.17	.95	.07
		AB	.19	2.96	2.23	1.91	1.80	.10
Post	Men	JB	.13	2.39	1.94	2.27	2.23	.11
		DF	.19	2.66	2.42	2.25	2.17	.04
		CB	.16	2.62	1.86	1.41	1.43	.35
		MM	.20	1.69	1.69	1.29	1.64	.17
		WOC	.19	3.25	3.73	3.24	3.02	.13
		VH	.27	1.85	1.89	2.02	1.91	.16
		LM	.65	3.95	3.76	3.55	3.05	.28
		HH	.17	2.56	2.39	2.62	3.05	.08
	Women	EC	.12	1.87	1.53	1.34	1.31	.02
		KS	.07	1.68	1.55	1.54	1.46	.10
		MB	.25	2.81	2.58	1.64	1.22	.23
		MBU	.26	1.75	1.70	1.46	1.56	.16
		AB	.44	1.66	1.25	1.32	1.49	0.00

Table 14-15. Fat oxidation rates (g/min) throughout the low CHO intervention during the two hour fixed intensity ride.

			Low CHO					
			Pre	T10	T40	T80	T120	Post
Baseline	Men	JB	.07	.11	.28	.37	.21	.12
		DF	.11	.60	.75	.88	.98	.08
		CB	.05	1.20	1.22	1.32	1.34	.24
		MM	.14	.94	.93	1.04	.69	.19
		WOC	.19	1.05	1.29	1.35	1.37	.34
		VH	.07	.61	.63	.69	.69	.20
		LM	0.00	.65	.52	.57	.57	.08
		HH	.14	.84	1.55	1.31	1.44	.24
	Women	EC	.14	.60	.66	.61	.60	.14
		KS	.07	.25	.31	.55	.49	0.00
		MB	.01	.19	.29	.29	.42	0.00
		MBU	.01	.20	.24	.31	.35	.09
		AB	.11	.57	.58	.65	.60	.16
Mid	Men	JB	.14	.35	.50	.53	.63	.13
		DF	.19	.48	.71	.70	.78	.25
		CB	.27	1.51	1.70	1.74	1.89	.36
		MM	.16	1.07	1.11	1.18	1.20	.25
		WOC	.21	1.60	1.68	1.61	1.65	.36
		VH	.09	.98	1.10	.97	1.21	.18
		LM	.15	.72	.69	.97	.81	.13
		HH	.17	1.39	1.44	1.22	1.62	.18
	Women	EC	.17	.66	.65	.74	.85	.18
		KS	.11	.48	.62	.57	.61	.14
		MB	0.00	.11	.40	.27	.39	0.00
		MBU	.06	.23	.16	.28	.31	.09
		AB	.14	.41	.43	.39	.71	.20
Post	Men	JB	.15	.20	.50	.48	.44	.20
		DF	.19	.44	.54	.53	.65	.29
		CB	.25	1.49	1.73	1.71	1.83	.34
		MM	.14	1.05	1.12	1.24	1.29	.23
		WOC	.19	1.52	1.66	1.79	1.97	.24
		VH	.07	.73	.93	.82	.79	.15
		LM	.09	.75	.82	.78	.78	.11
		HH	.18	1.30	1.61	1.45	1.36	.24
	Women	EC	.18	.98	.99	1.08	1.05	.20
		KS	.12	.52	.83	1.13	.99	.28
		MB	.12	.77	.85	.67	.73	.17
		MBU	.06	.27	.32	.35	.37	.08
		AB	.12	.44	.60	.57	.67	.15

Table 14-16. Fat oxidation rates (g/min) throughout the moderate CHO intervention during the two hour fixed intensity ride.

			Moderate CHO					
			Pre	T10	T40	T80	T120	Post
Baseline	Men	JB	.11	.16	.31	.35	.47	.20
		DF	.15	.56	.70	.90	1.02	.23
		CB	.20	1.21	1.36	1.35	1.43	.06
		MM	.06	.57	.72	.76	.92	.13
		WOC	.18	1.00	1.15	1.12	.97	.22
		VH	.03	.41	.53	.47	.47	.11
		LM	.15	.43	.44	.43	.83	.14
		HH	.05	.75	.84	.62	.73	.10
	Women	EC	.08	.31	.45	.50	.56	.15
		KS	.06	.36	.40	.37	.45	.10
		MB	0.00	.39	.52	.47	.43	0.00
		MBU	.06	.19	.31	.32	.43	.08
		AB	.11	.29	.48	.55	.65	.16
Mid	Men	JB	.09	.55	.66	.82	1.62	.25
		DF	.18	.95	1.10	2.02	2.05	.32
		CB	.10	.70	.94	.92	1.00	.03
		MM	.07	.37	.47	.55	.50	.10
		WOC	.15	.89	1.10	1.11	1.18	.19
		VH	0.00	.04	.13	.19	.20	.12
		LM	.03	.24	.35	.13	.32	.06
		HH	.09	.82	.86	.78	.98	.18
	Women	EC	.10	.47	.60	.63	.59	.14
		KS	.05	.15	.18	.39	.28	.09
		MB	0.00	.36	.55	.53	.76	.17
		MBU	.09	.29	.30	.32	.43	.10
		AB	.10	0.00	.14	.28	.50	.10
Post	Men	JB	.12	.40	.58	.55	.58	.18
		DF	.15	.65	.81	.88	1.01	.24
		CB	.15	.81	.96	1.15	1.18	.15
		MM	.06	.47	.49	.64	.55	.14
		WOC	.15	.69	.58	.82	.94	.20
		VH	.06	.47	.41	.39	.47	.12
		LM	0.00	.05	.08	.19	.37	.07
		HH	.17	1.01	1.13	1.10	.91	.23
	Women	EC	.09	.39	.49	.57	.55	.13
		KS	.12	.46	.48	.52	.61	.14
		MB	.07	0.00	.09	.33	.51	.11
		MBU	.05	.10	.17	.22	.22	.09
		AB	.09	.32	.49	.53	.46	.17

Table 14-17. Heart rate (bpm) throughout the low CHO intervention during the two hour fixed intensity ride.

			Low CHO					
			Pre	T10	T40	T80	T120	Post
Baseline	Men	JB	53.00	123.00	131.00	132.00	147.00	108.00
		DF	47.00	118.00	122.00	127.00	130.00	73.00
		CB	71.00	134.00	134.00	134.00	143.00	77.00
		MM	58.00	138.00	145.00	145.00	148.00	80.00
		WOC	47.00	147.00	157.00	167.00	175.00	76.00
		VH	49.00	125.00	137.00	135.00	145.00	66.00
		LM	75.00	144.00	148.00	151.00	156.00	92.00
		HH	53.00	134.00	139.00	143.00	147.00	78.00
	Women	EC	43.00	107.00	111.00	113.00	111.00	39.00
		KS	60.00	136.00	137.00	144.00	149.00	74.00
		MB	49.00	110.00	124.00	127.00	139.00	67.00
		MBU	61.00	132.00	145.00	142.00	142.00	73.00
		AB	61.00	125.00	130.00	130.00	133.00	71.00
Mid	Men	JB	58.00	125.00	139.00	143.00	146.00	94.00
		DF	49.00	125.00	143.00	146.00	153.00	80.00
		CB	60.00	135.00	137.00	134.00	141.00	78.00
		MM	55.00	136.00	141.00	143.00	149.00	78.00
		WOC	45.00	152.00	160.00	168.00	171.00	71.00
		VH	53.00	128.00	134.00	140.00	146.00	77.00
		LM	53.00	141.00	147.00	151.00	145.00	82.00
		HH	50.00	140.00	134.00	133.00	142.00	66.00
	Women	EC	40.00	118.00	120.00	118.00	126.00	54.00
		KS	57.00	132.00	139.00	135.00	139.00	73.00
		MB	57.00	113.00	124.00	122.00	131.00	84.00
		MBU	67.00	143.00	127.00	133.00	135.00	82.00
		AB	59.00	145.00	147.00	150.00	154.00	78.00
Post	Men	JB	51.00	122.00	133.00	131.00	131.00	85.00
		DF	44.00	122.00	138.00	138.00	144.00	76.00
		CB	57.00	135.00	138.00	134.00	144.00	76.00
		MM	52.00	135.00	143.00	147.00	147.00	78.00
		WOC	45.00	136.00	143.00	147.00	157.00	68.00
		VH	61.00	127.00	130.00	136.00	143.00	87.00
		LM	69.00	139.00	142.00	144.00	150.00	87.00
		HH	45.00	137.00	142.00	137.00	144.00	65.00
	Women	EC	47.00	122.00	125.00	130.00	129.00	47.00
		KS	52.00	143.00	146.00	150.00	147.00	67.00
		MB	51.00	133.00	128.00	125.00	125.00	77.00
		MBU	57.00	130.00	136.00	133.00	148.00	78.00
		AB	57.00	119.00	124.00	118.00	124.00	68.00

Table 14-18. Heart rate (bpm) throughout the moderate CHO intervention during the two hour fixed intensity ride.

			Moderate CHO					
			Pre	T10	T40	T80	T120	Post
Baseline	Men	JB	68.00	130.00	144.00	152.00	163.00	100.00
		DF	52.00	111.00	121.00	136.00	141.00	73.00
		CB	52.00	138.00	132.00	136.00	136.00	75.00
		MM	50.00	133.00	142.00	139.00	141.00	88.00
		WOC	45.00	133.00	140.00	154.00	149.00	52.00
		VH	54.00	107.00	112.00	117.00	123.00	64.00
		LM	72.00	154.00	153.00	148.00	147.00	74.00
		HH	51.00	137.00	141.00	147.00	150.00	80.00
	Women	EC	45.00	116.00	118.00	120.00	120.00	45.00
		KS	64.00	130.00	131.00	132.00	137.00	75.00
		MB	69.00	116.00	112.00	121.00	125.00	75.00
		MBU	53.00	134.00	134.00	140.00	148.00	74.00
		AB	57.00	124.00	133.00	136.00	143.00	90.00
Mid	Men	JB	71.00	139.00	144.00	152.00	159.00	98.00
		DF	61.00	126.00	136.00	138.00	140.00	77.00
		CB	57.00	136.00	137.00	138.00	148.00	87.00
		MM	51.00	136.00	141.00	135.00	144.00	79.00
		WOC	46.00	127.00	134.00	132.00	140.00	50.00
		VH	50.00	109.00	115.00	119.00	122.00	68.00
		LM	72.00	147.00	137.00	141.00	143.00	82.00
		HH	47.00	139.00	144.00	140.00	145.00	84.00
	Women	EC	36.00	115.00	114.00	117.00	119.00	37.00
		KS	53.00	124.00	129.00	136.00	139.00	65.00
		MB	59.00	116.00	117.00	121.00	123.00	62.00
		MBU	50.00	129.00	132.00	140.00	129.00	76.00
		AB	55.00	112.00	115.00	117.00	117.00	60.00
Post	Men	JB	71.00	132.00	148.00	154.00	165.00	104.00
		DF	58.00	123.00	134.00	143.00	151.00	84.00
		CB	52.00	133.00	130.00	146.00	150.00	82.00
		MM	52.00	129.00	130.00	142.00	146.00	82.00
		WOC	46.00	142.00	146.00	152.00	160.00	73.00
		VH	49.00	105.00	114.00	125.00	125.00	71.00
		LM	76.00	141.00	141.00	145.00	147.00	77.00
		HH	62.00	145.00	143.00	150.00	154.00	83.00
	Women	EC	41.00	120.00	107.00	112.00	116.00	47.00
		KS	55.00	127.00	132.00	134.00	130.00	70.00
		MB	64.00	118.00	119.00	133.00	139.00	85.00
		MBU	75.00	140.00	131.00	141.00	152.00	80.00
		AB	59.00	116.00	119.00	123.00	132.00	69.00

Table 14-19. Rating of perceived exertion (RPE) throughout both dietary interventions during the two hour fixed intensity ride.

			Moderate CHO				Low CHO			
			T10	T40	T80	T120	T10	T40	T80	T120
	Men	JB	12.00	13.00	15.00	15.00	9.00	12.00	13.00	14.00
		DF	12.00	12.00	12.00	13.00	12.00	12.00	12.00	13.00
		CB	14.00	13.00	14.00	15.00	12.00	12.00	13.00	13.50
		MM	10.00	11.00	11.00	12.00	11.00	12.00	13.00	18.00
		WOC	12.00	12.00	12.00	13.00	13.00	14.00	15.00	15.00
		VH	13.00	14.00	14.00	14.00	13.00	13.50	14.00	15.00
Baseline		LM	12.00	12.00	13.00	17.00	11.00	11.00	11.00	12.00
		HH	13.00	13.00	13.00	14.00	12.00	12.00	12.00	13.00
	Women	EC	9.00	11.00	13.00	12.00	11.00	11.00	11.00	15.00
		KS	12.00	12.00	12.50	13.00	12.00	12.00	13.00	12.00
		MB	12.00	14.00	14.00	15.00	11.00	12.00	14.00	13.00
		MBU	12.00	12.00	13.00	13.00	12.00	13.00	13.00	13.00
		AB	14.00	14.00	14.00	14.00	13.00	15.00	16.00	16.00
	Men	JB	9.00	11.50	12.00	12.00	9.00	12.00	13.00	13.00
		DF	9.00	11.00	12.00	13.00	11.00	12.00	13.00	13.00
		CB	11.00	11.50	13.00	14.50	12.00	12.00	12.50	13.00
		MM	10.00	11.00	11.00	11.00	10.00	11.00	11.00	11.00
		WOC	12.00	12.50	13.50	14.00	13.00	15.00	15.00	15.00
		VH	13.00	13.00	13.50	14.00	13.00	13.50	14.00	14.00
Mid		LM	12.00	13.00	13.00	13.00	12.00	13.00	13.00	13.00
		HH	12.00	12.00	13.00	13.00	12.00	12.00	12.00	12.00
	Women	EC	11.00	11.00	11.00	11.00	11.00	11.50	12.50	12.50
		KS	11.00	12.00	12.00	12.00	13.00	13.00	13.00	13.00
		MB	12.00	13.00	14.00	14.00	13.00	16.00	16.00	17.00
		MBU	12.00	13.00	13.00	13.00	12.00	12.00	13.00	14.00
		AB	14.50	14.50	15.00	16.00	13.00	14.00	14.00	15.00
	Men	JB	9.00	11.00	11.00	11.00	8.00	10.00	10.00	11.00
		DF	9.00	10.00	12.00	13.00	9.00	11.00	11.00	12.00
		CB	12.00	12.00	12.50	13.50	13.50	13.00	13.50	13.50
		MM	10.00	11.00	12.00	12.00	11.00	11.00	11.00	11.00
		WOC	12.00	13.00	13.00	14.00	12.00	12.00	12.00	13.00
		VH	12.50	13.00	13.00	13.00	13.00	13.00	13.50	13.00
Post		LM	12.00	12.00	13.00	13.00	12.00	12.00	12.00	13.00
		HH	12.00	13.00	13.00	13.00	12.00	12.00	12.00	12.00
	Women	EC	12.00	12.00	11.50	12.00	12.00	12.00	12.00	14.00
		KS	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
		MB	11.00	13.00	13.00	14.00	12.00	13.00	16.00	15.00
		MBU	12.00	13.00	13.00	14.00	12.00	12.00	13.00	13.00
		AB	15.00	16.00	17.00	17.00	13.00	13.00	14.00	15.00

14. 4. APPENDIX C

Table 14-20 Individual Macronutrient intake during Study Five

		Habitual				Moderate CHO				Ketosis			
		Calories		S.D	%	Calories		S.D	%	Calories		S.D	%
WOC	CHO	1244.00	±	498.08	39%	1238.46	±	483.77	39%	398.91	±	253.73	12%
	Fat	1355.63	±	380.11	42%	1428.23	±	567.96	45%	2555.18	±	470.91	75%
	Pro	455.00	±	127.95	14%	422.77	±	128.78	13%	487.64	±	93.43	14%
	Total	3193.75	±	356.74		3180.62	±	996.17		3410.55	±	446.69	
TM	CHO	982.67	±	205.80	49%	1680.00	±	264.06	52%	364.67	±	207.35	15%
	Fat	544.50	±	186.47	27%	1054.64	±	163.80	33%	1429.50	±	583.07	58%
	Pro	263.67	±	85.83	13%	416.00	±	69.99	13%	634.33	±	181.10	26%
	Total	2018.33	±	345.12		3240.45	±	362.90		2467.83	±	634.15	
MM	CHO	447.75	±	172.48	18%	1372.92	±	269.55	60%	295.60	±	159.88	15%
	Fat	1546.88	±	303.62	62%	600.23	±	291.34	26%	1117.80	±	248.79	56%
	Pro	532.75	±	103.28	21%	314.15	±	98.58	14%	550.80	±	199.52	27%
	Total	2478.56	±	407.13		2305.77	±	500.86		2006.80	±	416.95	
JH	CHO	1144.33	±	443.14	42%	1819.47	±	402.25	60%	239.64	±	75.06	8%
	Fat	834.75	±	383.97	31%	749.40	±	158.10	25%	2134.64	±	454.83	72%
	Pro	476.00	±	188.91	17%	448.00	±	164.33	15%	572.73	±	162.29	19%
	Total	2729.75	±	585.25		3015.13	±	572.82		2946.73	±	459.90	
JD	CHO	1522.00	±	420.59	55%	1811.08	±	394.15	59%	372.57	±	326.53	15%
	Fat	779.14	±	306.86	28%	752.54	±	221.23	24%	1411.71	±	326.95	58%
	Pro	455.43	±	148.03	16%	492.92	±	84.16	16%	688.86	±	140.20	28%
	Total	2790.21	±	573.74		3083.15	±	521.67		2437.14	±	508.40	
DF	CHO	1199.20	±	357.07	51%	1532.53	±	204.59	54%	461.54	±	218.77	31%
	Fat	750.60	±	241.26	32%	834.60	±	288.12	29%	650.77	±	262.38	43%
	Pro	363.60	±	68.38	15%	445.33	±	149.61	16%	314.77	±	134.65	21%
	Total	2358.20	±	405.40		2858.67	±	344.10		1511.69	±	405.64	

Table 14-21. Participant overview study five: Ketone Supplementation

Participant	Peak Power (W)	Mass (kg)	VO2peak (L/min)	VO2peak (mL/min/Kg)	heart rate (bpm)	Age (yrs)	Height (cm)
WOC	487	77.7	5.75	74.01	181	27	187
MM	355	63.7	3.71	58.44	191	29	165
JH	441	69.3	4.51	65.09	189	18	178
JD	435	67.28	4.60	68.37	196	21	177
TM	422	83.2	4.78	57.42	191	39	182
DF	409	83.2	5.10	61.31	171	42	185
Average	424.83	74.06	4.74	64.10	186.5	29.33	179
SD	39.51	7.71	0.62	5.80	8.24	8.73	7.19

Table 14-22. Individual self-pace TT results for Ketone Supplement Study (Five) presented as time (seconds) and watts per kg (W/kg).

		Moderate CHO						Ketosis					
		Baseline		PLB		KS		Baseline		PLB		KS	
	Order	Time (sec)	W/Kg	Time (sec)	W/Kg	Time (sec)	W/Kg	Time (sec)	W/Kg	Time (sec)	W/Kg	Time (sec)	W/Kg
DF	MC1	1144	3.19	1113	3.27	1148	3.16	1127	3.19	1133	3.19	1181	3.06
JD	MC1	1049	4.56	1092	4.31	1057	4.45	1041	4.49	1074	4.61	1100	4.48
JH	MC1	848	4.78	819	4.86	855	4.66	946	4.18	868	4.8	917	4.57
MM	K1	1013	3.57	997	3.61	1050	3.42	934	3.91	953	3.84	1030	3.57
TM	K1	713	4.31	749	4.15	741	4.19	743	4.02	752	4.08	770	4.02
WOC	K1	1078	4.3	1154	3.98	1079	4.3	1049	4.38	1064	4.38	1082	4.28

Table 14-23. Individual physiological data from Ketone Supplement Study (Five). Beta-hydroxybutyrate [β-HB], Lactate [Lac], Glucose [Glu].

		BASE								PLB								SUP							
		Carb				Keto				Carb				Keto				Carb				Keto			
		rest	Pre	75%	TT	rest	Pre	75%	TT	rest	Pre	75%	TT	rest	Pre	75%	TT	rest	Pre	75%	TT	rest	Pre	75%	TT
[β-HB]	DF	.10	5.80	.10	.10	.10	2.50	.10	.10	.10	4.00	.10	.10	.10	3.00	.10	.10	.10	2.00	.60	.50	.10	2.00	.60	.60
	JD	.10	2.80	.10	.10	.10	3.60	.10	.10	.10	1.90	.10	.10	.10	2.80	.10	.20	.10	2.70	.60	.80	.10	1.60	.60	.70
	JH	.10	2.30	.10	.10	.10	3.50	0.00	.10	.10	2.20	.10	.10	.10	2.50	0.00	.10	.10	2.40	.40	.30	.10	2.80	.40	.40
	MM	.10	2.20	.10	.10	.10	2.00	.10	.10	.10	3.20	0.00	.10	.10	2.00	.10	.10	.10	1.70	.60	.60	.10	3.20	.40	.60
	TM	.10	3.60	0.00	0.00	.10	2.10	0.00	.10	.10	7.80	.20	0.00	.10	2.30	.10	.10	.10	2.40	.40	.40	.70	1.50	.30	.30
	WOC	.10	2.10	.10	.20	.10	1.30	.10	.20	.10	1.30	.10	.10	.20	1.40	.10	.20	.10	1.50	.40	.40	.60	1.60	.70	.80
[Lac]	DF	2.30	2.30	3.70	6.00	3.50	3.50	3.50	11.90	2.70	2.70	6.00	5.80	2.10	2.10	7.60	7.20	3.10	3.10	3.00	5.50	3.90	3.90	3.70	3.90
	JD	1.20	1.20	2.10	7.10	3.50	3.50	3.40	16.70	1.60	1.60	4.40	5.70	1.70	1.70	3.00	8.60	2.00	2.00	1.70	6.30	2.70	2.70	2.50	5.10
	JH	1.50	1.50	2.10	7.00	1.80	1.80	2.00	3.60	2.10	2.10	1.70	7.30	1.40	1.40	1.60	6.80	2.00	2.00	2.00	5.70	2.90	2.90	1.10	5.00
	MM	1.50	1.50	3.00	12.10	2.50	2.50	2.90	15.20	1.70	1.70	2.20	14.90	1.90	1.90	6.30	16.40	1.50	1.50	3.30	11.60	1.80	1.80	3.10	7.70
	TM	1.20	1.20	1.20	8.70	1.30	1.30	1.10	12.50	1.50	1.50	1.10	6.20	2.30	2.30	1.50	8.40	2.50	2.50	3.50	7.50	1.60	1.60	2.30	5.20
	WOC	2.10	2.10	3.60	13.00	0.00	1.30	3.30	6.70	2.90	2.90	10.00	8.70	0.00	1.40	5.90	10.80	2.00	2.00	3.70	4.40	0.00	1.60	3.30	6.20
[Glu]	DF	6.10	6.70	4.90	4.40	5.60	6.80	4.60	4.40	5.30	5.30	4.30	5.40	6.10	5.40	4.20	4.30	4.30	5.50	3.70	4.10	4.60	5.20	4.00	4.90
	JD	5.10	6.40	3.70	3.50	4.80	3.40	5.20	4.10	6.20	5.70	4.00	4.10	5.10	5.20	4.20	3.90	5.70	4.90	5.10	4.10	6.40	3.80	3.20	3.80
	JH	5.70	5.10	4.40	4.80	4.40	3.10	4.00	5.70	4.90	6.20	4.70	5.30	5.30	5.10	4.70	5.00	4.50	4.90	4.20	4.60	5.70	4.20	4.40	4.30
	MM	5.90	3.90	3.70	4.40	5.90	4.90	4.50	5.10	5.90	6.10	4.80	4.60	6.60	6.60	5.30	5.90	6.20	4.70	5.30	4.90	4.60	5.40	3.60	3.70
	TM	5.80	4.40	5.10	3.90	5.80	4.80	4.90	4.50	5.30	4.40	4.90	0.00	6.00	5.10	3.90	5.00	5.00	4.90	4.40	3.80	4.80	5.30	4.50	5.10
	WOC	5.60	6.20	5.90	4.30	5.20	5.20	3.90	3.70	5.60	5.20	3.90	3.40	5.90	5.90	4.40	5.00	4.60	4.90	4.70	7.70	5.90	5.90	5.40	5.70

Table 14.22. continued. Individual physiological data from Ketone Supplement Study (Five). Absolute VO_2, Respiratory Exchange Ratio (RER)

		BASE								PLB								SUP							
		Carb				Keto				Carb				Keto				Carb				Keto			
		rest	Pre	75%	TT	rest	Pre	75%	TT	rest	Pre	75%	TT	rest	Pre	75%	TT	rest	Pre	75%	TT	rest	Pre	75%	TT
Absolute VO2	DF		.38	3.62	3.86		.43	3.80	3.81		.35	3.61	3.89		.39	3.61	3.75		.37	3.60	4.09		.44	3.61	4.06
	JD		.30	3.42	3.88		.21	3.55	4.02		.36	3.51	3.71		.35	3.86	4.27		.40	3.46	4.12		.35	3.73	4.29
	JH		.33	3.32	4.30		.36	3.05	3.55		.35	3.07	4.21		.39	3.18	4.13		.35	2.99	4.07		.37	3.15	3.99
	MM		.32	2.53	3.35		.37	2.58	3.66		.36	2.65	3.37		.35	2.85	3.66		.34	2.73	3.18		.36	2.67	3.49
	TM		.37	2.82	4.69		.40	.00	4.65		.43	2.94	3.98		.37	3.10	4.30		.44	2.68	4.56		.39	3.11	3.94
	WOC		.49	4.23	4.92		.48	4.57	5.61		.48	4.38	4.50		.48	4.25	5.09		.53	4.07	4.96		.44	4.05	5.27
RER	DF		1.09	1.04	1.04		1.10	1.02	1.05		.93	1.06	1.02		1.06	1.02	1.35		.91	1.05	.99		1.00	.96	.98
	JD		1.04	1.04	1.03		.93	.99	1.01		1.01	.98	1.03		.85	.89	.94		.93	.93	.96		.78	.86	.86
	JH		.91	.98	1.08		.93	.94	.95		1.04	1.01	1.07		.97	.96	1.01		1.00	.99	1.07		.85	.95	.97
	MM		.89	.92	1.03		.89	.88	1.03		.91	.92	.98		.84	.91	1.02		.88	.95	1.03		.83	.92	1.09
	TM		.85	.86	.95		.96	4.83	.99		.92	.90	.92		.84	.86	.93		.79	.86	.90		.77	.85	.90
	WOC		1.11	1.00	1.05		.75	.84	.85		1.00	.98	1.00		.94	.88	.92		1.01	1.03	1.03		.81	.88	.93

14. 5. APPENDIX D

Table 14-24. Profile of Mood State (POMS) test sheet.

Name:			Date:		Time:
Friendly	Not at all	A little	moderately	Quite a lot	Extremely
Tense	Not at all	A little	moderately	Quite a lot	Extremely
Angry	Not at all	A little	moderately	Quite a lot	Extremely
Worn Out	Not at all	A little	moderately	Quite a lot	Extremely
Unhappy	Not at all	A little	moderately	Quite a lot	Extremely
Clear Headed	Not at all	A little	moderately	Quite a lot	Extremely
Lively	Not at all	A little	moderately	Quite a lot	Extremely
Confused	Not at all	A little	moderately	Quite a lot	Extremely
Sorry for things done	Not at all	A little	moderately	Quite a lot	Extremely
Shaky	Not at all	A little	moderately	Quite a lot	Extremely
Listless	Not at all	A little	moderately	Quite a lot	Extremely
Peeved	Not at all	A little	moderately	Quite a lot	Extremely
Considerate	Not at all	A little	moderately	Quite a lot	Extremely
Sad	Not at all	A little	moderately	Quite a lot	Extremely
Active	Not at all	A little	moderately	Quite a lot	Extremely
On Edge	Not at all	A little	moderately	Quite a lot	Extremely
Grouchy	Not at all	A little	moderately	Quite a lot	Extremely
Blue	Not at all	A little	moderately	Quite a lot	Extremely
Energetic	Not at all	A little	moderately	Quite a lot	Extremely

Panicky	Not at all	A little	moderately	Quite a lot	Extremely
Hopeless	Not at all	A little	moderately	Quite a lot	Extremely
Relaxed	Not at all	A little	moderately	Quite a lot	Extremely
Unworthy	Not at all	A little	moderately	Quite a lot	Extremely
Spiteful	Not at all	A little	moderately	Quite a lot	Extremely
Sympathetic	Not at all	A little	moderately	Quite a lot	Extremely
Uneasy	Not at all	A little	moderately	Quite a lot	Extremely
Restless	Not at all	A little	moderately	Quite a lot	Extremely
Unable to Concentrate	Not at all	A little	moderately	Quite a lot	Extremely
Fatigued	Not at all	A little	moderately	Quite a lot	Extremely
Helpful	Not at all	A little	moderately	Quite a lot	Extremely
Annoyed	Not at all	A little	moderately	Quite a lot	Extremely
Discouraged	Not at all	A little	moderately	Quite a lot	Extremely
Resentful	Not at all	A little	moderately	Quite a lot	Extremely
Nervous	Not at all	A little	moderately	Quite a lot	Extremely
Lonely	Not at all	A little	moderately	Quite a lot	Extremely
Miserable	Not at all	A little	moderately	Quite a lot	Extremely
Muddled	Not at all	A little	moderately	Quite a lot	Extremely
Cheerful	Not at all	A little	moderately	Quite a lot	Extremely
Bitter	Not at all	A little	moderately	Quite a lot	Extremely
Exhausted	Not at all	A little	moderately	Quite a lot	Extremely
Anxious	Not at all	A little	moderately	Quite a lot	Extremely
Ready to Fight	Not at all	A little	moderately	Quite a lot	Extremely

Good Natured	Not at all	A little	moderately	Quite a lot	Extremely
Gloomy	Not at all	A little	moderately	Quite a lot	Extremely
Desperate	Not at all	A little	moderately	Quite a lot	Extremely
Sluggish	Not at all	A little	moderately	Quite a lot	Extremely
Rebellious	Not at all	A little	moderately	Quite a lot	Extremely
Helpless	Not at all	A little	moderately	Quite a lot	Extremely
Weary	Not at all	A little	moderately	Quite a lot	Extremely
Bewildered	Not at all	A little	moderately	Quite a lot	Extremely
Alert	Not at all	A little	moderately	Quite a lot	Extremely
Deceived	Not at all	A little	moderately	Quite a lot	Extremely
Furious	Not at all	A little	moderately	Quite a lot	Extremely
Efficient	Not at all	A little	moderately	Quite a lot	Extremely
Trusting	Not at all	A little	moderately	Quite a lot	Extremely
Full of Pep	Not at all	A little	moderately	Quite a lot	Extremely
Bad Tempered	Not at all	A little	moderately	Quite a lot	Extremely
Worthless	Not at all	A little	moderately	Quite a lot	Extremely
Forgetful	Not at all	A little	moderately	Quite a lot	Extremely
Carefree	Not at all	A little	moderately	Quite a lot	Extremely
Terrified	Not at all	A little	moderately	Quite a lot	Extremely
Guilty	Not at all	A little	moderately	Quite a lot	Extremely
Vigorous	Not at all	A little	moderately	Quite a lot	Extremely
Uncertain about things	Not at all	A little	moderately	Quite a lot	Extremely
Bushed	Not at all	A little	moderately	Quite a lot	Extremely

Table 14-25. MyFitnessPal® online diet recording software – moderate CHO

FOODS	Calories	Carbs	Fat	Protein	Cholest	Sodium	Sugars	Fiber
Breakfast								
Denny's - Coffee W/cream, 10 oz.	60	2g	5g	1g	0mg	0mg	0g	0g
Pams (Nz) - Rolled Oats, 90 g	288	51g	5g	12g	0mg	1mg	0g	8g
Arataki - Honey Clover Blend, 15 g	51	13g	0g	0g	0mg	3mg	13g	0g
Banana Raw - Banana, 2 banana (medium)	210	54g	0g	2g	0mg	2mg	28g	6g
Rice Dream - Rice Milk Original, 1.2 cup	144	28g	3g	1g	0mg	120mg	12g	0g
Lunch								
Z Energy - Flat White - Regular Size, 1 Regular Cup	62	5g	3g	3g	0mg	43mg	5g	0g
Tomatoes - Raw, 148 g (1 medium tomato)	25	5g	0g	1g	0mg	20mg	3g	1g
Onions - Raw, 1 small	29	7g	0g	1g	0mg	2mg	3g	1g
Farrah Wraps - Garden Spinach Wrap, 1 Wrap	171	29g	4g	5g	0mg	451mg	3g	3g
Anchor - Butter, 15 g	108	0g	12g	0g	0mg	90mg	0g	0g
Carrots - Vegetable	35	8g	0g	1g	0mg	65mg	5g	2g
Kiwi (Nz) - Shoulder Bacon, 75 g (1 slice)	137	0g	10g	11g	0mg	900mg	0g	0g
Dinner								
Healthy Chef - Nacho Beans With Mince, 1 bowl	318	23g	11g	32g	0mg	0mg	0g	11g
Farrah Wraps - Garden Spinach Wrap, 1 Wrap	226	36g	5g	8g	0mg	652mg	2g	3g
Bakers Delight - Date Scone, 2 Scone	550	106g	9g	11g	0mg	1,184mg	42g	8g
Snacks								
Go Native Raspberry & Apple - Fruit Bar, 8 bar	560	127g	1g	2g	0mg	80mg	117g	11g
TOTAL:	**2,974**	**494g**	**68g**	**91g**	**0mg**	**3,613mg**	**233g**	**54g**

Table 14-26. MyFitnessPal® online diet recording software – low CHO

FOODS	Calories	Carbs	Fat	Protein	Cholest	Sodium	Sugars	Fiber
Breakfast								
Solo's Choice - Coconut Cream, 61 mls	81	1g	8g	1g	0mg	13mg	1g	0g
Anchor - Pure Cream ~*~, 210 ml	731	6g	78g	5g	0mg	56mg	6g	0g
Fruzio (Nz) - Mixed Berries, Frozen, 50 g	21	4g	0g	0g	0mg	2mg	4g	0g
Denny's - Coffee W/cream, 30 oz	180	6g	15g	3g	0mg	0mg	0g	0g
Naturalea Acidophilus - Plain Unsweetened Yoghurt, 250 g	144	10g	8g	9g	0mg	100mg	5g	0g
Diamond - Chopped Nuts, 0.25 cup	200	4g	20g	5g	0mg	0mg	1g	2g
Lunch								
Mainland - Tasty Cheddar Cheese, 50 g	212	3g	18g	12g	0mg	334mg	0g	2g
Galaxy - Camembert Cheese, 50 g	160	0g	13g	10g	0mg	354mg	0g	0g
Generic - Hard Boild Egg, 2 large	140	2g	9g	12g	430mg	130mg	0g	0g
Dinner								
Spinach - Raw, 10 leaf	23	4g	0g	3g	0mg	79mg	0g	2g
Carrots, raw, 0.25 cup chopped	13	3g	0g	0g	0mg	22mg	2g	1g
Hellers Nz - Middle Bacon, 2 strip	229	1g	18g	16g	0mg	1,200mg	1g	0g
Onions - Onions Fried, 0.3 cup (210 gm)	28	6g	0g	1g	0mg	2mg	3g	1g
Cauliflower - Fried Rice, 2.0 cup	162	21g	5g	14g	71mg	1,302mg	2g	9g
Snacks								
Anchor - Milk - Light Blue (Nz), 250 ml	116	12g	4g	8g	0mg	103mg	12g	0g
TOTAL:	**2,440**	**83g**	**196g**	**99g**	**501mg**	**3,697mg**	**37g**	**17g**

Table 14-27. MyFitnessPal® online diet recording software – CHO loading.

FOODS	Calories	Carbs	Fat	Protein	Cholest	Sodium	Sugars	Fiber
Breakfast								
Pam's (Nz) - Rolled Oats, 90 g	288	51g	5g	12g	0mg	10mg	2g	8g
Anchor - Blue Standardised Milk, 250 mL	155	12g	8g	8g	0mg	106mg	12g	0g
Fresh Up (Nz) - Crisp Apple Juice, 500 mls	222	54g	0g	0g	0mg	28mg	0g	0g
Lunch								
Pams - Superfruit Strawberry & Blackberry, 62 g (1 bar)	234	37g	7g	4g	0mg	26mg	13g	3g
Pret - Flat White Coffee, 1 cup	75	7g	3g	5g	0mg	0mg	7g	0g
Wattie's (Nz) - Beef Lasagne, 250 g	250	21g	11g	16g	0mg	790mg	10g	0g
Dinner								
Pams - Creamed Rice Chocolate, 420 g	492	83g	12g	13g	0mg	150mg	51g	0g
Watties - Cottage Pie, 540 g	688	56g	37g	29g	0mg	1,128mg	13g	0g
Pams - Superfruit Strawberry & Blackberry, 62 g (1 bar)	234	37g	7g	4g	0mg	26mg	13g	3g
Snacks								
Fresh Up (Nz) - Crisp Apple Juice, 500 mls	222	54g	0g	0g	0mg	28mg	0g	0g
TOTAL:	**2,860**	**412g**	**90g**	**91g**	**0mg**	**2,292mg**	**121g**	**14g**

Table 14-28. Table of chemical shifts - metabolomics.

^{1}H shift (δ)	multiplicity	molecule	assignment	observed	^{13}C shift (δ)
0.66	m	cholesterol	C18 (in HDL)	1D, HMQC	12.6
0.70	m	cholesterol	C18 (in VLDL)	HMQC	
0.84	m	cholesterol	C26 and C27	HMQC	23.3
0.84	t	lipid (mainly LDL)	$CH_3(CH_2)_n$	1D, JRES, COSY, HMQC	14.7
0.87	t	lipid (mainly VLDL)	$CH_3CH_2CH_2C$=	1D, JRES, COSY	
0.91		cholesterol	C21	HMQC	19.4
0.93	m	lipid	CH_3CH_2	COSY	
0.93	t	isoleucine	δ-CH_3	1D, JRES, COSY	
0.95	d	leucine	δ-CH_3	1D, JRES, COSY	
0.97	d	leucine	δ-CH_3	1D, JRES, COSY	
0.97	d	valine	CH_3	1D, JRES, COSY, HMQC	19.6
1.00	d	isoleucine	β-CH_3	1D, JRES, COSY, HMQC	14.6
1.02	d	valine	CH_3	1D, JRES, COSY	
1.13	d	isobutyrate	CH_3	CPMG, JRES	
1.20	d	3-hydroxybutyrate	γ-CH_3	1D, CPMG, JRES	
1.22	m	lipid	$CH_3CH_2CH_2$	HMQC	32.7
1.25	m	lipid (mainly LDL)	$(CH_2)_n$	1D, CPMG, JRES, COSY, HMQC	30.6
1.26	m	lipid	$CH_3CH_2(CH_2)_n$	COSY, HMQC	23.2
1.28	m	isoleucine	half γ-CH_2	COSY	
1.29	m	lipid (mainly VLDL)	$CH_2CH_2CH_2CO$	1D, CPMG, JRES, COSY	
1.30	m	lipid	CH_2	COSY, HMQC	19.7
1.31	d	fucose	CH_3	CPMG, JRES, COSY	
1.32	d	threonine	γ-CH_3	JRES, COSY	
1.32	m	lipid	$CH_2CH_2CH_2CO$	COSY	
1.33	d	lactate	CH_3	JRES, COSY, HMQC	20.9
1.46	d	alanine	CH_3	1D, CPMG, JRES, COSY, HMQC	16.8
1.47	m	isoleucine	half γ-CH_2	JRES, COSY	
1.48	m	lysine	γ-CH_2	CPMG, COSY, HMQC	
1.57	m	lipid (mainly VLDL)	CH_2CH_2CO	JRES, COSY, HMQC	25.6
1.57	m	citrulline	γ-CH_2	ID, COSY	
1.68	m	arginine	γ-CH_2	ID, CPMG, COSY	
1.69	m	lysine	δ-CH_2	1D, COSY	
1.69	m	lipid	CH_2CH_2C=C	1D, JRES, HMQC	27.4
1.71	m	leucine	β-CH_2, γ-CH	COSY, HMQC	40.7
1.86	m	citrulline	β-CH_2	COSY	
1.91	m	lysine	β-CH_2	JRES, COSY, HMQC	30.3
1.91	m	arginine	β-CH_2	COSY	
1.91	s	acetate	CH_3	JRES, CPMG	
1.96	m	isoleucine	β-CH	COSY	
1.97	m	lipid	CH_2C=C	1D, COSY	
1.99	m	proline	γ-CH_2	COSY	
2.00	m	lipid	CH_2C=C	COSY, HMQC	27.8
2.00	m	lipid	CH_2C=C	COSY	
2.00	m	glutamate	half β-CH_2	JRES, COSY	
~2.04	s	glycoprotein[b] (acetyls)	$NHCOCH_3$	1D, JRES, HMQC	23.0
2.05	m	proline	half β-CH_2	COSY	
2.08	m	glutamine	half β-CH_2	1D, JRES, COSY	
2.09	m	glutamine	half β-CH_2	1D, JRES, COSY	
2.13	s	methionine	S-CH_3	CPMG, JRES	
2.14	m	glutamate	half β-CH_2	1D, JRES, COSY, HMQC	30.1
2.22	s	acetoacetate	CH_3	CPMG, JRES	
2.23	m	lipid	CH_2CO	COSY, HMQC	34.6
2.24	spt of d	valine	β-CH	COSY	
2.31	m	3-hydroxybutyrate	half α-CH_2	CPMG, JRES, COSY	
2.36	m	glutamate	half γ-CH_2	COSY, HMQC	34.5
2.36	s	pyruvate	CH_2	ID, CPMG, JRES, CPMG	
2.36	m	proline	half β-CH_2	COSY	
2.38	m	3-hydroxybutyrate	half α-CH2	JRES, COSY	
2.39	ABX	U1[c]	CH_2	COSY	
2.41	m	glutamine	half γ-CH_2	CPMG, JRES, COSY, HMQC	31.9
2.47	t	2-oxoglutarate	γ-CH_2	JRES	
2.52	d	citrate	half CH_2	JRES, COSY	
2.54	s	methylamine	CH_3	CPMG, JRES	
2.54	ABX	U1[c]	CH_2	COSY	
2.68	dd	aspartate	half β-CH_2	JRES	
2.69	d	citrate	half CH_2	JRES, COSY	
2.69	m	lipid	C=CCH_2C=C	COSY	
2.71	s	dimethylamine	CH_3	CPMG, JRES	
2.71	m	lipid	C=CCH_2C=C	1D, COSY	
2.72	m	lipid	C=CCH_2C=C	1D, COSY, HMQC	26.2
2.81	dd	aspartate	half β-CH_2	JRES	
2.83	s	trimethylamine	CH_3	1D, CPMG, JRES	
2.84	dd	asparagine	half β-CH_2	JRES	
2.89	t	albumin lysyl	ϵ-CH_2	1D, COSY, HMQC	40.3
2.94	dd	asparagine	half β-CH_2	JRES	

^{1}H shift (δ)	multiplicity	molecule	assignment	observed	^{13}C shift (δ)
2.96	t	albumin lysyl	ϵ-CH_2	1D, COSY, HMQC	40.3
3.01	t	albumin lysyl[d]	ϵ-CH_2	1D, JRES, COSY, HMQC	40.3
3.04	s	creatine	CH_3	CPMG, JRES	
3.05	s	creatinine	CH_3	1D, CPMG, JRES	
3.06	dd	tyrosine	half β-CH_2	JRES, COSY	
3.12	dd	phenylalanine	half β-CH_2	JRES	
3.14	dd	histidine	half β-CH_2	JRES	
3.15	t	citrulline	γ-CH_2	JRES	
3.16	dd	tyrosine	half β-CH_2	JRES, COSY	
3.21	s	choline	$N(CH_3)_3$	JRES, HMQC	55.0
3.24	t	arginine	δ-CH_2	COSY, HMQC	41.3
3.24	dd	β-glucose	H2	1D, JRES, COSY, HMQC	75.1
3.25	dd	histidine	half β-CH2	JRES	
3.25	t	taurine	CH_2NH	JRES	
3.26	s	TMAO	CH_3	JRES	
3.26	t	U2[c]	ABX	COSY	
3.26	dd	phenylalanine	half β-CH_2	COSY	
3.28	t	*myo*-inositol	H5	JRES	
3.34	m	proline	half δ-CH_2	COSY	
3.40	t	β-glucose	H4	1D, JRES, COSY, HMQC	70.6
3.41	t	taurine	CH_2SO_3	JRES	
3.42	t	α-glucose	H4	JRES, COSY, HMQC	70.6
3.45	m	proline	half δ-CH_2	COSY	
3.47	ddd	β-glucose	H5	JRES, COSY, HMQC	76.7
3.48	dd	threonine	α-CH	JRES, COSY	
3.48	t	β-glucose	H3	JRES, COSY, HMQC	76.7
3.54	dd	α-glucose	H2	JRES, COSY, HMQC	72.3
3.54	dd	U2[c]	CH	COSY, HMQC	
3.54	s	glycine	CH_2	CPMG, JRES	
3.56	dd	*myo*-inositol	H1, H3	JRES	
3.56	dd	glycerol	half CH_2	1D, JRES, COSY	63.5
3.57	d	valine	α-CH	JRES, COSY, HMQC	64.2
3.60	d	threonine	α-CH	JRES	
3.63	dd	*myo*-inositol	H4, H6	JRES	
3.64	dd	glycerol	half CH_2	1D, JRES, COSY	63.5
3.66	m	choline (lipid)	NCH_2	COSY, HMQC	66.7
3.68	t	glutamine	α-CH_2	JRES, COSY, HMQC	55.4
3.69	dd	leucine	α-CH	JRES, COSY, HMQC	55.1
3.70	m	citrulline	α-CH	COSY	
3.71	t	α-glucose	H3	JRES, COSY, HMQC	73.6
3.72	dd	α-glucose	half CH_2-C6	JRES, COSY, HMQC	61.6
3.75	m	U1[c]	α-CH	COSY	
3.76	m	α-glucose	half CH_2-C6	JRES, COSY, HMQC	61.4
3.76	q	alanine	α-CH	JRES, COSY	
3.83	ddd	α-glucose	H5	JRES, COSY, HMQC	72.3
3.84	m	α-glucose	half CH_2-C6	JRES, COSY, HMQC	61.4
3.87	m	glycerol	C2-H	JRES, COSY	72.6
3.90	dd	β-glucose	half CH_2-C6	JRES, COSY, HMQC	61.6
3.93	s	creatine	CH_2	JRES	
3.94	dd	tyrosine	α-CH	JRES	
3.97		phenylalanine	α-CH	JRES, COSY	
3.98	dd	histidine	α-CH	JRES	
3.98	ABX	U2[c]	CH	COSY, HMQC	
4.05	s	creatinine	CH_2	1D, JRES	
4.06	m	glyceryl of lipids	CH_2OCOR	HMQC	62.5
4.06	t	*myo*-inositol	H2	JRES	
4.11	q	lactate	CH	1D, JRES, COSY, HMQC	69.2
4.12	m	proline	α-CH	JRES, COSY	
4.13	m	3-hydroxybutyrate	β-CH	1D, JRES, COSY	
4.24	m	threonine	β-CH	1D, JRES, COSY	
4.25	m	glyceryl of lipids	CH_2OCOR	HMQC	62.5
4.29	m	choline (lipid)	OCH_2	HMQC	60.2
4.53	d	β-galactose	H1	JRES	
4.64	d	β-glucose	H1	1D, CPMG, JRES, COSY, HMQC	
5.20	m	glyceryl of lipids	CHOCOR	COSY	
5.23	d	α-glucose	H1	1D, CPMG, JRES, COSY, HMQC	92.9
5.23	m	unsaturated lipid	$CH{=}C\mathit{H}CH_2C\mathit{H}{=}CH$	1D, COSY	128.6
5.26	m	unsaturated lipid	$CH{=}C\mathit{H}CH_2C\mathit{H}{=}CH$	1D, COSY, HMQC	128.6
5.27	m	unsaturated lipid	$=C\mathit{H}CH_2CH_2$	1D, COSY	
5.29	m	unsaturated lipid	$CH{=}C\mathit{H}CH_2C\mathit{H}{=}CH$	1D, COSY	128.6
5.31	m	unsaturated lipid	$=C\mathit{H}CH_2CH_2$	1D, COSY, HMQC	130.1
5.33	m	unsaturated lipid	$=C\mathit{H}CH_2CH_2$	1D, COSY	
6.87	m	tyrosine	H3, H5	1D, CPMG, HMQC	116.7
7.01	s	3-methylhistidine	H4	1D, CPMG, JRES	
7.02	s	histidine	H4	ID, CPMG, JRES	
7.05	s	1-methylhistidine	H4	ID, CPMG, JRES	

^{1}H shift (δ)	multiplicity	molecule	assignment	observed	^{13}C shift (δ)
7.17	m	tyrosine	H2, H6	CPMG, JRES, COSY	
7.33	m	phenylalanine	H2, H6	1D, CPMG, COSY	
7.38	m	phenylalanine	H4	1D, CPMG, COSY	
7.43	m	phenylalanine	H3, H5	1D, CPMG, COSY	
7.61	s	3-methylhistidine	H2	1D, CPMG, JRES	
7.73	s	histidine	H2	ID, CPMG	
7.77	s	1-methylhistidine	H2	CPMG, JRES	
8.45	s	formate	CH	1D, CPMG	

[a] Abbreviations and Key: s, singlet; d, doublet; t, triplet; q, quartet; spt, septet; m, complex multiplet; dd, doublet of doublets; ddd, doublet of doublets of doublets. Chemical shifts all referenced to H-1 and C-1 of α-glucose at 5.233 for ^{1}H and at 92.9 ppm for ^{13}C. [b] Mainly α_1-acid glycoprotein. [c] U1 and U2 refer to unidentified metabolites. [d] Indicates overlap of free lysine with albumin lysyl and α_1-acid glycoprotein signals.

"Never ask someone to do something you haven't tried yourself"

Professor Steve Stannard

Thank you for reading.